studies in physical and theoretical chemistry 26

AGGREGATION PROCESSES IN SOLUTION

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ELSEVIER SCIENTIFIC PUBLISHING COMPANY
Amsterdam – Oxford – New York 1983

Chapter 16

THE USE OF NMR SPECTROSCOPY IN THE STUDY OF HYDROGEN BONDING IN SOLUTION

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16.1 INTRODUCTION

What can we learn about hydrogen bonding in solution from NMR spectroscopy? Some years ago the answer would probably have been that NMR chemical shift studies provide in, favourable cases, knowledge of the type and the thermodynamics of hydrogen bonding between proton donors and acceptors. Arnold and Packard [1] and Liddel and Ramsey [1] had shown in 1951 that the position of the NMR signal of the hydroxylic protons of alcohols shifted to lower magnetic fields or to higher frequencies upon the formation of hydrogen bonded associates as the temperature was lowered or the concentrations increased. Since then there have been numerous NMR studies of hydrogen bond formation and the subject has often been reviewed. We mention the books by Pople [2] in 1959, Vinogradov and Linnell [3] in 1971, and Joesten and Schaad [4] in 1974. The last survey of this topic was - to our knowledge - Tucker and Lippert's article [5] in 1976.

Today the answer to our question is much more difficult because the measurement of chemical shifts in proton donor containing systems - though still important - is no longer the only way of studying hydrogen bonding in solution by NMR spectroscopy. A number of systems are now known where the lifetimes of the hydrogen bonded species are long enough to permit a direct observation of these species by NMR spectroscopy. These are systems which are able to form intramolecular as well as intermolecular hydrogen bonds, especially biologically important molecules such as peptides and ribonucleic acids. Their study was made possible by the development of multinuclear high field Fourier Transform NMR spectroscopy [6,7]. Further insight into the dynamics of hydrogen bonding and the related proton exchange was gained from the study of NMR lineshapes and relaxation times. Surveys of several of these topics have appeared and are cited below.

A complete account of all recent work concerning hydrogen bonding in solution and NMR spectroscopy is beyond the scope of this article. Our aim is simply to show how NMR methods can contribute today to answering the above question. As this is still a wide field the description of hydrogen bond effects in NMR spectroscopy will be mainly of a qualitative nature. For more quantitative descriptions the reader will be referred to the original literature. Section 16.2 gives

Studies in Physical and Theoretical Chemistry Vol. 26
AGGREGATION PROCESSES IN SOLUTION
edited by E.WYN-JONES and J.GORMALLY
Elsevier Scientific Publishing Company Amsterdam-Oxford-New York 1983

a general introduction into the NMR spectroscopy of proton donors in solution as influenced by hydrogen bonding and proton exchange. Section 16.3 is devoted to chemical shift studies of hydrogen bond equilibria in solution. The association behaviour of systems containing one as well as several proton donor groups, especially biologically important systems, are reviewed. The last section 16.4, is concerned with recent progress in the study of systems with extended intramolecular hydrogen bonding such as H-chelates, peptides and ribonucleic acids.

16.2 NMR-SPECTROSCOPY OF PROTON DONORS IN SOLUTION

This section deals with the influence of the kinetics and the thermodynamics of hydrogen bonding and of proton exchange on the NMR spectra and the NMR relaxation behaviour of proton donors in solution.

16.2.1 The influence of hydrogen bond formation on the NMR-spectra of proton donors

A proton donor AH can exert an attractive force on a base B containing free electron pairs and form a hydrogen bond complex with a defined AB distance and a defined AHB angle [1-5]:

$$AH + B \rightleftharpoons AH\cdots B \qquad (1)$$

In solution the solvent S may act as a base, which leads to hydrogen bonding with the solvent [1-5]:

$$AH + S \rightleftharpoons AH\cdots S. \qquad (2)$$

If the molecule AH has basic properties self-association occurs [1-5]:

$$n\ AH \rightleftharpoons (AH)_n \qquad (3)$$

The shielding of the hydrogen bond proton is decreased during these processes and the corresponding ^1H NMR signal is shifted to lower magnetic fields or higher Larmor frequencies [1-5]. For a theoretical understanding of this effect the reader is referred to the review of Kollman and Allen [8]. The typical frequency shifts are in the order of 100 to 1000 Hz.

However, the rates of interconversion between the species are much higher. The dissociation rate constant [9] of benzoic acid dimer in CCl_4 at 20^oC has, for example, a value of $3.3 \cdot 10^5\ s^{-1}$, and the bimolecular association rate constant a value of $6.6 \cdot 10^9\ l\ mol^{-1}\ s^{-1}$. Such kinetic parameters are obtained by ultrasonic absorption and dielectric relaxation techniques [9-12]. It is a general spectroscopic rule that only an averaged spectrum is observed for a molecule in different

environments when the interconversion rates between these environments are higher than the frequency differences between the corresponding lines. Therefore, only one NMR line or group of NMR lines is generally observed for the associating AH molecule. If δ_i is the chemical shift of a nucleus in the environment i, and x_i the mole fraction of this environment the average chemical shift δ of the nucleus is given according to Gutowsky and Saika [13] by

$$\delta = \sum_i x_i \delta_i \tag{4}$$

The chemical shifts δ_i are related to the absorption frequencies ν_i by

$$\delta_i = (\nu_i - \nu_{ref}) \cdot 10^6 / \nu_{ref} \tag{5}$$

where ν_{ref} is the absorption frequency of a reference compound.

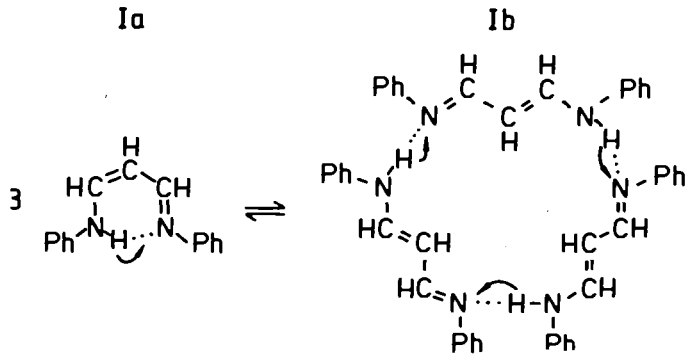
However, hydrogen bond substitution reactions of the type

$$AH\cdots B + B' \rightleftharpoons AH\cdots B' + B \tag{6}$$

are in some cases so slow that AH···B and AH···B' can be observed directly by NMR spectroscopy. The first part of this section deals with this aspect, whereas in the second part problems associated with NMR studies of the thermodynamics of hydrogen bonding in the fast hydrogen bond exchange range based on eq. (4) are treated.

16.2.1.1 Proton donor systems in the slow hydrogen bond exchange range and hydrogen bond exchange kinetics

There are only few examples in the literature where molecules have been observed directly by NMR spectroscopy in different competing hydrogen bonded states.



$\Delta H = -52\,kJ\,mol^{-1}$ $\Delta S = -203\,JK^{-1}mol^{-1}$

One of the first examples was provided by Limbach and Seiffert [14-16]. They showed that the monomer and the trimer of N,N'-diphenyl-1-amino-3-iminopropene (I) interconverted slowly in CS_2 at low temperatures. The low field NH region of the ^1H NMR spectra of I in CS_2 contained two signals at low temperatures as shown in Figure 1.

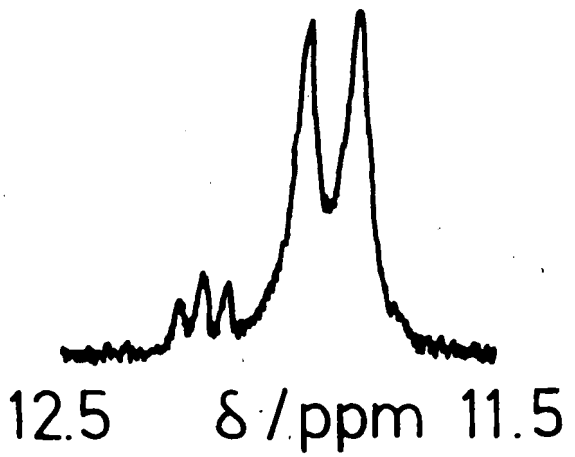


Fig. 16.1. Low field NH-region of the 100 MHz ^1H-NMR-spectra of I in CS_2 at -82°K and 0.29 mol l^{-1}. (Reproduced with permission from ref. (15)).

The triplet was due to the monomer Ia, the doublet due to the cyclic trimer Ib which has trans-conformation as proved by the analysis of the whole NMR spectrum. These line splittings arise from the coupling of the NH protons with the adjacent CH groups. The triplet in Ia indicates delocalisation of the NH proton between the two nitrogens within the NMR timescale. However, the NH protons in Ib are localised on one nitrogen at low temperature as shown by the doublet splitting.. The mean association number n = 3 for Ib was obtained from the dependence of the ratio Ia/Ib on the concentration of I. n did not change with temperature. The interconversion rates [17] between Ia and Ib were of the order of 10^{-1} s^{-1} and did not affect the lineshapes. These were, however, affected by intermolecular proton exchange. As shown in section 16.2.2.2 evidence for the cyclic structure of Ib came from the activation parameters of this exchange process.

Fratiello et al. [18] reported two hydroxyl proton signals for mixtures of $(CF_3)_2CH$-OH (II) with triethylamine (TEA) in ether, when the samples were cooled to -125°C. The two signals can be ascribed to the two complexes of II with TEA and ether. The energy of activation for the corresponding hydrogen bond exchange process according to eq. (6) was found to be of the order of 18 kcal mol^{-1}. Proton transfer to TEA and ion pair formation was excluded from IR spectroscopic results.

An important contribution to the study of hydrogen bond exchange kinetics by dynamic NMR spectroscopy comes from the work of Golubev, Denisov et al. [19-21].

In a study of o-formylphenol (III) with hexamethylphosphoramide (HMPA) in CHF_2Cl they obtained ^1H NMR spectra as shown in Figure 2 [19]:

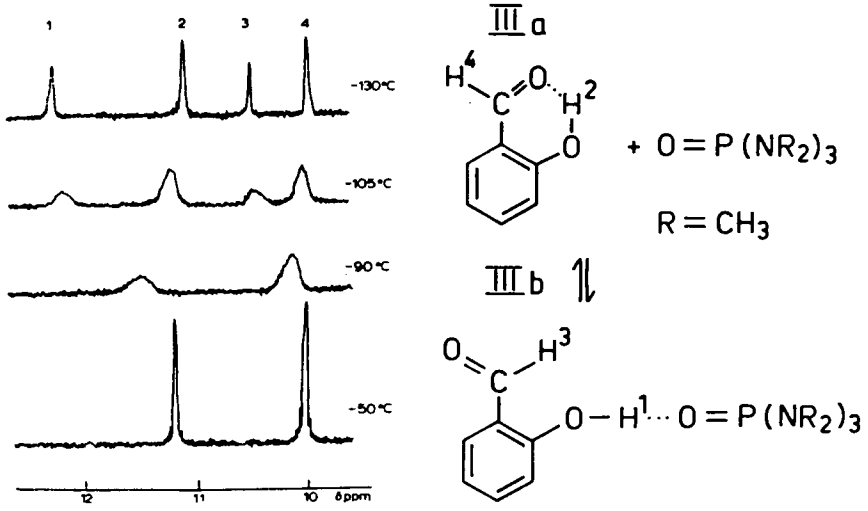


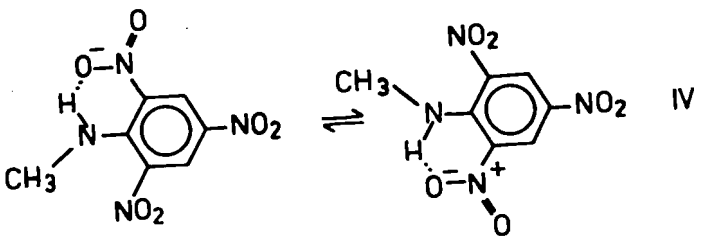
Fig. 16.2. 100 MHz ^1H NMR-spectra of III (0.06 mol l^{-1}) in CHF_2Cl. Reproduced with permission from ref. (19).

At low temperatures the two environments IIIa and IIIb give well resolved sharp NMR signals. As the temperature is raised the exchange between the environments becomes faster and the lines broaden. At higher temperatures line 1 has coalesced with line 2 and line 3 with line 4 due to fast exchange. The coalesced lines are not in the middle between the non coalesced lines. This is due to the fact that at higher temperatures IIIa dominates which is about 13 kJ mol^{-1} less stable than IIIb. As the chemical shifts δ_i were known from the low temperature spectra, the molefractions of the environments could be determined from the line positions using eq. (4). The mole fractions in the slow exchange range could be determined by integration. The rate constants were determined by lineshape analysis using NMR lineshape theory for exchange between two different sites [22]. From the high activation energy of 46 kJ mol^{-1} needed for the formation of IIIa from IIIb the authors postulate a non hydrogen bonded form of III as intermediate and discard a hydrogen bond substitution mechanism. The thermodynamics and the kinetics of similar reactions of 2-nitrophenol and 2-acetylphenol with HMPA and collidin were similarly reported. Similar experimental findings and conclusions were obtained by the same authors [20] by measuring the NMR spectra of carboxylic acids in the presence of HMPA in CHF_2Cl. The kinetics and thermodynamics of the following reaction were studied.

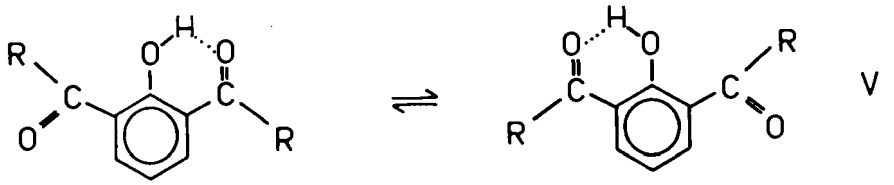
$$2\ RCOOH\cdots B \rightleftharpoons (RCOOH)_2 + 2B \qquad (8)$$

Care had to be taken to exclude complications arising from proton exchange with residual water in the samples and from the formation of more complicated aggregates at higher concentrations.

This section would not be complete if we did not mention the purely intramolecular rearrangements of molecules IV and V containing intramolecular hydrogen bonds studied by NMR lineshape analysis:



IV



V

As shown by Heidberg et al. [23,24] the energy of activation of the rearrangement of IV is 61 kJ mol^{-1} and log A = 15. This indicates a considerable loss of hydrogen bond energy in the transition state. In picric acid where the $NHCH_3$ group of IV is replaced by an OH group the rearrangement is immeasurably fast [23]. Koelle and Forsen [25] showed that the energy of activation for the rearrangement of V was of the same order as the values found for the formyl group rotation in reference compounds which did not contain an OH group i.e. an intramolecular hydrogen bond.

