A HYPOTHESIS EXPLAINING THE ACTIVATED COMPLEX IN PRIMARY PHOTOSYNTHETIC ELECTRON TRANSFER AS A DISSIPATIVE STRUCTURE

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Abstract:

A far from equilibrium thermodynamic kinetic model treatment of the kinetics of primary electron transfer considering autocatalytic feedback reveals relevant mechanistic properties: a small portion of the excitation energy is used to build up microscopic order in an activated complex which thus facilitates efficient electron transfer and capture. On the molecular basis, monomeric bacterio-chlorophyll energetically activated by the excited bacterio-chlorophyll pair and inducing non-linear feedback via the protein environment is assumed to play a key role. A negative effective activation energy, for a given temperature range, is obtained - in agreement with experimental data. Formally the system may be understood as exhibiting a negative reorganization energy with the energy for the activated complex provided by the reaction itself. The model also explains why the rate of electron transfer can be extremely fast and why the rate of forward reaction largely exceeds the rate of reverse reaction. The concept of activated complexes as dissipative structures may lead to a more general new approach in understanding efficient irreversible mechanisms.

1. Introduction

Biological long-range electron transfer has long been a matter of controversy (see [1]). One extreme view is that distance, driving force and reorganization energy are the main factors determining biological electron transfer (see e.g. [2]). An opposite view is that specific protein structures and environments have significant influence either on stimulating or impeding electron transfer (see e.g. [3]). A clarification of the nature of long range electron transfer has a special significance for the primary process of photosynthesis.

The primary processes of electron transfer and charge separation in isolated reaction centers (RCs) have recently intensively been investigated (e.g. [4]). The investigated reaction centers contain two cofactor (pigment)/ protein branches, denoted L and M [5] with the bacterichlorophyll (BChl) special pair P, as well as a monomeric BChl, denoted B and a bacteripheophytin, denoted H in each branch for purple bacteria. A quinone (Q_A) in the L branch acts as further electron acceptor. However the interpretation of the electron transfer mechanism remains controversial essentially because of the following properties [6]:

a) electron transfer is strongly unidirectional proceeding virtually exclusively in the L branch from P* via H_L to Q_A.
b) back reactions are strongly suppressed
c) the fast charge separation (approx. 3 ps at room temperature for purple bacteria) speeds up significantly at low temperatures (0.9 ps at 1 K) [7, 8]
d) when the slight asymmetry in the two branches is essentially neutralized through genetic techniques electron transfer is still asymmetric, but much slower [9].

Imaginative models have been suggested to explain the primary photosynthetic electron transfer, such as the superexchange mechanism [10, 11] or the consecutive two-step electron transfer [12]. However agreement on the nature of electron transfer is not yet reached. Especially the property c) of the RC is astonishing and apparently in contradiction with classical theory, provided very complicated mechanisms are not developed for explanation.

The present approach is based on the simple observation that nature has solved essential structural and kinetic problems with mechanisms of irreversible thermodynamics. Selforganization of biological structures, stabilization of membranes, oscillations, all-or-non responses, multiple state behaviour (e.g. Lac Operon) are well known examples. Oscillations of light scattering and luminescence are also known from photosynthetic membranes [13]. Why should primary charge separation in photosynthesis not also be a non-linear irreversible process. The authors have recently demonstrated on the basis of a phenomenological model that stimulated and co-operative electron transfer are possible and exhibit hitherto unrecognized properties [14, 15, 16]. A key to these phenomena is autocatalysis, that is a feedback between subsequent electrons or electron densities. In contrast to the above mentioned views on long-range electron transfer, dynamic electronic and (or) structural changes with autocatalytic (positive feedback) effects will be considered as

new elements to evaluate the above electron transfer properties of photosynthetic reaction centers a) to d).