There are more examples in the literature of long lived hydrogen bonded species such as FHF^-, H-chelates, and biomolecules with extended intramolecular hydrogen bonding. Because these systems are characterized by the presence of only one hydrogen bonded state we shall deal with them in section 16.4. We may conclude that hydrogen bond exchange is, in general, very fast. Exchange between different environments can, however, be slow enough for detection by NMR spectroscopy if the hydrogen bond exchange is coupled to other processes such as rotations, helix coil transitions or other rearrangements.

16.2.1.2 Thermodynamics of hydrogen bond formation from chemical shift measurements in the fast hydrogen bond exchange range

In this section we shall examine the determination of equilibrium constants by hydrogen bond equilibria in the fast exchange range from the position of NMR lines using eq. (4). For the simplest hydrogen bond reaction

$$AH + B \rightleftharpoons AH\cdots B \tag{9}$$

eq. (4) can be written in the form:

$$\delta = \frac{c_A}{C_A} \cdot \delta_A + \frac{c_{AB}}{C_A} \cdot \delta_{AB} \tag{10}$$

where c_A is the concentration of the monomeric AH molecules, c_{AB} the concentration of complexed AH molecules, and $C_A = c_A + c_{AB}$ the total AH concentration. By introducing the equilibrium constant

$$K = \frac{c_{AB}}{c_A c_B} = \frac{c_{AB}}{(C_A - c_{AB})(C_B - c_{AB})} \tag{11}$$

into eq. (10) one obtains δ as a function of C_A and C_B which are determined by the preparation of the samples:

$$\delta = \delta_A + \frac{\delta_{AB} - \delta_A}{2C_A} \left[C_A + C_B + \frac{1}{K} - \left((C_A - C_B)^2 + \frac{2}{K}(C_A + C_B) + \frac{1}{K} \right)^{\frac{1}{2}} \right]. \tag{12}$$

Eq. (12) was first used by Wiley and Miller [26]. The limiting cases are $\delta = \delta_A$ for $C_B \to 0$ and $\delta = \delta_{AB}$ for $C_B \gg C_A$. The determination of δ as a function of C_A and C_B allows one to calculate the three unknowns K, δ_A, and δ_{AB} using a non-linear least squares fitting procedure.

In the past when modern computer facilities were not available, several approximations to eq. (12) were used. By combination of eq. (10) and (11) Higuchi et al. [27] found

$$y = \frac{C_B}{\delta - \delta_A} = \frac{1}{\delta_{AB} - \delta_A} (C_A + C_B - c_{AB}) + \frac{1}{K(\delta_{AB} - \delta_A)} \tag{13}$$

which is still exact. In the Higuchi method one plots y against $C_A + C_B - c_{AB}$ where c_{AB} is neglected in first order. From the slope and the intercept K is calculated which yields c_{AB} using eq. (11). The plot is now repeated with the improved values of c_{AB} and K calculated until selfconsistency is achieved. Another approximation of eq. (12) is the equation of Benesi-Hildebrand [28]

$$\frac{1}{\delta-\delta_A} = \frac{1}{\delta_{AB}-\delta_A} + \frac{1}{KC_B(\delta_{AB}-\delta_A)} \qquad (14)$$

which has frequently been used, though also severely criticised [29,30]. This equation shows that at low C_A the chemical shifts δ no longer depend on C_A. By plotting δ as a function of C_B one obtains convex curves which are linear at low C_B where $KC_B << 1$ as shown by Shoolery et al. [31,32]:

$$\delta = (\delta_{AB}-\delta_A)\ K\ C_B + \delta_A \qquad (15)$$

Though K cannot be determined from the limiting slope because $\delta_{AB}-\delta_A$ is unknown, as a rule the association enthalpy can be obtained by measuring the slopes as a function of the temperature and by neglecting the dependence of $\delta_{AB}-\delta_A$ on the temperature.

Eq. (10) to (15) can be easily extended to the case of self-association

$$AH + AH \rightleftharpoons AH\cdots AH \qquad (16)$$

by setting A=B and $C_A = C_B = C/2$, where C is the total concentration of AH. δ_{AA} is then the average chemical shift of the two protons in the dimer. In general, the self-association is not limited to dimers; higher aggregates are also formed according to eq. (3):

$$n\ AH \rightleftharpoons (AH)_n$$

By introducing the appropriate mass action law into eq. (4) one obtains

$$\delta = (c_1\ \delta_1 + nc_1^{\ n}\ \delta_n K_n)/C \qquad (17)$$

where c_n is the concentration of $(AH)_n$. $C=c_1+nc_n$ the total AH concentration, and δ_n the average chemical shift in the associate. By rearrangement of eq. (17) Lippert [33] obtained the equation

$$\left(\frac{\delta-\delta_1}{C}\right)^{1/n} = ((\delta_n - \delta_1)n\ K_n)^{1/n} - ((\delta_n - \delta_1)^{1-n}\ n\ K_n)^{1/n}\ (\delta-\delta_1) \qquad (18)$$

If the association process can be described by the simple eq. (3) a plot of eq. (18) is linear only for one n value which can be determined in this way. However, in general more than one type of associate is present in solutions of OH and NH proton donors, and the curve $\delta = \delta(C)$ depends on at least five parameters:

two equilibrium constants and three chemical shifts. As Tucker and Lippert [5] discussed in their review, these chemical shift dilution curves do not contain enough information for the determination of these parameters. According to these authors the NMR method may give equivocal results if more than two species influence the chemical shifts, especially in the case of alcohols and phenols. The evaluation of equilibrium constants for the AH···B association according to eq. (1) may also be complicated by AH self-association as a side reaction. If dimerisation dominates, the corresponding equilibrium constant can, however, be determined in the absence of the base B and introduced into a formalism which permits the determination of the AH···B association constant. This procedure was successfully applied by Zimmermann et al. [34,35] in NMR studies of the association of phenols with bases.

Many of these problems of multiple association may be circumvented by using non inert solvents such as tetrahydrofuran which act as hydrogen bond acceptors. In such solvents, S, the number of hydrogen bonds is retained during the association process:

$$AH\cdots S + B \rightleftharpoons AH\cdots B + S \qquad (19)$$

B may be another AH molecule or a base. The formation of true monomers or higher associates does not affect the chemical shifts because the great concentration c_S of free solvent molecules shifts all the equilibria on the side of the quasi monomers which are hydrogen bonded to the solvent. Another advantage of the use of hydrogen bond acceptors is that in biological systems association is better described by eq. (19), where $S=H_2O$, than by eq. (1). Additionally, basic solvents make proton exchange reactions, which could affect the chemical shifts, very slow. Since the solvent takes part in the association process it must be taken into account in calculating the chemical shift dilution curves. This problem has been solved by Gerritzen and Limbach [36] using the dimerisation and mixed 1:1 association of, in their example, methanol and acetic acid in tetrahydrofuran. They treated the general case

$$A\cdots S_1 + B\cdots S_m \rightleftharpoons A\cdots B\cdots S_{1+m-n} + n\ S \qquad (20)$$

where the equilibrium constant is given by

$$K^* = \frac{c_{AB} c_S^{\,n}}{c_{AS} c_{BS}} = K \cdot c_S^{\,n} \qquad (21)$$

The apparent equilibrium constant K is as defined in eq. (11). By combining

eqs. (12)-(15) with eq. (21) one obtains the chemical shifts as a function of the total reactant concentrations C_A, C_B, the free solvent concentration c_S and the number n of liberated solvent molecules. c_S decreases as C_A and C_B are increased, which favours the dimerisation process. The following expression was derived [36]:

$$c_S = \bar{V}_S^{-1} - C_A(1 + \frac{\bar{V}_A}{\bar{V}_S}) - C_B(m + \frac{\bar{V}_B}{\bar{V}_S}) \qquad (22)$$

The partial molar volumes $\bar{V}_i$ are, in principle, unknown. However, it was shown that the error introduced into the calculation of the chemical shifts was negligible if the $\bar{V}_i$ were approximated by the molar volumes $V_i^o = (C_i^o)^{-1}$ of the pure compounds. The effect of the solvent correction on the chemical shift dilution curves eq. (12) is shown in Figure 3 for the case of dimerisation at different values of

$$K_o = K^*/(C_S^o)^n \qquad (23)$$

and different n values. The parameters were taken for the dimerisation of acetic acid in tetrahydrofuran where n=1 for the formation of linear and n=2 for the formation of cyclic dimers.

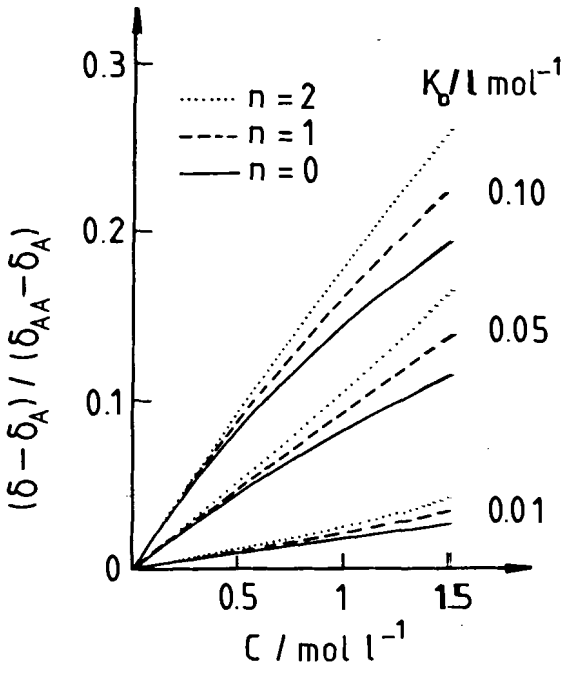


Fig. 16.3. Effect of the free solvent concentration correction on eq. (12) for the case of dimerisation with V_S^o = 81.6 .10^{-3}l mol^{-1} and V_A^o/V_S^o = 0.706.

At the higher K_o values the uncorrected curves with n=0 have a convex curvature above 1 mol l^{-1}. The corrected curves, however, are linear or even concave. The observation of a linear curve is, therefore, not necessarily the proof of the validity of eq. (15) but may be explained by the solvent participation. In fact, the proton chemical shift dilution curves of acetic acid and of methanol in tetrahydrofuran could be explained only in this way [36].

The results of some recent chemical shift studies on hydrogen bonded systems will be discussed in section 16.3.

16.2.2 The influence of proton exchange on the NMR-spectra of proton donors

The NMR spectra of proton donors are not only influenced by the formation and breaking of hydrogen bonds but also of A-H bonds, i.e. by proton exchange

$$AH + BH^* \rightleftharpoons AH^* + BH \qquad (24)$$

reactions. There are several reasons to discuss such reactions at this stage. Fast proton exchange influences the AH chemical shifts as well as hydrogen bond formation. The proton transfer process takes place in a hydrogen bonded complex which has to be formed beforehand. The study of the proton exchange kinetics gives, therefore, information either on the proton motion along hydrogen bonds or on the hydrogen bond formation process depending on the rate determining step. Knowledge of the proton motion in AH/BH complexes allows one to decide whether cyclic or linear complexes have been formed.

The section deals with the kinetic aspects of the problem. We shall concentrate on proton exchange in aprotic solvents which are the preferred media for NMR studies of hydrogen bond formation. The thermodynamic aspects of proton transfer in AH···B complexes studied by chemical shift measurement will be reserved for section 16.3.1.

16.2.2.1 Dynamic NMR-spectroscopy of proton exchange including lineshape and pulse techniques

The effects of proton exchange according to eq. (24) on the NMR-spectra of proton donors have been known for a long time [2] and have been used to study mainly proton exchange in protic solvents. We mention here especially the work of Meiboom and Grunwald et al., reviewed by Grunwald and Ralph [37]. These effects are the apparent loss of spin-spin coupling of the AH proton to the nuclei in the group A and the averaging of the chemical shifts of the AH and the BH protons. The AH selfexchange rate and the exchange rate of AH with BH can be obtained from the NMR lineshape analysis. This lineshape analysis has to be carried out using the quantum-mechanical density matrix formalism developed by

Kaplan [38], Alexander [39] and Binsch [40]. The lineshape equations of these authors were, however, dependent on the reaction mechanism. They differed, for example, in the case of a dissociative and a non dissociative reaction mechanism discussed below. Limbach [41] has found a general formulation which avoids assumptions about the reaction mechanism at the stage of extracting the kinetic data from the NMR lineshape. Limbach and Seiffert [42] applied this formalism to the system acetic acid/methanol/tetrahydrofuran-d_8. Some typical superposed experimental and theoretical ^{1}H-NMR-spectra are shown in Figure 4.

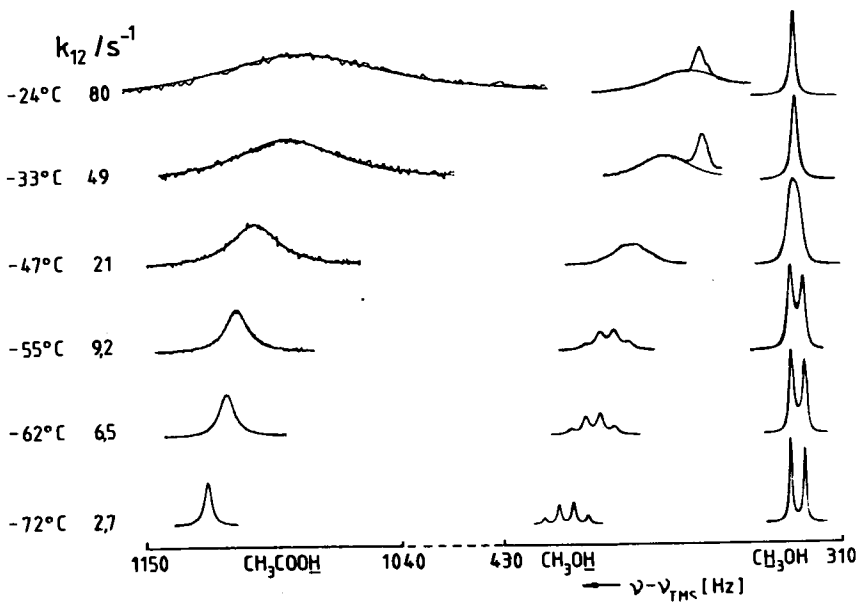


Fig. 16.4. Superposed experimental and calculated 100 MHz ^{1}H NMR spectra of a mixture of 0.29 mol l^{-1} acetic acid and 0.80 mol l^{-1} methanol in tetrahydrofuran-d_8. k_{12} is the inverse proton lifetime in acetic acid. TMS:Tetramethylsilan. Reproduced with permission from ref. (42).

At low temperatures the case of slow proton exchange is reached with separate OH/COOH lines and spin-spin coupling of the methanol signals. At higher temperatures proton exchange between acetic acid and methanol is faster, the methyl doublet of methanol coalasces and the OH and COOH lines broaden. The coalescence point of these two signals can be reached in tetrahydrofuran-d_8 only at high concentrations and temperatures. The proton lifetimes in the two species were determined by adapting the theoretical and experimental spectra. No methanol selfexchange was found in pure samples. However, samples which contained acid impurities showed acid catalyzed methanol selfexchange [41]. The chemical shifts in this system depended on the concentrations and the temperature [36],

a significant case of fast hydrogen bond and slow proton exchange. The slow proton exchange is mainly due to the hydrogen bond formation of the reactants with the solvent, which hinders the formation of the encounter complex. The absence of hydrogen bonding to the solvent leads to very fast proton exchange between the reactants in methylcyclohexane-d_{14} (MCH) and coalescence of the OH/COOH signals as found by Limbach et al. [43]. This effect is shown in Figure 5a. The position of the coalesced line depends mainly on the mole fractions of the exchanging species. In chemical shift studies of proton donors it must, therefore, be checked that fast proton exchange with small amounts of impurities, for example water does not affect the line positions. The chemical shift of a non exchanging proton donor depends linearly on the temperature [44] because of a temperature dependent A-H distance in the hydrogen bond associates and because of the change of the thermodynamic parameters of the association equilibria. If proton exchange with an impurity becomes important as the temperature is raised the curves become s-shaped at intermediate exchange rates and linear again at high rates. Thus proton exchange may be identified even if no lineshape effects on the proton donor NMR lines are observable.

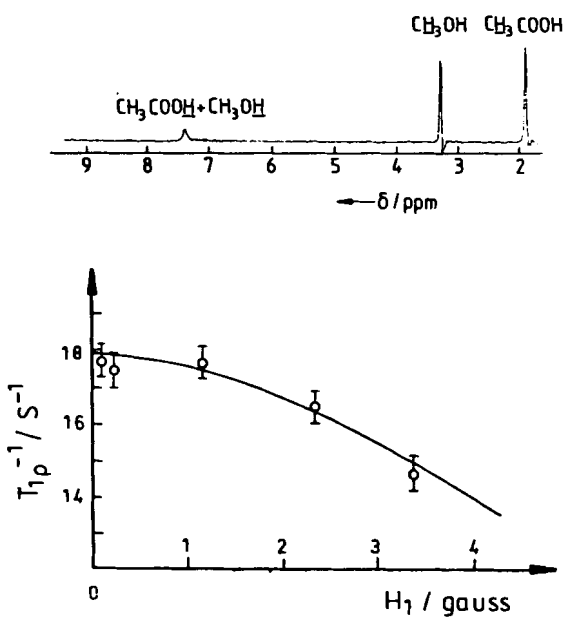


Fig. 16.5a: ^{1}H-NMR spectrum of 0.08 mol l^{-1} acetic acid and methanol in MCH at 100 MHz and 299 K. b: $T_{1\rho}$-relaxation experiment at 90 MHz, where T_1 (COOH/OH) = 6.2 s. (See ref. 43).

The coalesced signal in Figure 5a still contains an exchange broadening of about

6 Hz. In order to extract an exchange rate from this quantity the frequency difference $\delta\nu$ of the coalesced lines have to be known. $\delta\nu$ can be obtained by measuring the longitudinal relaxation time in the rotating frame, $T_{1\rho}$ as a function of the so called spin locking pulse strength H_1 [45-47]. The results of the experiment on the sample in Figure 5a are shown in Figure 5b. By non-linear least squares fitting procedure a value of $\delta\nu$ (100 MHz) = 660±20 Hz and an exchange rate of $k_{12} = 8.8 \cdot 10^4\ s^{-1}$ was obtained. $\delta\nu$ is of the same order as in THF [36] whereas k_{12} is about 400 times greater. In principle, similar results could be obtained by the spin-echo method [45], which has, however, been quantitatively applied only very recently by Frahm [48] to systems with more than one line in the NMR spectrum.

The knowledge of the kinetic hydrogen/deuterium isotope effects is important for the discussion of proton exchange mechanisms. Limbach et al. [43] have presented ^{2}H-NMR-lineshape measurements of the system $CH_3COOD/CH_3OD/THF$ and have established the deuteron exchange rates. They found a kinetic isotope effect of about 11 which indicates that the proton exchange is the rate determining step in this system. The complete HH/HD/DH/DD kinetic isotope effects of double proton exchange reactions according to eq. (24) can be obtained by an appropriate combination of ^{1}H and ^{2}H NMR lineshape analysis.

A problem in the study of slow exchange reactions arises if the exchange rates are in the order of seconds but too fast for detection by real time measurements of the H/D substitution rates. In this range the exchange does not affect the observed line widths any more. Forsen and Hoffmann [49] have proposed a solution to this problem. Saturation of one of two slow exchanging NMR lines using double irradiation produced also saturation of the other line. By measuring the saturation transfer rate they were able to determine the chemical exchange rates. The saturation transfer technique has been extended for use with Fourier Transform Pulse NMR spectrometers [50-52]. Selective pulses are applied to one of the slowly exchanging lines and the relaxation of this line as well as the magnetisation transfer to other lines used to determine the exchange rates. The saturation transfer technique has been frequently applied, for example to the study of proton exchange between nucleic acid bases in organic solvents by Iwahashi et al. [53]. The method was used by Redfield et al. for the determination of proton exchange rates between water and amides [54] as well as ribonucleic acids [55]. Kearns et al. [56] recently reported similar studies for DNA fragments. The different methods for the study of slow exchange rates have been compared by Bovee [57] and Baine et al. [58]. If the nuclei in the exchanging sites are coupled by dipole-dipole interaction a complication arises from cross relaxation which leads to an enhancement of the non irradiated line due to the Nuclear Overhauser Effect (NOE) [59]. Campbell et al. [52] have studied this

problem. The use of the NOE in the study of hydrogen bonding is discussed in section 16.2.3.2. Hennig and Limbach [47] have proposed the use of the $T_{1\rho}$ technique in the slow exchange region. As they showed recently [60] this method in an improved version is especially useful when the exchanging lines are very close together. In this case the saturation transfer method fails.

We may conclude that NMR-spectroscopy as a kinetic tool covers a wide range of exchange rates. It is the task of the next section to discuss the information provided by the measurement of proton exchange rates on the reaction mechanism.

16.2.2.2 Single and double proton exchange mechanisms

So far we have not yet discussed the ways in which the protons of two molecules AH and BH may be exchanged, and how it is possible to distinguish between the different pathways by NMR. As has been shown by Grunwald [37] one has to take into account dissociative and non dissociative mechanisms. The dissociative mechanism consists of a series of single proton transfer steps in hydrogen bonded intermediates involving the formation of ions:

$$AH + BH \rightleftharpoons A^- + BH_2^+ \qquad (25)$$

$$AH + BH_2^+ \rightleftharpoons AH_2^+ + BH \qquad (26)$$

$$AH + B^- \rightleftharpoons A^- + BH \qquad (27)$$

These single proton transfers between AH and BH are, however accompanied by self-exchange

$$AH + A^- \rightleftharpoons A^- + AH \qquad (28)$$

$$BH + B^- \rightleftharpoons B^- + BH \qquad (29)$$

$$AH + AH_2^+ \rightleftharpoons AH_2^+ + AH \qquad (30)$$

$$BH + BH_2^+ \rightleftharpoons BH_2^+ + BH \qquad (31)$$

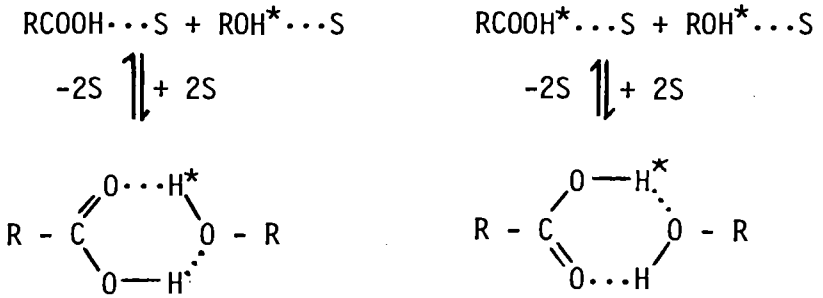
The non dissociative mechanism consists of a double proton transfer reaction in a cyclic hydrogen bonded intermediate:

$$AH + BH^* \rightleftharpoons A\overset{H}{\cdots}B \rightleftharpoons A\overset{H}{\cdots}B \rightleftharpoons AH^* + BH \qquad (32)$$

In the buffered protic solvents studied by Grunwald et al. [37] the ionic exchange dominates. The ions are, however, not formed by dissociation of the reactants but provided by the buffer. The rate constants of the reactions eq. (28) and (30) were measured for AH = water [61] and methanol [62] by dynamic NMR-spectroscopy. The rate constants are of the order of 10^{10} s^{-1} and the processes have a very low activation energy of the order of 2 kcal/mol. Gerritzen and Limbach [63] found a much higher energy of activation for proton exchange in pure methanol and methanol-d_1. In pure methanol the ions A^- and AH_2^+ which catalyze the exchange have to be formed first by autoprotolysis. Their concentration is of the order of 10^{-9} mol/l which yields, with the known rate constants, a proton lifetime of about 10^{-1}s. The energy of activation in the pure solvent is the sum of the activation energy of the proton exchange and half the dissociation energy. The observed primary H/D kinetic isotope effect is mainly the equilibrium isotope effect of the autoprotolysis [63]. In solutions of biopolymers in water even higher energies of activation may be found though the exchange is of the ionic type. This situation occurs when the labile protons of proton donors are blocked in a very long lived hydrogen bond as is the case in peptides [64-66] or nucleic acids [67] of higher molecular weights. In these forms BH is not accessible to the solvent. The rate determining step of the proton exchange is then the unfolding process of the biopolymer, for example, a helix coil transition. The study of proton exchange is, therefore, a convenient way to study the kinetics of such processes.

Non dissociative proton transfer processes have been discussed in the literature for a long time, for example in nucleophilic addition reactions to double bonds which are catalyzed by bifunctional compounds such as water, carboxylic acids, heterocycles [68,69] or enzymes [70]. Grunwald et al. [37,62] have found evidence for cyclic proton exchange between carboxylic acids and alcohols as a side reaction of the ionic exchange processes. Ralph et al. [71] recently established a similar process in solutions of histamine in water. The characteristic feature of these processes shown in eq. (32) is the transfer of two or more protons during one encounter of the reactants. No dissociation into free solvated ions is necessary for this exchange to occur. These non dissociative mechanisms should, therefore, be dominant in aprotic solvents of low dielectric constant. Cyclic proton exchange mechanisms have been postulated in a number of dynamic NMR [16,72-76] and kinetic studies [77] of proton exchange involving alcohols, carboxylic acids, or heterocyclic compounds in aprotic solvents. However, small traces of bases or acids as well as water are able to catalyze the proton exchange even in aprotic solvents which leads to low energies of activation and very negative activation entropies. Consequently, it is very difficult to prove a cyclic double proton exchange mechanism. For the system acetic acid (AH)/

methanol (BH)/THF (S) (Fig. 4) the proof was given by Limbach et al. [41,42] as follows. If proton exchange took place according to the dissociative mechanism eq. (25)-(31) the ions BH_2^+ and B^-, which could also be created by reaction with impurities, would catalyze the proton exchange between methanol molecules. In fact, methanol selfexchange was observed as well as higher rates of exchange between methanol and acetic acid when small traces of sulfuric acid were added to the samples [41]. However, in pure samples the spectra (Fig. 4) could be simulated only with a vanishing methanol selfexchange rate indicating the absence of ions. The bimolecular rate law indicated that the exchange takes place in a 1:1 complex:



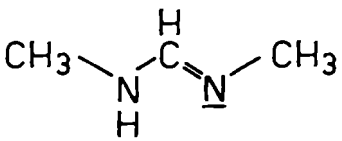
(33)

Hydrogen bond studies showed [36] that the reactants are dominantly in a hydrogen bond complex with the solvent which shifts the fast pre-equilibrium on the side of the quasimonomers. The observed rate constant k_{obs} depends on the pre-equilibrium constant K, the solvent concentration C_S and the exchange rate constant k_{ex} in the complex [42]:

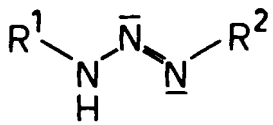
$$k_{obs} = K\, k_{ex}\, C_S^{-2} \qquad (34)$$

From the observed frequency factor $\log A_{obs} = 8.1$ one obtains $\log A_{ex} = 10.3$ by setting K=1. For pure methanol, in which the exchange takes place by autoprotolysis, a frequency factor of log A=6.1 was found [63]. This has to be divided by the methanol concentration for comparison with $\log A_{obs}$ which gives a value of 4.7. One can conclude that a cyclic exchange mechanism is very probable if high frequency factors are found for the exchange. Low frequency factors either indicate a dissociative mechanism or a very high loss of entropy for the complex formation between the reactants. In this way one can explain why proton exchange between the molecules of Ib had a frequency factor of log A=13, whereas a value of 6.9 was found for Ia. A first order rate law of the intermolecular proton exchange was obtained for Ia and Ib. These findings are understandable only if the exchange between the Ib molecules takes place in the cyclic trimer in a non dissociative way. The exchange between the Ia molecules, however, is probably a catalyzed exchange. Halliday et al. [73] found that the frequency

factor of proton exchange between molecules of N,N'-dimethylformamidine (VI) changed from a value of log A = 10 in the pure liquid to a value of about 14 in $CDCl_3$. The order of log A is consistent with a cyclic mechanism.



VI



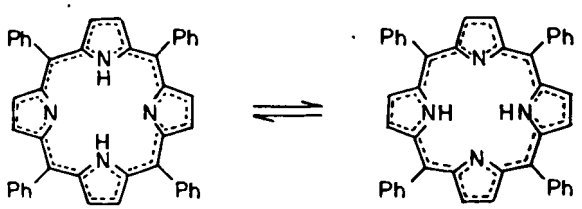
VII

a: $R_1=R_2=CH_3$

b: $R_1=CH_3$

$R_2=Ph$

However, it is difficult to comment on the change in log A because the exact concentration dependence of the rate constants was not measured. Lunazzi et al. [75] found a first order rate law for proton exchange between molecules of VIIb in aprotic solvents with a frequency factor of log A = 12. This indicates a cyclic mechanism in cyclic complexes which have a relatively high concentration. By constrast, a second order rate law with a frequency factor of log A = 7.5 was observed for VIIa. If there is no catalytic exchange one can explain this value with the dominance of non-hydrogen bonded monomers which lose entropy by complex formation. In order to prove the hypothesis that the central proton exchange step in eq. (32), (33) is characterized by a normal frequency factor of about log A = 13 Hennig and Limbach [47,78] have studied the hydrogen migration in mesotetraphenylporphine (VIII), where the intramolecular i.e. cyclic character of the exchange was proved by observing the coupling between the inner hydrogens and the ^{15}N atoms of ^{15}N substituted VIII.