2. Simple and co-operatively stimulated electron transfer: basic mechanisms .

We proposed some models of stimulated electron transfer, which can be, for the sake of simplicity, reduced to two basic mechanisms (where the reacting species can either be electron transferring molecules or electron densities within macromolecules):

Simple stimulated electron transfer:

$$A \underset{k'}{\overset{k}{\rightleftarrows}} X \ , \ X \underset{k'}{\overset{k}{\rightleftarrows}} F \qquad (1)$$

$$X \underset{a'}{\overset{a}{\rightleftarrows}} M \qquad (2)$$

$$A + M \underset{b'}{\overset{b}{\rightleftarrows}} X + M \qquad (3)$$

with A=const., F=const, K=k/k'=b/b', κ_a=a/a' and α=b/k=b'/k'.
Here, the electron transfer is mediated by an intermediate state X, activation of which leads to the generation of an intermediate state M catalyzing the first reaction of (1).

Co-operative stimulated electron transfer:

$$A \underset{k'}{\overset{k}{\rightleftarrows}} X \ , \ X \underset{k'}{\overset{k}{\rightleftarrows}} F \qquad (1)$$

$$X + M \underset{a'}{\overset{a}{\rightleftarrows}} 2M \qquad (2a)$$

$$A + M \underset{b'}{\overset{b}{\rightleftarrows}} X + M \qquad (3)$$

The only difference to the first model is reaction (2a) representing here an autocatalysis of 2nd order involving a higher degree of non-linearity. Both models are, for the sake of simplicity, constructed in a way that the solutions describing the stimulated electron transfer formally coincide. Therefore the differences of both models are restricted to the dynamic properties of the solutions (stability, co-operativity of relaxation processes and correlation of the fluctuations). It should be noted that the discrete description (The concentrations of some "species" A, X, F and M can be interpreted as electron densities at discrete sites in the macromolecule) used here can be extended to a more realistic model describing the electron density changes along the molecular chain continuously (see [14]). This continuous model has qualitatively the same features as the model in the present paper, but it is less transparent due to the occurrence of non-linear partial differential equations.

The molecular electronic situation for electron transfer described by the reaction steps (1), (2a) and (3) is depicted in Fig. 1.

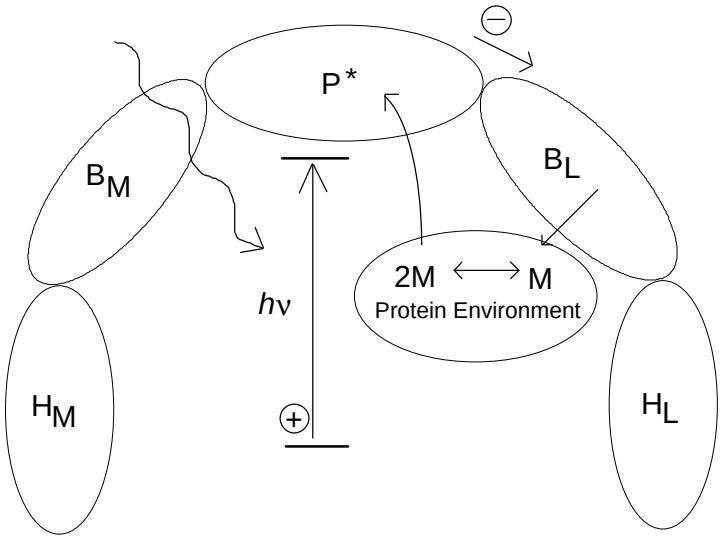


Fig. 1 Schematic description of the electronic processes and feedback loops in our model system according to the eqs. (1), (2a) and (3).

To facilitate intuitive understanding the two branches (M and L) of the BC reaction center are schematically depicted. They show the activated bacterio-chlorophyll pair (P^*), the monomeric bacterio-chlorophyll (B) and the pheophytine (H). According to our concept, B - as a neighbouring molecule to P^* - must play a special role.