VIII

In fact, a normal frequency factor of log A^{DD} = 12.9 was found for the double deuteron migration, whereas for the HH-migration a value of log A^{HH} = 11.5 was obtained. These observations, non-linear Arrhenius curves, and anomalous HH/HD/DD kinetic isotope effects were explained in a vibrational model of tunnelling along a tight double minimum potential with discrete NH stretching states characterized by IR spectroscopy. In this model the DD and HD migration proceed between the second excited ND-stretching states which lie near the top of the

barrier. The HH-migration proceeds at high temperatures between the first excited NH-stretching states by tunnelling through the barrier which lowers the frequency factor [79,80]. HH/HD/DD isotope effects on the IR spectra of VIII show that the motion of the two protons is a coupled motion which was previously predicted on the grounds of the vibrational model of tunnelling [47,78]. Of course, in intermolecular proton exchange the situation may be quite different. Due to the intermolecular flexibility a distribution of double minimum potentials better describe the central exchange step. However, a coupled proton motion avoids ion pairs with a highly ordered solvation shell as intermediates which would result in low frequency factors or negative activation entropies. We expect that knowledge of the intermolecular HH/HD/DD kinetic isotope effects will allow one to say more about the mechanism of these intermolecular double proton exchange reactions, especially on the role of tunnelling*.

We may conclude that the existence of non dissociative proton transfer in aprotic solvents has now been well established. The study of these reactions by dynamic NMR spectroscopy including their kinetic isotope effects is, therefore, not only a tool to learn more about proton motions in hydrogen bonds, which is important in bifunctional and enzymatic analysis, but also a method to establish whether two molecules are able to form cyclic hydrogen bonded complexes or not.

16.2.3 NMR relaxation studies of hydrogen bonded proton donors

An NMR line is not only characterized by a frequency, an intensity and a line width $W=1/\pi T_{2eff}$), where T_{2eff} is the transverse relaxation time, but also by a time T_1, the longitudinal relaxation time [2,45,59,82-85]. T_1 is the time constant of the process which leads to an equilibrium Boltzmann population of the spin states of the nuclei in a static external field after a perturbation. During this relaxation process energy is transferred between the spin system and the electromagnetic field at the nucleus of interest created by the random motion of the molecules in space. In order to discuss how relaxation studies can contribute to an understanding of hydrogen bond formation we have to summarize some results of the relaxation theory.

The total relaxation rate can always be written as the sum of an intramolecular and an intermoelcular term [59,81]:

$$\frac{1}{T_1} = \left(\frac{1}{T_1}\right)^{intra} + \left(\frac{1}{T_1}\right)^{inter} \qquad (35)$$

---

* The mechanism of intramolecular proton tunnelling is discussed further in section 16.4.1. See also Note added in proof.

The two terms can be separated in the case of solute-solute relaxation by varying the concentration of the solute and in the case of solute-solvent relaxation by the use of deuterated solvents because protons are in general responsible for the intermolecular term, apart from paramagnetic impurities whose presence can be detected by ESR spectroscopy. Each term in eq. (35) is itself the sum of different contributions which represent different relaxation mechanisms r [45]:

$$\frac{1}{T_1} = \sum_r \frac{1}{T_{1r}} \tag{36}$$

The most important relaxation mechanism for spin 1/2 nuclei is the dipole-dipole relaxation between two nuclei i and j. The dipole-dipole relaxation times T_{1d} (i) depnds on whether the T_1 experiment is performed selectively on i or non-selectively on i and j at the same time. The selective relaxation time $T^i_{1d}(i)$ and the non-selective relaxation time $T^{NS}_{1d}(i)$ are given in good approximation [82-84] by

$$\frac{1}{T^i_{1d}(i)} = \sum_j \rho_{ij} \quad , \quad \frac{1}{T^{NS}_{1d}(i)} = \sum_j \rho_{ij} + \sigma_{ij} \tag{37}$$

$$\rho_{ij} = 2\,W^i_1 + W_o + W_2 \tag{38}$$

where σ_{ij} is the cross relaxation rate

$$\sigma_{ij} = W_2 - W_o \tag{39}$$

W_n is the probability of an n-quantum transition in the two spin system [85] given by

$$W_o = \frac{1}{10} \frac{\gamma_i^2 \gamma_j^2 \hbar^2}{r_{ij}^6} \left(\frac{\tau_{ij}}{1 + (w_i - w_j)^2 \tau_{ij}^2}\right) \tag{40a}$$

$$W_1 = \frac{3}{20} \frac{\gamma_i^2 \gamma_j^2 \hbar^2}{r_{ij}^6} \left(\frac{\tau_{ij}}{1 + w_i^2 \tau_{ij}^2}\right) \tag{40b}$$

$$W_2 = \frac{3}{5} \frac{\gamma_i^2 \gamma_j^2 \hbar^2}{r_{ij}^6} \left(\frac{\tau_{ij}}{1 + (w_i + w_j)^2 \tau_{ij}^2}\right) \tag{40c}$$

w_i is the Larmor frequency of the nucleus i, r_{ij} the distance between i and j, γ_i the gyromagnetic ratio. We assume $\bar{R}_{ij}$, the vector sum of $\bar{r}_{ij}$ for an ensemble of molecules rotating at different frequencies to have a non zero value at a time t=0. Then $\bar{R}_{ij}$ decays to its equilibrium value of zero. If this process is exponential it can be characterized by a time τ_{ij}, the rotational correlation time of the vector $\bar{r}_{ij}$. The numerical value of $\gamma_H^4 \hbar^2$ in eq. (40) is $0.5695.10^{-49}$ $m^6 s^{-2}$ for protons. In the case of "extreme narrowing" one obtains:

$$\rho_{ij} = \frac{\hbar^2 \gamma_i^2 \gamma_j^2}{r_{ij}^6} \tau_{ij} \qquad (41)$$

$$\sigma_{ij} = \frac{1}{2} \rho_{ij} \qquad (42)$$

For isotropic reorientating rigid molecules all τ_{ij} are equal to the isotropic correlation time τ of the molecule given by the modified [81] Debye-Einstein equation:

$$\tau = 4 \pi \eta a^3 / 3kT \qquad (43)$$

where k is the Boltzmann constant, a the hydrodynamic radius and η the microviscosity which is given in approximation by the viscosity of the solvent. If the motion of the molecule is anisotropic, i.e. characterised by three different correlation times τ_x, τ_y, τ_z about the principal axes, then the τ_{ij} depend on the τ_x, τ_y, τ_z in a complicated way. For the case of a symmetric rotator such as benzene [86] appropriate expressions were derived by Woessner. Eq. (37)-(43) show that the measurement of T_{1d} can lead to a knowledge of the geometry and the motional behaviour of the molecule and, therefore, also of its hydrogen bonded state. T_{1d} can be obtained in different ways. Since in most cases the nucleus j is a proton, the replacement of j by a deuteron leads to T_{1d} [86]:

$$\left(\frac{1}{T_{1d}}\right) = 1.06 \left[\left(\frac{1}{T_1}\right)_i^{j=H} - \left(\frac{1}{T_1}\right)_i^{j=D}\right] \qquad (44)$$

Another way is to measure the cross relaxation rate σ_{ij} directly by performing additionally selective T_1 measurements on the line of the nucleus i without irradiating the nucleus j. A simpler method is to measure the "Nuclear Overhauser Enhancement"

$$NOE_{ij} = \frac{I_i - I_i(o)}{I_i(o)} \qquad (45)$$

of the signal intensity of the line of the nucleus i by saturating the line of the nucleus j. The enhancement is given by [59]

$$NOE_{ij} = \frac{\sigma_{ij} I_j(o)}{\rho_{ij} I_i(o)} = \frac{\sigma_{ij}}{\rho_{ij}+\rho_i^{res}} \cdot \frac{\gamma_j}{\gamma_i} \qquad (46)$$

where ρ_i^{res} is the contribution of other relaxation mechanisms to the total relaxation rate. For the case of extreme narrowing $w_i \ll 1$ one obtains by combination of eq. (41), (42), (46)

$$NOE_{ij} = \frac{1}{2 + cr_{ij}^6} \frac{\gamma_j}{\gamma_i}, \quad c = \frac{2\rho^{res}}{\gamma_i \gamma_j \hbar^2} \qquad (47)$$

The maximum NOE is obtained when $T_1 = T_{1d}$, i.e. $\rho_i^{res} = 0$:

$$NOE_{ij}^{max} = \frac{1}{2} \frac{\gamma_j}{\gamma_i}$$

The use of eq. (47) for the determination of relative distances was proposed by Bell and Sauders [87].

A discussion of other than dipole-dipole relaxation mechanisms, especially quadrupolar relaxation, chemical shift anisotropy, spin rotation, and relaxation by paramagnetic impurities, may be found in the appropriate NMR-textbooks [45, 81]. The information which can be obtained from a study of these relaxation mechanisms, for example by measuring the T_1 value of deuterons, is the determination of τ_x, τ_y, τ_z and the detection of intramolecular flexibility. In the following sections we shall discuss the contribution of relaxation studies to an understanding of hydrogen bond association in solution. We distinguish between the case of small molecules in the fast hydrogen bond exchange limit and systems with a long lifetime of hydrogen bonded states as encountered in H-chelates and molecules of higher molecular weight.

16.2.3.1 Relaxation studies in the fast hydrogen bond exchange range

As the subject has been reviewed by Hertz and Zeidler [88] and very recently by Hertz et al. [89] we shall be brief. One has to distinguish between the effect of hydrogen bonding on the intramolecular and on the intermolecular relaxation times. We shall discuss first the intramolecular part. As shown in eq. (43) the isotropic correlation time increases if a rigid molecule forms a hydro-

gen bond because of the increase in the hydrodynamic radius a. For every nucleus, for example, a proton i relaxed by a proton j, this leads to a decrease in the dipolar relaxation time $T_{1d}(i)$ as shown by a combination of eq. (37), (41), (42). The same is found for the corresponding deuteron relaxation times [88]. The effect is particularly obvious in ^{13}C-T_1 values of CH groups. For example, the association of uracils with adenines (see section 16.3.2.2) in $CHCl_3$ leads to a decrease of the ^{13}C-T_1 values of the aromatic carbons as found by Iwahashi et al. [53]. Similarly, Ruterjahns et al. [90] could explain a sudden increase of the ^{15}N-T_1 values of the imidazole (Im) residue in histidine dissolved in water at pH 6-8 with a dimerisation of the type ImH^+...Im between two histidines. This association is possible only in this pH region. The association constant could be obtained from the T_1 measurements. If the intramolecular geometry of a complex is known the measurement of the intramolecular relaxation rates leads to an effective hydrodynamic radius a which indicates the size of the associates which have been formed [88]. This information is especially important in the case of small self-associating molecules. The assumption of isotropic tumbling associates is, however, only a rough approximation and studies of intramolecular relaxation rates have been undertaken [91-96] in order to explore to what extent individual rotational correlation times τ_x, τ_t, τ_z are affected by hydrogen bonding. It was found that hydrogen bond formation of substituted phenols [91,96], chloroform [95] and pyridine [92-94] had the effect of slowing down molecular reorientation of both proton donor and acceptor about axes perpendicular to the hydrogen bond while leaving motion about the hydrogen bond unaffected.

How can the measurement of intermolecular proton-proton relaxation contribute to an understanding of hydrogen bonding in solution? Intramolecular relaxation between two protons i and j was caused by a random reorientation of the vector $\bar{r}_{ij}$ whose length did not change. Intermolecular relaxation of i is caused by a rapid random change of the vector r_{ij} from infinity to a value a, the mean distance of closest approach between the atoms i and j through the molecular diffusion process, characterized by the diffusion coefficient D. In the basic model of intermolecular relaxation of Torrey [97] and Abragam [81] the intermolecular proton-proton relaxation rate is given in the extreme narrowing case where the typical rate of intermolecular motions is much higher than the Larmor frequency by:

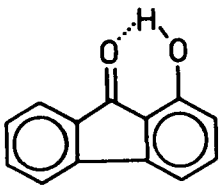
$$\left(\frac{1}{T_1}\right)_{ij}^{inter} = A_{ij}\,\frac{c_j'}{D}\,,\quad A_{ij} = \frac{2}{5}\,\pi\,\gamma_H^4\,\hbar^2\,\frac{1}{a_{ij}} \qquad (48)$$

The model assumes a uniform concentration c_j' of spins beyond the distance a_{ij}, whereas the probability of finding the spin j at distances shorter than a_{ij} is

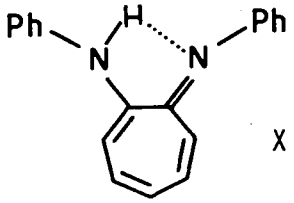
zero. The association factor A_{ij} is higher for a sample with hydrogen bonded molecules as compared to a reference sample where only van der Waals interaction occurs because a_{ij} is smaller. A change in A_{ij} as the concentration of a solute is varied indicates, therefore, a hydrogen bond formation process. Hertz has developed a theory of intermolecular relaxation [98] in which a more realistic atomic pair distribution function is used. The interaction between molecules can be described either by a set of atomic pair distribution functions, one for each pair of atoms, or by an orientational dependent molecular distribution function. Therefore, as many parameters of the last function can be determined, the A_{ij} are known. Using this method Hertz et al. [99-103] and Helm et al. [95] were able to determine mean geometric structures of associates of small proton donors in binary mixtures, especially at medium and high proton donor concentrations where the occurence of a great number of different associates makes the interpretation of chemical shift data in terms of chemical reactions very difficult. It was shown, for example, that the nonpolar head of carboxylic acid associates experiences a hydrophobic interaction [99]. The side-by-side configuration of the CH-groups in pure formic acid is disturbed by chloroform [102]. Acetic acid forms, in cyclohexane, dimers whose geometry is close to the classical cyclic dimer structure [103] $CHCl_3$ [102,95] forms hydrogen bonds with acetone and dioxan but also self-associates in mixtures of these solvents. A more detailed account of intermolecular relaxation and hydrogen bonding can be found in the review of Hertz et al. [89].

16.2.3.2 Relaxation studies in the slow hydrogen bond exchange

So far we have not yet considered the possibility of directly determining internuclear distances r_{ij} in molecules from relaxation data using eq. (36)-(47). The method cannot be applied to systems in the fast hydrogen bond exchange range because the correlation times are different in the various associates and unknown. The method can, however, be applied for intramolecular hydrogen bonded rigid molecules such as H-chelates or peptides whose motion is approximately isotropic. In this case the τ_{ij} of all vectors r_{ij} in the molecule are equal and the measurement of the values of ρ_{ij}, σ_{ij} or NOE_{ij} j=1, n leads to an exact determination of the position of i with respect to the atoms j. Jackman and Trewella [104] established the position of the intramolecular hydrogen bonded proton in 1-hydroxyfluorenon (IX) and 1-phenylamino-7-phenylimino-1,3,5-cycloheptatriene (X) by measuring the ^{13}C-T_1 values and in the case of (X) also the ^{15}N-T_1 values of these compounds as well as the reference compounds where the labile protons were replaced by deuterons. The anisotropic motion was taken into account as follows. The τ_{ij} for the different ^{13}C-H vectors were calculated from the ^{13}C-T_1 values using eq. (41) and the known C-H distances. The τ_{ij} were a function



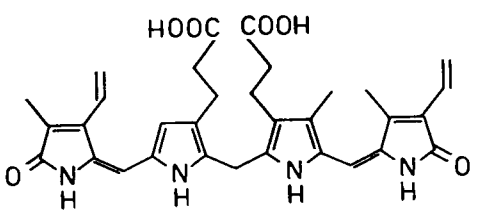
IX



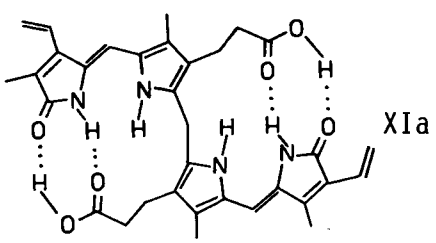
X

of the known angle between the C-H vectors and the principal axes of the molecule. From this function [86] the unknown τ_{ij} and r_{ij} involving the hydrogen bonded proton could be calculated from eq. (41) in an iterative way. This method is very precise. It turned out that in (X) the proton is localised on the nitrogen atoms, which establishes a double minimum potential for the very fast proton motion between the nitrogens. We come again to this problem of a single or double minimum potential in H-chelates as (X) in section 16.4.1.

The use of relaxation data for establishing the structure of biomolecules in water has been recently reviewed by Bothner-By [105]. Some more recent examples are the studies of Gibbons et al. [106] who established the geometry of peptides such as gramicidine and tyrocidine A in organic solvents on the base of NOE and selective T_1-measurements. Kaplan et al. [107] have shown that bilirubin (XI) has in chloroform the structure XIa with hydrogen bonds between the COOH and the lactam units.



XI



XIa

As mentioned at the end of section 16.2.2.1 care has to be taken in interpretating NOE and selective T_1 results carried out on labile protons such as the COOH, the lactam NH and the pyrrole NH protons in X which show three different lines. The irradiation of line i produces a Nuclear Overhauser enhancement on line j i.e. an increase in the intensity but a saturation transfer to line j, i.e. a decrease in the intensity if i and j exchange their protons. The two effects may cancel each other as found in part for XI The relaxation theory of exchange in a dipolar coupled spin system was published by Campbell et al. [52]. If the

change of the NOE with temperature is much smaller than the proton exchange rates the two effects can be separated by temperature dependent experiments as did Redfield et al. [54,55] in the study of biomolecules in water. In these biomolecules of high molecular weight the proton-proton NOE is not positive but negative, i.e. saturation of one line causes saturation of other lines. The reason for this is that τ is long because of the great hydrodynamic radius of the molecule and that $w\tau \approx 1$ or $w\tau > 1$. In this case $W_2 << W_0$ in eq. (39) and the cross relaxation rate becomes negative, i.e. a saturation transfer occurs which has nothing to do with chemical exchange. As τ is increased a point is reached where cross relaxation σ_{ij} is faster than the relaxation rate σ_{ij} itself. In this case the longitudinal relaxation times of all protons in the macromolecule are equal. The theory of such spin diffusion processes has been published by Kalk et al. [108] for the case of proteins. We have stressed in this section the quantitative use of relaxation experiments. However, the qualitative use is equally important for the assignment of NMR lines in complicated ^1H NMR spectra of biomolecules [54,55,64,65] on which we comment in section 16.4.

16.3 CHEMICAL SHIFT STUDIES OF HYDROGEN BOND EQUILIBRIA IN SOLUTION

In this section we want to discuss chemical shift studies of hydrogen bond equilibria in the fast hydrogen bond exchange range carried out since the review of Tucker and Lippert [5]. For methodical reasons we treat systems containing one proton donor group and systems with more than one group separately.

16.3.1 Systems containing one proton donor group AH

As discussed in section 16.2.1 proton donors AH are able to self-associate and to associate with added bases or with the solvent. In principle the three processes are interrelated. However, the experimental conditions are, in general, chosen in such a way that one process dominates so that the other processes can be neglected in first order approximation. We, therefore, discuss first systems with extended self-association, then association with neutral and negatively charged bases and finally the problem of proton transfer to these bases and its influence on the chemical shifts.

16.3.1.1 AH-self-association

The self-association of carboxylic acids in aprotic solvents is a classical reaction whose thermodynamics were studied by NMR chemical shift measurements [5]. Jentschura and Lippert [109] had shown for acetic and propionic acid that the chemical shift dilution curves can be well described by a monomer-dimer-polymer (1-2-n) model. There is no ambiguity in the description of these curves because at concentrations below 0.05 mol l^{-1} the monomer and the dimer predominate

with respect to the polymer. In this concentration range the COOH line shifts to lower field because of the formation of the dimer. Above 0.05 mol l^{-1}, however, the line position is shifted upfield because the COOH proton resonates at higher field in the polymer than in the dimer [109,110]. The minimum was found both for cyclohexane and for the less inert CCl_4 as solvent. In thorough studies [111] Kimtys et al. have confirmed these results and this interpretation for trimethylacetic acid, propionic acid, n-butyric acid, 2-methylpropionic acid, and adamantane carboxylic acid. The chemical shifts of the COOH protons in the cyclic dimer depended on the type of the acid. The authors explained this difference by an interaction of one cyclic dimer with another dimer, the interaction being weaker for those acids RCOOH with a bulkier group R. The association properties are different in basic solvents which act as hydrogen bond acceptors. Fujiwara [112] has studied chemical shift dilution curves of carboxylic acids at $35^{o}C$ in DMSO. At low concentrations the AH···S (S=solvent) complex dominates, whereas at higher concentrations the dimer $(AH...)_2S$ or AH...AH···S is formed. The data could be explained without consideration of the true monomer. This assumption is justified as shown by Balevicius and Kimtys [113] who determined the true monomer concentration of trimethylacetic acid in acetone as well as the quasimonomer and the dimer concentration by adding cyclohexane and measuring the chemical shifts. They showed that care must be taken in using acetone as solvent because drying agents such as molecular sieves induce condensation reactions of acetone, for example the formation of diacetonealcohol $CH_3COCH_2C(OH)(CH_3)_2$. Muller and Rose [114] purified acetone by distillation at $-22^{o}C$ after drying. A proton acceptor solvent which can be easily dried is tetrahydrofuran (THF) Gerritzen and Limbach [36] described the chemical shift dilution curves of acetic acid and of methanol in THF by simple equilibria between the quasimonomers and linear dimers:

$$AH\cdots S + AH\cdots S \rightleftharpoons AH\cdots AH\cdots S + S \qquad (49)$$

taking into account the change of the free solvent concentration according to eq. (22). The linear dimer formation is a slightly exothermic process for methanol but a slightly endothermic process for acetic acid in THF. During the formation of linear trimers or cyclic dimers two solvent molecules are liberated. Therefore, their formation can be neglected in the discussion of the chemical shift dilution curves because the solvent effect of shifting the equilibria on the side of the quasimonomers is much increased. This effect is also the reason why basic solvents decrease rates of proton exchange. In summary, one can say that the NMR method is of special use for hydrogen bond association studies in basic solvents because the chemical shift data can be easily interpreted due to

the absence of higher associates and because IR spectroscopy is not very sensitive to these solvents. The association of alcohols and phenols in inert media is nowadays studied preferentially by IR - spectroscopy using the matrix isolation technique [115] or by vapor-spectral methods [116].

There are, however, in the literature, some interesting self-association studies of different molecules which we should like to mention. Sergeev et al. [117] have studied the self-association of HCl in 1,2-dibromotetrafluoroethane and CH_2Cl_2 as a function of the temperature. Using the Lippert plot, eq. (18) the authors could describe the association at concentrations below 0.5 mol l^{-1} by a monomer-dimer equilibrium. At higher concentrations higher associates are formed. The dimerisation enthalpy was -8 kJ mol^{-1}. Complexation of HCl with CH_2Cl_2 was also observed. Silin et al. [118] have studied the association of sterically hindered lactams in CCl_4. The enthalpy of the lactam dimerisation was found to be smaller than that for the corresponding carboxylic acids, whereas the reverse was found for the polyassociation. Stassinopolou et al. [119] have carried out chemical shift measurements of acetonphenylhydrazones Ph-NH-N=CR_1R_2 in n-heptane, CS_2 and hexachlorbutadiene. A Lippert plot showed that linear trimeric aggregates predominate in n-heptane. The hydrogen bond in formed between the NH proton and the iminic nitrogen. In the other solvents complexation with the solvent competes with the self-association. Kobets et al. [120] have studied the association of o- and m-toluidine ($CH_3C_6H_4NH_2$) in cyclohexane. The NH_2 line is shifted to lower field for m-toluidine and to higher field for o-toluidine as the concentration is increased. This effect was explained by the formation of NH···N bonds in the case of m-toluidine and of NH hydrogen bonding to the aromatic ring in the case of o-toluidine, because an upfield shift is expected due to aromatic ring currents in such a complex. Finally, we mention the chemical shift study of the self-association of imidazole in $CDCl_3$ carried out by Wang et al. [121]. The best fit of experimental and theoretical shift curves was obtained for the assumption of a trimerisation process. However, association of imidazole to dimethylacetamide, tri-n-butyl phosphate and tri-n-octylphosphinoxide were shown to be stronger than the self-association process.

16.3.1.2 AH association with bases

The last example showed that in principle all processes, self-association, association with bases and association with the solvent have to be considered in explaining chemical shifts of proton donors in solution. In order to suppress the undesired self-association in the study of AH association with bases, proton donors with bulky residues have frequently been used and the bases have been added in excess. A problem arises if the acid strength of AH and the base strength of B are high. In this case the hydrogen bond formation of AH with B

is followed by proton transfer, ion pair formation and, depending on the solvent, dissociation into free ions according to the Eigen scheme of proton transfer [122]:

$$AH + B \rightleftharpoons AH\cdots B \rightleftharpoons A^-\cdots HB^+ \rightleftharpoons A^- + HB^+ \qquad (50)$$

The ions may form homoconjugates of the form $AH\cdots A^-$, $BH\cdots B^+$ and higher associates. We want to discuss here to what extent it is possible to distinguish between the different phenomena by chemical shift measurements.

Gramstad et al. [123,124] have studied the solvent influence on the association of 2-methyl-6-t-butylphenol (XI) with phosphoryl bases of the type $O{=}PR_1R_2R_3$. They were able to verify that the difference in the H-bond energy $AH\cdots B$ in cyclohexane and in CH_3CN corresponded to the enthalpy of association of XI with CH_3CN (S) in cyclohexane:

$$\begin{aligned} AH + B &\rightleftharpoons AH\cdots B && , \Delta H_1 \\ AH\cdots S + B &\rightleftharpoons AH\cdots B + S && , \Delta H_2 \\ AH + S &\rightleftharpoons AH\cdots S && , \Delta H_3 \end{aligned} \qquad \Delta H_1 - \Delta H_2 = \Delta H_3 \qquad (51)$$

A particularly large solvent effect was found for the association in $CHCl_3$ as compared to CCl_4 [124]. Figure 6 shows the chemical shift of the OH proton of (XI) in CCl_4 and $CHCl_3$ as a function of the base concentration. Since the chemical shifts in the monomer and in the complex are not very different in the two solvents the ordinate in Figure 6 is a measure of the extent of complex formation. One immediately sees that $CHCl_3$ hinders the complex formation*. Futsaeter and Gramstead could explain this effect by hydrogen bonding of $CHCl_3$ to B and to the π-electron system of (XI). In this context we should like to mention the study of Lin et al. [125] on association of 2-t-butylphenol with THF and acetone in cyclohexane, which is an extension of former studies [34,35].

The interaction of CH acids as chloroform with bases is a well known phenomenon which has been widely studied. We refer to the book by Green [126] on hydrogen bonding of CH groups which appeared in 1974. The hydrogen bond enthalpies are in the order of 8-10 kJ mol^{-1}. As some more recent studies we mention the paper by Wong and Ng [127] on the association of $CHCl_3$ with THF in cyclohexane, the paper by Borodin et al. [128] on the association of $(CF_3)_2CHR$ with dimethylsulphoxide in CCl_4, the paper by Meille et al. [129] on the association of halogenoalkanes with trisdimethylaminophosphine and the paper by Pogorelyi et al.

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* The equilibrium constants differ by a factor of 20 in the two solvents.

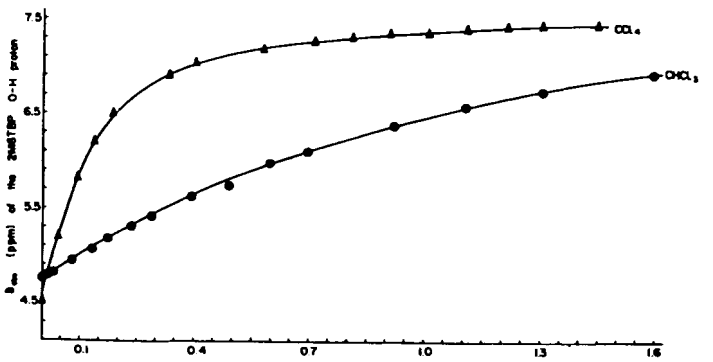
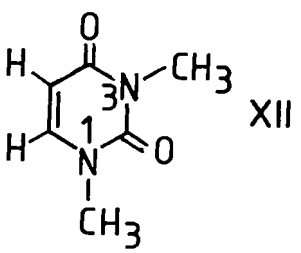


Fig. 16.6. δ_{OH}/ppm of (XI) in CCl_4 and $CHCl_3$ as a function of the base concentration. (Reproduced with permission from ref. 124) at 25°C.

[130] on the association of $(RSO_2)_2CH(COR)$ with acetone and dimethylsulphoxide. Bruskov et al. [131] have presented a study of 1,3-dimethyluracil (XII) in $CHCl_3$. They found evidence for CH···O hydrogen bonds between XII and solvent molecules



XII

as well as dimerisation of (XII) via C(6)-H···O bonds.

We turn now to the problem of how to distinguish hydrogen bonding between AH and B from proton transfer to A^- and BH^+. Hadzi and Smerkolj [132] have studied the thermodynamics of complex formation between mono-, di-, and trichloracetic acid with bases in CCl_4. The hydrogen bond enthalpies varied from 20 to 100 kJ mol^{-1}. Qualitatively, these enthalpies were related to the acid and base strength of the reactants. The chemical shift of the OH proton in the complexes increased with increasing hydrogen bond enthalpy. The absence of a CO_2^- band in the IR spectra proved the absence of proton transfer although the shift of the OH-stretching frequency to very low values indicated the formation of very strong

hydrogen bonds. In a chemical shift study of substituted phenols with triethylamine, Ilczyszyn et al. [133] investigated the question of what happens to the chemical shifts in the complex if the acid strength of AH and the base strength is increased to such an extent that proton transfer occurs. They found that the chemical shift increases until the point of 50% proton transfer and then decreases again. The same effect was found by Brycki et al. [134] in the study of the interaction of trifluoracetic acid (AH) with p-substituted pyridine-N-oxides. The reactants form crystalline 1:1 complexes. The chemical shifts of the H-bond protons are shown in Figure 7.

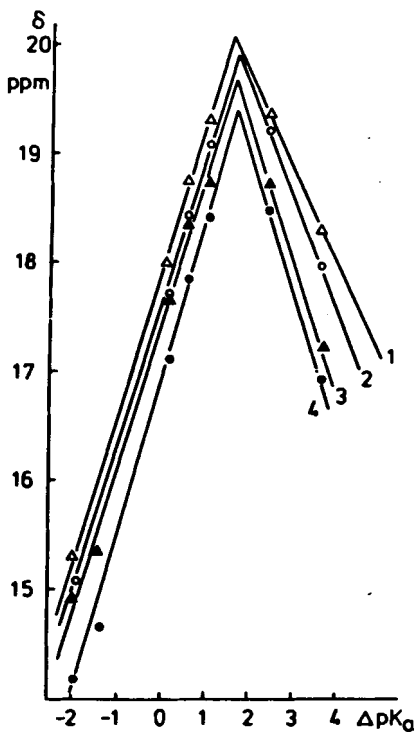


Fig. 16.7. Chemical shifts of the H-bond proton in complexes of AH with B (0.3 mol l^{-1}) as a function of the difference in the acid strengths Δ pK_a of AH and BH^+. 1:benzene, (2) chlorobenzene, (3) 1,2-dichlorethane, and (4) chloroform. Reproduced with permission from ref. (134).

The plot consisted of two intersecting straight lines. The left line corresponds to AH···B, the right line to $A^-\cdots HB^+$ formation. The strongest hydrogen bonds and highest chemical shifts are found for the systems with 50% proton transfer as indicated by the maximum. These results show that the NMR method can compete very well with other methods such as UV or IR spectroscopy in detecting ionization. If the Δ pK_A value is very large the ionized species can sometimes be

directly determined. It has been known for a long time, for example, that species such as $H\text{-}N^{+}R_1R_2R_3$ can be identified by the observation of couplings of the proton to nitrogen or adjacent CH groups [135]. By observing a coupling constant $J_{H\text{-}N\text{-}C\text{-}H}$ = 5 Hz Samoilenko et al. [136] established ionization in mixtures of triethylamine and CF_3COOH.

A question currently under investigation is the fate of the ions formed by proton transfer in the different solvents. The ion pair $A\cdots HB^{+}$ may dissociate into free solvated ions or can react with the reactants in order to form $(AH)_n\cdots A^{-}$ and $BH\cdots B^{+}$ or even more complicated complexes. Fujiwara et al. [137,138] have identified the long lived FHF^{-} ion in acetonitrile. Mateescu et al. [139] have proved the existence of the H_3O^{+} ion in SO_2. They found a quartet of the ^{17}O signal due to coupling with the three protons. Fritsch and Zundel [140] have studied the association of pyridine(Py) with pyridinium (PyH^{+}) in aprotic solvents by combining NMR chemical shift measurements with IR experiments. The topic of very strong hydrogen bonding between proton donors and acceptors has been recently reviewed by Emsley [141] including the problem of a double and a single minimum potential for the proton motion. We come again to this problem in section 16.4.1.

16.3.2 Systems containing different proton donors

There are not many studies of the thermodynamics of hydrogen bond equilibria in systems which contain more than one proton donor group because the chemical shifts are affected by proton exchange between these groups as discussed in section 16.2.2. We shall treat association in aprotic and in protic solvents separately.

16.3.2.1 Association between different proton donors, especially biomolecules in aprotic solvents

In order to obtain information on the association of proton donors AH with BH from the chemical shifts of the hydrogen bond protons, the case of slow proton exchange must be studied. For the association of carboxylic acids with alcohols this condition has so far been realized only by Limbach et al. [36,41 42] in the study of the system acetic acid/methanol/tetrahydrofuran (Fig. 4) at low temperatures. The thermodynamic parameters of the self-association of acetic acid and of methanol in THF were obtained from the measurements of the chemical shifts in the binary systems. Using this information the thermodynamic parameters of the mixed 1:1 association of acetic acid with methanol were obtained from the measurement of the COOH and OH chemical shifts in the ternary system. The enthalpy of the association of acetic acid with methanol calculated from the COOH chemical shifts and the enthalpy of the association of methanol

with acetic acid calculated from the OH chemical shifts were found to be different. This result proves the formation of two different linear associates which are still hydrogen bonded to the solvent S:

$$\begin{aligned} \mathrm{RCOOH\cdots S + ROH\cdots S} &\rightleftharpoons \mathrm{RCOOH\cdots \overset{R}{\underset{|}{O}}H\cdots S + S} \\ &\rightleftharpoons \mathrm{ROH\cdots O{=}\overset{R}{\underset{|}{C}}\text{-}OH\cdots S + S} \end{aligned} \qquad (52)$$

The number of hydrogen bonds is still retained during the association process. Therefore, the enthalpies are very small, i.e. below 4 kJ mol^{-1}, and, consequently, also the entropies. The high solvent concentration, however, shifts the equilibria far to the left side. The formation of the cyclic dimer in which the proton exchange takes place does not affect the chemical shifts any more because of its low concentration.

The range of slow proton exchange between different proton donor groups is much easier to obtain in aprotic solvents if non dissociative proton exchange is impossible between these groups. This is, for example, the case for alcohols or amines. As discussed in section 16.2.2.2 proton exchange in pure methanol takes place by dissociation and not by double proton transfer. It is, therefore, not surprising that the slow proton exchange condition has been found for solutions of nucleic acid bases in aprotic solvents. Thus, several authors have used the chemical shift method to study the question of whether the specific base pairing according to Watson and Crick [142] and Hoogsteen [143] shown in Figure 8 is also realized in solution.

Katz et al. [144,145] had already measured proton chemical shifts of well resolved NH_2 and NH signals of nucleosides (R = 2-deoxyribose in Fig. 8) in aprotic solvents. The chemical shifts of guanosine (G) and of cytidine (C) showed a much greater dependence on the concentration when they were dissolved together than when they were dissolved separately in dimethylsulphoxide-d_6 (DMSO). The shifts of the two NH and the NH_2 signals were parallel which could only be explained by specific base pairing. Adenosine (A) and uridine (U) as well as A and thymidine (T) showed no base pairing in DMSO but did in $CDCl_3$, where competitive hydrogen bonding to the solvent is much less pronounced. Newmark and Cantor [146] measured a dimerisation constant of 0.18 l mol^{-1} for G and a value of 3.7 l mol^{-1} for the mixed association of G with C in DMSO at 32°C. No evidence for higher aggregates was obtained. Similar values were found very recently by Petersen and Led [147] for the GC interaction in DMSO/methanol mixtures by measuring ^{13}C-chemical shifts. ^{1}H-NMR measurements are not suitable for this solvent because of proton exchange with the methanol. The ^{13}C method has, however, many more problems because of the long measuring times and the relatively small changes in the chemical shifts on hydrogen bonding. The authors showed

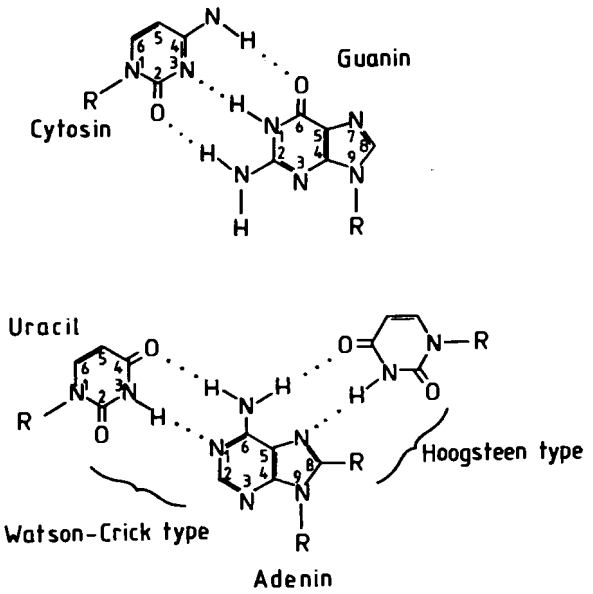


Fig. 16.8. Specific base pairing between nucleic acid bases according to Watson and Crick [142] and Hoogsteen [143] (R=H). Thymin: 5-methyluracil.

that in the case of cytidine a syn/anti conformational change of the ribose unit occurred on base pairing, in agreement with the Watson-Crick model. Katz [145] has measured by ^{1}H NMR association constants of 3.1 l mol^{-1} and 6.1 l mol^{-1} for AA and UU dimerisation and of 100 l mol^{-1} for AU base pairing. Evidence was, however, found for a 1:2 complex UAU which was formed with an equilibrium constant of 40 l mol^{-1}. The GC base pairing constant in $CDCl_3$ should exceed the AU value to a high degree but has not yet been measured because of the low solubility of G and C in $CDCl_3$. Kyoguku et al. have studied the structure of AU and UAU base pairs by ^{13}C [53,148], ^{1}H [149,150], and ^{15}N [150] chemical shift measurements. Because of the better solubility R in Figure 8 was cyclohexyl for U and T and ethyl for A. The ^{15}N experiments were carried out with R = 2'-3'-5'-tri-O-acetyl-2-deoxyribose for A and R = cyclohexyl for U where A and U were 95% enriched in ^{15}N. The equilibrium constants were measured by ^{1}H NMR because of the relatively small ^{13}C and ^{15}N shifts. The constants were of the same order as those found by Katz [145] for the A,U nucleosides. The ^{15}N chemical shifts, however, gave the information that about 2/3 of the AU dimer is a Hoogsteen and 1/3 a Watson-Crick pair. This result was confirmed by low temperature ^{1}H NMR

where a singlet was found for each of the two NH_2 protons in A due to slow rotation about the CN group. They showed a different dependence of the position on a change in the concentrations. The limiting ^{15}N chemical shifts in the base pairs could be obtained by extrapolation. Their values will be important for base pair identification in polynucleotides using ^{15}N NMR.

Engel et al. [151] have studied the influence of the methylation of amino-groups on the stability of AU and GC base pairs in $CDCl_3$. In 6-N-methyl-9-methyl-adenine the N-methyl group was found to be dominantly in the syn form with respect to N1 which induced the formation of Hoogsteen base pairs. Only 4% was found in the antiform.

Lancelot has measured the chemical shifts of the NH_2 protons of substituted adenines in $CDCl_3$ in the presence of p-cresol (p-$CH_3C_6H_4OH$) [152] and of butyric acid [153]. Equilibrium constants of the 1:1 association were obtained taking into account the self-association of the reactants. Butyric acid showed interaction with 9-ethyladenine in the order of the AU interaction. The analogue to the Watson-Crick pair was preferred to the Hoogsteen analogue as shown by comparison with 6-N-methyl-9-methyladenine. This was consistent with the observation at low temperatures under condition of restricted NH_2 rotation that one of the two NH_2 signals was shifted more than the other to lower field in the presence of the acid. The interaction of butyric acid with other nucleic acid bases could not be studied in a quantitative way using the chemical shift method because of line broadening due to proton exchange. These problems were not encountered by Lancelot et al. [154] in a study of the interaction of acetamide (CH_3CONH_2) with 1-cyclohexyluracil and 9-ethyladenine in $CDCl_3$. The thermodynamic parameters were found to be of the same order as the AA and the UU interaction, i.e. butyric acid forms much more stable complexes with the bases than acetamide. Lancelot et al. [155] similarly showed that 9-ethylguanine forms a 1:1 complex with the acetate ion in DMSO/water mixtures, not the other bases. All these studies were undertaken in order to learn something about the interaction of amino acids with nucleic acids. In a recent study [155] the same authors found a specific interaction of the $(NH_2)CNHR^+$ unit of n-methylguanidiniumperchlorate (XIII) and of the arginine side chain of a dipeptide in DMSO with G and C as shown in Figure 8 using the chemical shift method.

The last example leads us to the question of chemical shift studies of the association behaviour of peptides in organic solvents S where intramolecular NH $\cdots$O=C hydrogen bonds compete with intermolecular bonds or NH$\cdots$S or SH$\cdots$O=C bonds or with the formation of non hydrogen bonded NH groups. Urry et al. [156] showed that the proton chemical shifts of NH groups and the ^{13}C shifts of the carbonyl groups depended strongly on the temperature when this competition occurred whereas the chemical shifts of strongly intramolecular hydrogen bonded groups

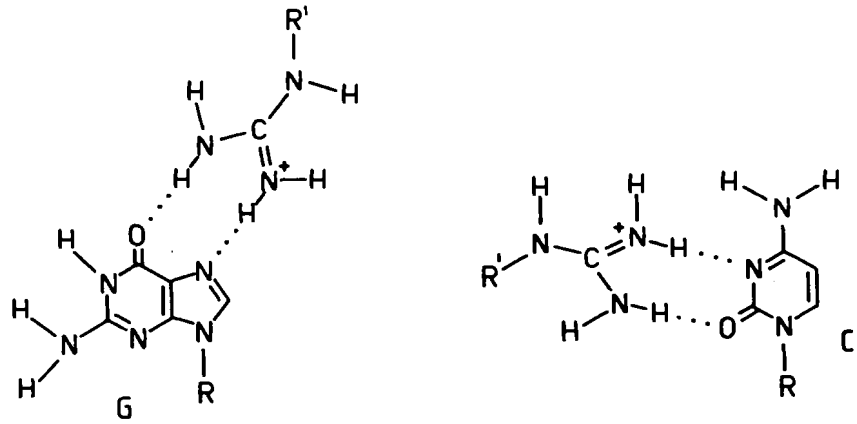


Fig. 16.8.a Models of the interaction of guanidinium with guanosine and cytidine according to ref. (156).

which are not "accessible" to the solvent were much less affected by a change in temperature. Thus, temperature dependent chemical shift studies can lead to a conformational analysis of peptides in solution. We cite as a recent example the paper by Toniolo et al. [157]; Toniolo also reviewed the topic at the same time [159]. Therefore, this topic will not be discussed further here.

16.3.2.2 Association of proton donors in water

If one dissolves proton donors in water fast proton exchange between solute and solvent generally takes place. This prevents chemical shift studies of solute association by monitoring the proton donor ^{1}H NMR signals. There are, however, some cases in the literature where the slow proton exchange conditions were reached. Raszka and Kaplan [159] reported broad but resolved singlets for the NH_2 protons of mononucleotides (R = ribose-5'-monophosphate) in water at $0^{o}C$ and pH = 7.5. Waelder et al. [50] observed a signal for the indole NH proton of tryptophan in water. Redfield et al. [53,160] measured resolved NH_2 amide proton signals in water. Ruterjahns et al. [161,162] obtained the slow proton exchange condition for the NH_2 and NH groups of ribonucleoside-3'-phosphates in water as monitored by the observation of ^{15}N-H couplings using ^{15}N NMR. These findings enabled the authors to study the mechanism of acid-base catalyzed proton exchange with the solvent. Downfield shifts of the proton donor signals as a function of the concentration were reported only be Raska and Kaplan

[159]. These were explained by hydrogen bond association between the nucleotides studied. The signals of the aromatic CH protons of the nucleotides were, however, shifted to higher field in agreement with previous studies by Chan et al. [163] and Jardetzky [164], who had explained these upfield shifts with stacking of the bases, i.e. association of the aromatic rings. According to Katz et al. [144] these upfield shifts are not observed if nucleosides undergo base pairing in aprotic solvents. Base pairing and stacking in water need not, however, exclude each other [159]. In fact, as the concentration of 5'-guanosine monophosphate is raised the ^{1}H NMR spectra show dramatic changes, as found by Pinnavaia et al. [165]. In the guanosine H-8 resonance (Fig. 8), a sharp singlet at low concentrations, was replaced by four broad lines which were assigned to different hydrogen bonded environments of the guanosine residue which exchanged only slowly. Similar observations were made also for the NH and the NH_2 resonances. These results are evidence of the formation of regular, ordered, hydrogen bonded associates, probably tetramers assembled in helically arranged stacks.

At the end of this section we should mention that the old observation of the upfield CH shifts of nucleic acid bases has been used by many authors for the study of the association of the bases by stacking [160,166,167] among themselves or with amino acid esters [168]. The NMR work on conformations of nucleic acid derivatives in water has been reviewed recently by Davies [169]. We refer further to the article of Hemmes in this book on aggregation properties of nucleosides.

16.4 NMR STUDIES OF MOLECULES IN ONE DOMINANT HYDROGEN BONDED STATE

In the preceding section we have discussed NMR studies of systems in which proton donors exchange very quickly between competing hydrogen bonded on non hydrogen bonded states leading to concentration dependent averaged NMR spectra. In this section we deal with NMR studies of systems characterized by one dominant hydrogen bonded state.

16.4.1 Proton localisation in hydrogen bonds by NMR

The problem of proton localisation in hydrogen bonds AH···B is very old [141, 170,171] and still a subject of current interest [172-178]. We discuss first symmetrical hydrogen bonds AH···A. The question is whether a single or double minimum potential with discrete vibrational levels splitted by tunnelling is realized for the proton motion as depicted in Figure 9, whether the potential is symmetrical only if the heavy atoms are allowed to move to new positions, as in malondialdehyde (XIV), or whether a continuum of vibrational states is present due to solute solvent interactions or due to a distribution of complexes

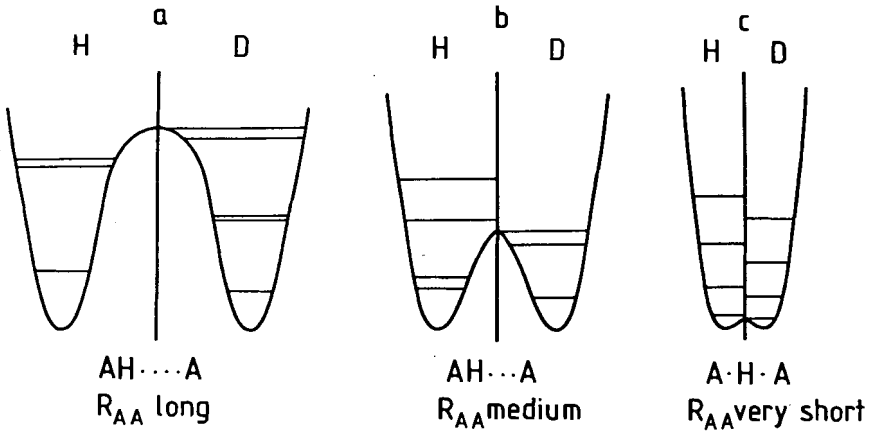
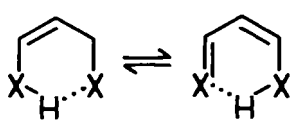


Fig. 16.9. One dimensional potential curves (schematically) for the proton motion in AH···A hydrogen bonds as a function of the distance R_{AA} between the heavy atoms.

with different heavy atom distances R_{AA}. Many spectroscopic techniques have been applied in studying this problem. They are cited in recent papers by Rosetti et al. [172] and Kreevoy et al. [173] who established ground state tunnel splittings according to Figure 9b in molecules of the malondialdehyde type XIVa. We are concerned here only with the contribution of NMR to this problem.



XIVa X=O
Ia X=NPh

In section 16.2.3.2 we have already made acquaintance with one NMR method of localising the proton position in symmetrical and asymmetrical H-bonds, the measurement of the dipole-dipole longitudinal relaxation times applied by Jackman et al. [103] to VIII and IX. A second method of Forsen et al. [174] is based on the fact that the chemical shifts of the H-bond proton in AH···A depend strongly on the average AH distances $\bar{r}_{AH}$. In a symmetrical double minimum potential with

a high barrier as shown in Figure 9a and in a single minimum potential (Fig. 9c) r_{AH} and r_{AD} are equal. Therefore, the chemical shifts δ_{AH} measured by ^1H NMR and δ_{AD} measured by ^2H NMR are equal, i.e. $\Delta\delta = 0$. In a potential with a medium barrier (Fig. 9b), however, the anharmonicity in the potential wells leads to a greater value for r_{AH} than for r_{AD}, i.e. $\delta_{AH} > \delta_{AD}$ or $\Delta\delta > 0$. The potential is more anharmonic at the height of the AH ground state than at the height of the AD ground state due to the different zero point energy. For a series of intramolecular hydrogen bonded compounds, mainly of the malondialdehyde type XIV Forsen et al. [174] found that $\Delta\delta$ increased to values of 0.7 ppm and decreased again as the heavy atom distances R_{AA} decreased in this series. The measurement of the corresponding ^3H chemical shifts brought additional information on the potential function. According to Blinc and Hadzi [175] the measurement of the quadrupole coupling constants of hydrogen bonded deuterons (DQCC) also carry information on the potential function. The DQCC are proportional to the electric field gradient at the ^2H nucleus which is high for a double minimum potential and very small for a single minimum potential. Forsen et al. [176] established a double-minimum potential for acetylacetone (R=CH_3, X=O in XIV), Jackman et al. [177] studied a great number of H-chelates using the DQCC method.* and Seiffert [15] had used the value of the $^1J_{^{15}N-H}$ coupling constant in order to settle the question of a single or double minimum potential in the H-chelate Ia. A value of 88 Hz was found for Ia and for Ib in which the NH proton was localised on one N-atom as shown by the ^1H NMR spectra. Thus, it was concluded that the NH distance and the CNH angle were identical for Ia and Ib, proving the double minimum potential for Ia. In fact, $^1J_{^{15}NH}$ in hydrogen bonded systems is lowered as the NH stretching frequency is shifted to smaller values as the r_{NH} distance is increased if hydrogen bonding occurs [178]. For example, in meso-tetraphenylporphine (VIII) $^1J_{^{15}NH}$ = 100 Hz and ν_{NH} = 3315 cm^{-1}. For Ia ν_{NH} = 3100 cm^{-1}. The establishment of such a correlation is important for localising protons in ^{15}N-nucleic acids in which these coupling constants can now be measured [179]. Similar observations were made by Fujiwara et al. [137] in an NMR study of HF and the FHF^- ion in acetonitrile. The high value of 476 Hz for the coupling constant between F and H in HF is a consequence of the short HF distance. A much lower value of 120 Hz was obtained for the FHF^- ion which indicates a larger HF distance typical for a single minimum potential according to Figure 9c.

A direct proof of a double minimum potential for 2,5-dihydroxy-p-benzochinone XVa has been given by Graf [180] and later by Bren et al. [182]. At low temperatures two ^{13}C signals were observed for XVa which can only be explained with proton localisation on one oxygen. The energies of activation [180,181] of the re-arrangement varied between 4.7 and 8 kcal mol^{-1} depending on the type of the solvent. The possibility of solute solvent influence on the rate constants [180]

*Some time before these studies were carried out Limbach

or of intermolecular proton exchange [181] was not excluded. Rumpel and Limbach [182] have measured ^1H NMR spectra of XVb which are shown in Figure 10 as a function of the temperature. Proton localisation is observed at low temperatures because the signal of the H-bond protons is a doublet with a coupling constant of 90 Hz due to coupling with the ^{15}N atom. At high temperature a triplet is observed which indicates fast entirely intramolecular proton jumps between the nitrogen atoms. From the calculation of the lineshape the rate constants of the

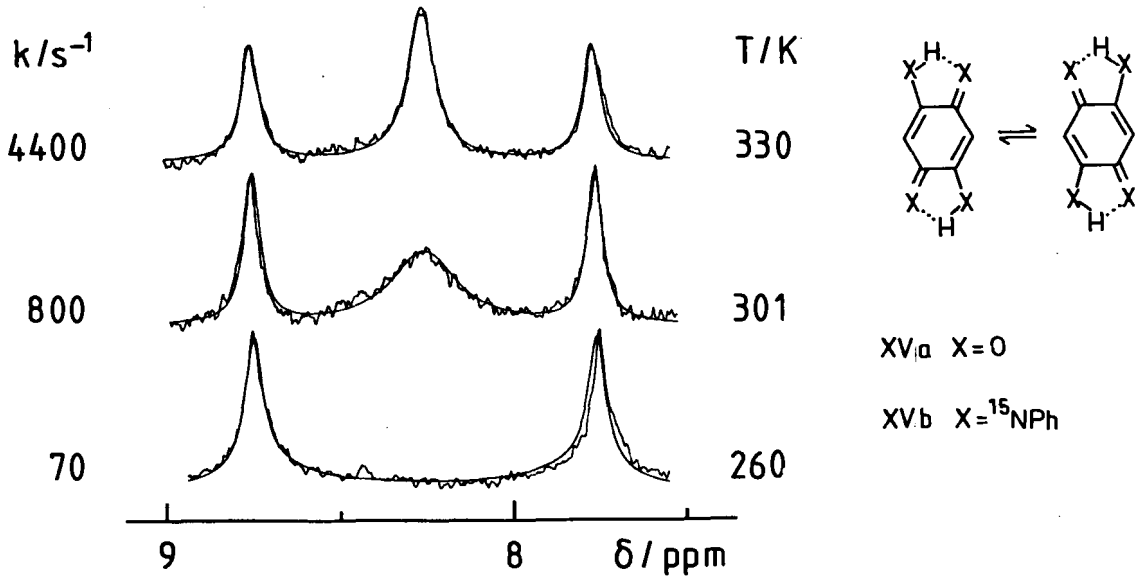
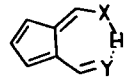


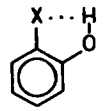
Fig. 16.10. Superposition of experimental and calculated ^1H NMR signals of H-bond protons in XVb as a function of temperature in $CDCl_3$ at 90 mHz [182].

HH migration are obtained as shown in Figure 10. The determination of the HH/HD/DD isotope effects of the reaction is in progress. The corresponding values for the hydrogen migration in meso-tetraphenylporphine (VIII) had enabled Hennig and Limbach [47,48] to prove a vibrational model of tunnelling in a very tight double minimum potential with discrete NH stretching levels corresponding to the situation in Figure 9a.

Proton localisation in asymmetrical complexes AH···B is easier to determine by NMR than in symmetrical complexes, though the question of an asymmetrical single or double minimum potential is more difficult to answer. We have already dealt with this question in section 16.3.1.2 for the case of intermolecular complexes. The ^{15}N-H coupling constants in unsymmetrical H-chelates XV, where X=N has been used in order to decide whether the proton is localised on the nitrogen or the atom Y. This work has been reviewed by Axenrod [183] and recently by Martin et al. [184]. In summary, if a coupling constant $^1J_{^{15}N-H}$ of the order of 90 Hz is found the H-bond proton is localised at the nitrogen atom. Couplings of the H-bond proton to adjacent CH protons have also been used for the proton localisation. Hafner et al. [185] localised the proton at the nitrogen atom in

XVI  XVI a X = N, Y = O

XVI b X = NR, Y = NR'

XVII  XVII a X = OCH_3

b X = SH

XVI a using this method. Muller-Westerhoff [186] found temperature dependent $^3J_{HH}$ in slightly asymmetric chelates of the type XVI b. He explained his findings with a symmetrical double minimum potential. Schaefer et al. [187] have proved an intramolecular hydrogen bond in compounds of the type XVII by analysing the long range coupling constants between the chelate proton and the aromatic protons.

16.4.2 NMR studies of biopolymers in water

In a way one may regard proteins or nucleic acids as the polymer analogues of the low molecular H-chelates because of the intramolecular-hydrogen bonds which are sometimes extremely stable. The stability of these bonds is such that the labile protons are protected from the solvent water molecules which results in very low proton exchange rates with the solvent. Shulman and co-workers [188] discovered in 1971 the hydrogen bonded imino proton signals of the GC and AU pairs in t-RNA dissolved in H_2O as quite sharp singlets in the range of 11 - 16 ppm where Katz et al. [144,145] and Shoup et al. [189] had previously located the corresponding signals of the monomeric base pairs dissolved in DMSO. Shulman et al. found that the chemical shift of the imino proton of a GC (and also AU) base pairs depended on the type of the nearest neighbour base pairs. This discovery opened the possibility of studying the type of base pairing in t-RNA or fragments of DNA by measuring the low field NMR spectra of these compounds dissolved in water, and a number of papers have appeared on this topic. As an example, Figure 11 shows the low field imino GC/AU region of E.coli $tRNA^{His}$ taken from the review of Reid and Hurd [190]. The number of long lived base pairs can be obtained by integration of the spectrum or, more precisely, by calculating the spectrum with a superposition of lorentzian lines of equal intensity. In general, more lines than expected are found for the secondary base pairs predicted by the cloverleaf model of tRNA (Fig. 11). These are tertiary Watson Crick or other base pairs which link different parts of the tRNA and are, thus, responsible for the tertiary structure. A very difficult task is now the assignment of the signals to specific nucleic bases. Several techniques have been applied to this problem, and reviewed by Robillard and Reid [191] and Hilbers [67]. The first is the study of tRNA modified chemically in specific positions, for example, by the replacement of oxygen by sulphur. A second method is study of oligonucleotides as references. A third method which has been widely applied

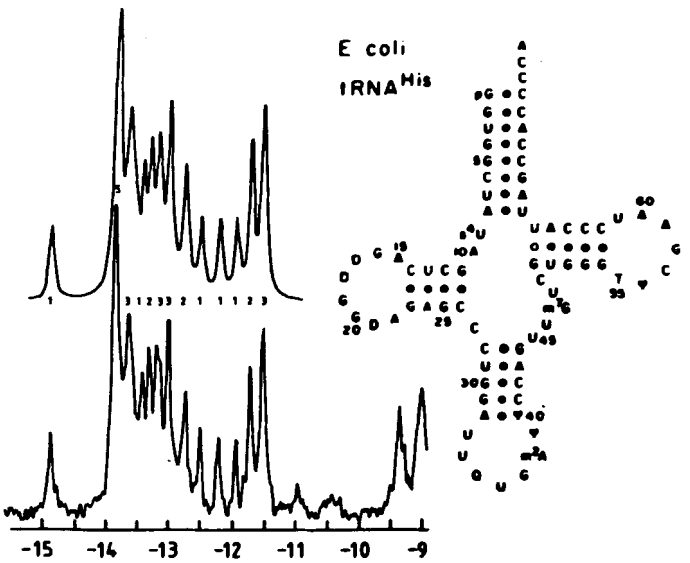
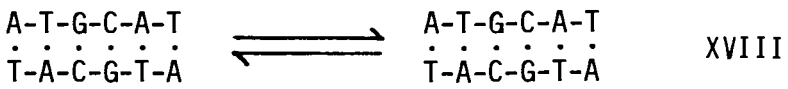


Fig. 16.11. The low field 360 MHz NMR spectrum of E.coli $tRNA^{His}$ at 39°C in the presence of excess $MgCl_2$ at pH 7.0. Lower curve: experimental spectru, upper curve: calculated spectrum consisting of a superposition of Lorentzian lines. Reporduced with permission from ref. (190).

[191] is the theoretical calculation of chemical shifts [192-194] which are mainly influenced by the aromatic ring currents induced by the magnetic field in the nucleic acid bases. Nowadays, the positions of the different imino signals can be well prediced on the base of the molecular geometry known from the crystallographic data. In a series of recent papers Redfield et al. [55,194, 195,196] have used the study of Nuclear Overhauser effects between thymidine CH_3 groups (Fig. 8), aromatic CH groups and the imino hydrogen bonded protons for the unambigous assignments of several imino resonances to specific base pairs. At the same time, the imino proton-solvent exchange rates were measured for the different lines as a function of the temperature and the $MgCl_2$ concentration. Mg^{2+} stabilizes the tertiary tRNA structure. At lower mg^{2+} concentrations first the tertiary base pairs break, as the temperature is raised, which is manifested by broadening and the disappearance of specific lines in the spectra, and then the secondary base pairs. Such melting studies also contribute to an assignment because first the outer and then the inner pairs are broken. These studies show that in tRNA the proton exchange rates are determined by the rate of base pair opening. The rates are then independent of the pH or the buffer concentrations. This is not so in oligonucleotides. Patel and Hilbers [197] found that the proton exchange rates of the terminal AT imino proton in the double stranded self-



XVIII

complementary oligonucleotide XVIII depended on the pH and were higher than for the imino protons of the inner base pairs. They explained this result with a rapid opening and closing of the terminal base pairs before the imino proton is abstracted by a OH^- ion. The equilibrium constants of this "fraying" process for XIX were obtained. If phosphate ions are added the proton exchange rates can be enhanced to the point where the opening and closing is again the rate limiting step. As discussed by Hilbers [67] the corresponding rate constants of the helix dissociation of $(AAGCUU)_2$ in water measured by Kan et al. [198] agree well with the rates measured by Porschke and Eigen [199] with the temperature jump relaxation method. Similar fraying processes have been studied recently by Kearns et al. [56] in a 12 base pair DNA fragment. Yamada et al. [200] and Young et al. [201] have studied the imino protons in polyriboguanylic acid (polyG). This molecule exists in a particular rigid multistranded form. The imino-solvent proton exchange is, therefore, so slow that the NH signal of the hydrogen bonded protons can be observed even in D_2O.

The hydrogen bonded imino protons are not the only labile protons whose signal can be observed in water. Bolton and Kearns [202] have reported a singlet at 6.8 ppm for solutions of tRNA which could be attributed to the 2'OH of the deoxyribose part of the tRNA [203]. The presence of this 2'OH group in tRNA is the only difference in the primary structure of RNA and DNA. The authors showed that this OH group must be hydrogen bonded in a water molecule but, furthermore, be protected from proton exchange with the bulk water. In consequence, they proposed a water molecule as link between the 2'OH group and the phosphate as shown in Figure 12.

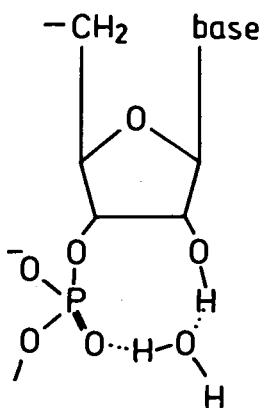


Fig. 16.12. Internal water bridge in tRNA according to Boltan et al. [202].

This water bridge could in part determine the conformation of RNA in solution.

E. Kaun et al. [179] have measured the ^{1}H 400 MHz NMR spectra of a variety of ^{15}N substituted tRNA's dissolved in H_2O. The spectra are of the type shown in Figure 11. The signals were split into doublets by coupling with ^{15}N. However an asymmetry of the doublets was observed in a number of signals. Figure 13 shows, for example the $^{15}N\underline{H}$ signal of the 11-AU base pair in E.coli $tRNA_{f1}^{Met}$

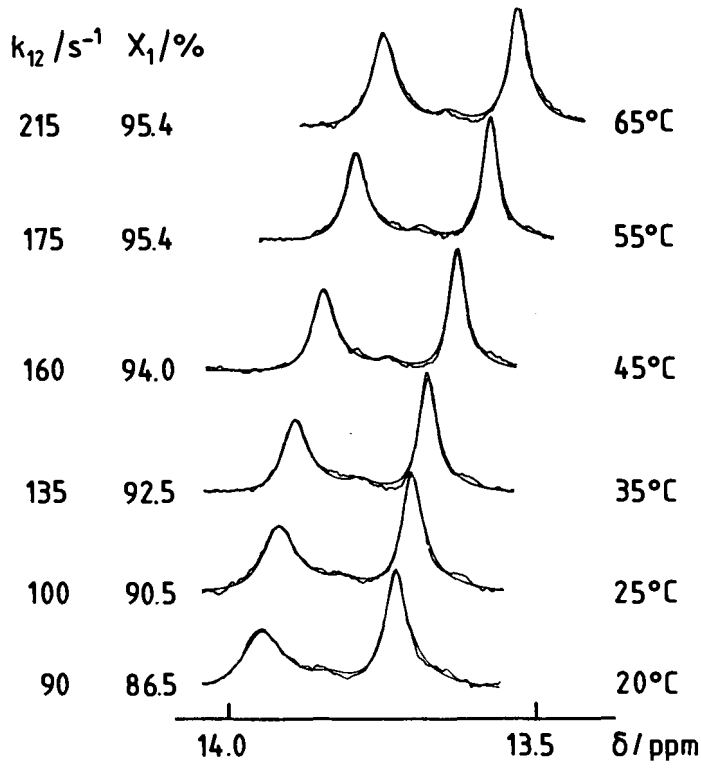


Fig. 16.13. Superposed experimental and calculated ^{1}H NMR signal of the $^{15}N^{H}\cdots{}^{15}N$ AU-11 base pair in E.coli $tRNA_{f1}^{Met}$ according to Kaun et al. [179] at 400 MHz in the presence of 15mMol $MgCl_2$. x_1 is the percentage of the dominant tautomer, k_{12} the rate constant.

as a function of the temperature. The asymmetry of the signal disappears at higher temperatures. The lineshapes of these spectra can only* be explained by the presence of two tautomeric forms. The rate constants, populations, static line widths, chemical shifts and coupling constants in the two forms could be obtained by the simulation of the spectra. The spectra prove that the proton exchange takes place within an $^{15}NH\cdots{}^{15}N$ hydrogen bond and not with water molecules, i.e. from the uracil N-3 atom to the adenine N-1 atom and from the adenine-

* A second differential line broadening mechanism not based on the tautomerism was additionally proposed in ref. 179

NH_2 to the uracil O (see Figure 8). The spectra of the H-chelate in Figure 10 and the $tRNS_{f1}^{Met}$ spectra in Figure 13 were calculated with the same computer program. The population of the dominant tautomer is increased as the temperature is raised. These results are the first demonstration of tautomers in nucleic acid base pairs which have been postulated for a long time. Much of the previous inconsistencies in NMR studies of tRNA's appear now under a different light.

We turn now to the second class of biopolymers, the polypeptides, proteins, and enzymes. The problems encountered in the study of these molecules resemble those of the tRNA's [204]. The lifetimes of the peptide hydrogen bonds NH···O=C depend on the stability of the secondary structure. In molecules such as basic bovine pancreas trypsin inhibitor (BPTI) which have been studied by Wuthrich et al. [65,205-207] the hydrogen bonded peptide NH groups appear as singlets in the range 7-11 ppm. The proton exchange between these labile protons and the solvent water is so slow - because of the great stability of the structure - that these signals can be observed even in D_2O. As shown in Figure 14 pairs of NH protons are much closer than in the nucleic acids so that they can be identified by their mutual Nuclear Overhauser Effect. An example provided by Wagner [66] is given in Figure 14. The lines marked Q 31 and F 22 correspond to the protons A and B. In order to replace these protons slowly by deuterons the sample temperature had to be raised to $60^{o}C$ and the pH to 8.0. The NOE of the residual protons after the exchange experiment is still retained, which proves that the exchange rates are determined by the rate of generating an "open" state from the "closed" state and not by the proton exchange rates in the open states. Similar results were obtained by the tritium-hydrogen exchange technique [208], which can also provide information on the folding process. NH proton exchange rates with water are much faster for oligo- or polypeptides. Small cyclic peptides have to be dissolved in DMSO in order to observe the NH protons [105]. ^{15}N substituted polyaminoacids show slow proton exchange with water only at very low pH values as was shown by Kricheldorf et al. [209,210] using ^{15}N-spectroscopy. NH_3^+ groups in side chains also exchange slowly under these conditions. Poly-L-lysine [209] and poly-L-ornithine [210] are in a stable helix form only at pH > 10, otherwise in a coil form in which the labile protons are exposed to the solvent. The formation of the helix is manifested in a decrease of the ^{13}C T_1 values [211], a line broadening and an upfield shift of the ^{15}N signals [209,210].

The last question we deal with in this review is the detection of H-bonded protons of imidazole residues in the side chains of histidines. The behaviour of these protons is of particular interest because of their catalytic activity. Blow et al. [212] showed in their x-ray studies that the active site of serine proteases such as trypsin and chymotrypsin consisted of a so called "charge relay

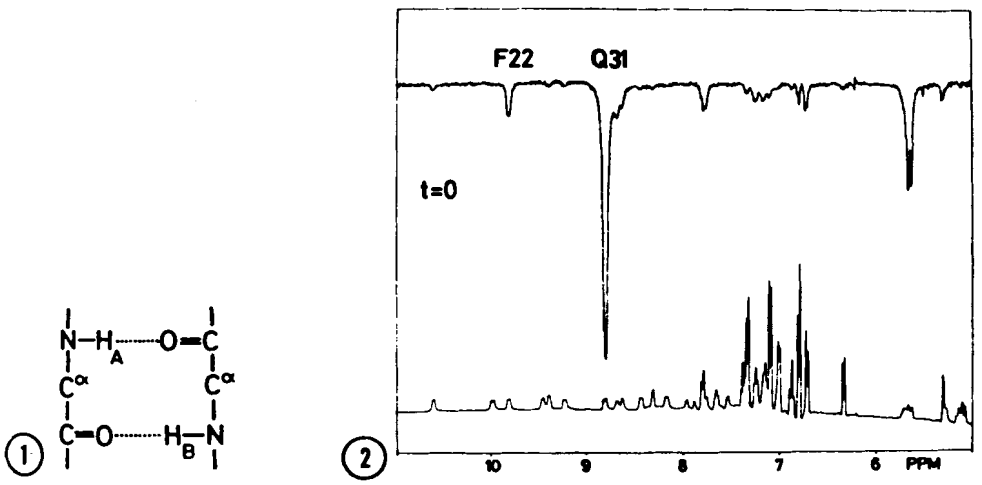
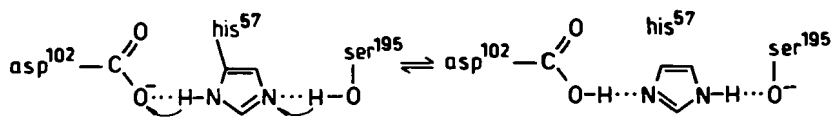


Fig. 16.14. Left: Schematic representation of two adjacent hydrogen bonds connecting opposite strands of an antiparallel β-sheet. The two amide protons A and B are separated by approximately 2.6 Å. Right, lower trace: 360 MHz ^{1}H NMR spectrum of BPTI in D_2O (PD 4.6, 24°C). The peaks between 8 and 11 ppm correspond to peptide NH bonded protons. Right, upper trace: NOE difference spectrum. Reproduced with permission from Wagner [66].

chain" in which the CO_2^- of asp^{102}, the imidazole group of his^{57} and the OH group of ser^{195} are assembled together (eq. (53)). A double proton transfer should



(53)

enable the formation of ser^{195}-O^-, which acts as nucleophile in the hydrolysis of the peptide bond. Studies of the primary H/D kinetic isotope effects of such reactions, reviewed recently by Schowen et al. [70], showed the motion of more than one proton in the rate determining step. Robillard and Shulman [213] discovered in α-lytic protease a peak at 18 ppm which was ascribed to the NH proton between asp^{102} and his^{57}. The question of the localisation of this proton and

the question whether the other nitrogen of the histidine is protonated or not has been a subject of interest which was reviewed by Markley [214]. Using ^{15}N NMR Bachovchin and Roberts [214] have shown that the imidazole residue of his^{57} shows a normal pk_a value of the order of 7, as also found for histidine itself by Ruterjahns et al. [90]. Since no $^1J_{15NH}$ coupling constant could be observed due to proton exchange NMR work could not establish the type of proton motion in the charge relay chain during the catalytic process until now. Finally, we mention the paper by Stoesz et al. [64] who detected low field histidine side chain NH resonances of reduced bovine superoxide dismutase. Using the NOE they could assigne two lines to the N(1)H and N(3)H protons of the same histidine [44], which in consequence, was charged positively. The NH protons exchanged with water only at high pH.

16.5 CONCLUSION

We have shown in this survey that our initial question:"what can we learn about hydrogen bonding in solution?" is a very wide question whose answers have evolved in the past and will continue to do so in the future. Many new techniques have opened the possibility of studying complex biological systems. Therefore, this paper cannot be complete. We have tried to point out the problems associated with NMR studies of hydrogen bonding and the type of information one can obtain. In conclusion, we hope that the reader will be convinced that it is still worthwhile studying hydrogen bonding in solution by means of NMR spectroscopy.

ACKNOWLEDGEMENTS

The author acknowledges a grant from the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg. I would like to thank Dr. U. Hennig, D. Gertizen and H. Rumpel, Freiburg, for their help. Similarly, I thank Dr. J. Stulz, Freiburg, for drawing my attention to several important papers. Finally, financial aid of the Fonds der Chemischen Industrie, Frankfurt, is gratefully acknowledged.

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Note added in proof: For the first time, primary kinetic HH/HD/DD isotope effects have been measured [216] for the tautomerism of VIII and XVIb as well as for the double proton exchange in the system acetic acid/methanol/tetrahydrofuran. In the last system an additional non dissociative triple HHH/HHD/HDD/DDD exchange was detected. In all cases a tunnelling reaction mechanism could be proved.