However, it is clearly different from that in the superexchange mechanism, since B must first be energetically activated and exert an influence on the protein environment, which exerts a positive feedback on P^*. Energetically this means, that a small fraction of energy liberated in the excited bacterio-chlorophyll dimer (relaxation energy) must find its way to the protein environment. From there, a back reaction on P^* will induce irreversible electron transfer. The following simple molecular reaction model may visualize the required sequence of steps:

$$(P) \rightarrow (P)^*$$

$$(P)^* (B) (H) (QA) \rightarrow$$
$$<PE>$$

$$\rightarrow (P)^{*}\text{-}\delta^* (B)^{\delta^*} (H) (QA) \rightarrow$$
$$<PE>$$

$$\rightarrow (P)^{*}\text{-}\delta^* (B) (H) (QA) \rightarrow$$
$$<PE>^{\delta^*}$$

$$\rightarrow (P)^{+} (B) (H)^{-} (QA) \rightarrow$$
$$<PE>$$

$$\rightarrow (P)^{+} (B) (H) (QA)^{-}$$
$$<PE>$$

where <PE> symbolically denotes the protein environment, δ^* a small part of excitation energy and (QA) the quinone electron acceptor.

The proposed (autocatalytic) feedback loop, which is considered in our model, is only introduced into the L-branch. In the frame of our hypothesis the role of the protein environment in the electrochemistry of the RC is crucial, since it must mediate the feedback mechanism. Individual amino acids have indeed been shown to influence charge separation (see, e.g.[17]). An interesting amino acid pair with the

residues positioned in proximity to the macrocycles of the dimeric and monomeric bacterio-chlorophylls and the bacteriopheophytin molecule associated with its particular side of the RC is L181Phe - M208Tyr. It influences the rate of electron transfer, as shown with mutants, and also influences the redox potential of the bacterio-chlorophyll dimer.[18] The fact that these amino acids influence the redox potential and thus the free energy of electron in the bacterio-chlorophyll dimer (which changes during excitation) means that there is a kind of energetic communication between the bacterio-chlorophyll monomer and dimer. This is what is needed for a working feedback loop. Interestingly an asymmetry of the spin density distribution in favour of the L-branch dimer fraction has been interpreted to be the consequence of an energetic difference between the dimer halfes of a magnitude comparable to that of the interdimer interaction energy.[19] The key task in determining the mechanism of feedback will consist in tracing down an asymmetric loop along which a small fraction of energy (extracted from the excitation process) can be transduced.
Much more detailed experimental an theoretical studies will be needed along this line.

The kinetic equations derived from (1) - (3) are:

$$\frac{dX}{dt} = kA - k'X - kX + k'F - f(X,M) + bMA - b'MX$$

$$\frac{dM}{dt} = f(X,M), \qquad (4)$$

(5)

where only f(X,M) depends on the kind of model:

$f(X,M) = aX - a'M$, for reaction (2), and (6)

$f(X,M) = aXM - a'M^2$, for reaction (2a) (6a)

If we introduce dimensionless variables (with A as concentration unit and 1/k' as time unit) we obtain a more compact formulation of equations:

(7)

$$\frac{dX}{dt} = (K - X)(1 + \alpha M) - KX + F - f^*(X, M)$$
$$\frac{dM}{dt} = f^*(X, M), \quad \text{where}$$
$$f^* = \eta \cdot (\kappa_a X - M) \quad \text{or} \quad f^* = \eta' \cdot (\kappa_a X - M) \cdot M \qquad (7)$$
$$\textit{with} \quad \eta = \text{a'/k'} \quad \text{or} \quad \eta' = \text{a'A / k'} .$$

One stationary solution of this system of differential equations, common for both models, is:

$$X_s = \frac{1}{2\alpha\kappa_a}\left[(\alpha\kappa_a K - 1 - K) + \sqrt{(\alpha\kappa_a K - 1 - K)^2 + 4\alpha\kappa_a (K + F)}\right] \qquad (8)$$
$$M_s = \kappa_a X_s$$

(the corresponding complementary solution is always negative and therefore without physical sense). However, the second model has in addition another nonnegative solution:

$$M_S = 0, \; X_S = K/(1+K) \qquad (9)$$

But this solution changes the stability at κ_a=0 and is unstable for all positive κ_a.

3. The nature of high irreversibility of charge separation

The dependence of the stationary value of X_S on the catalytic activity of M according to equ. (8) shows a characteristic S-shape (Fig. 2).

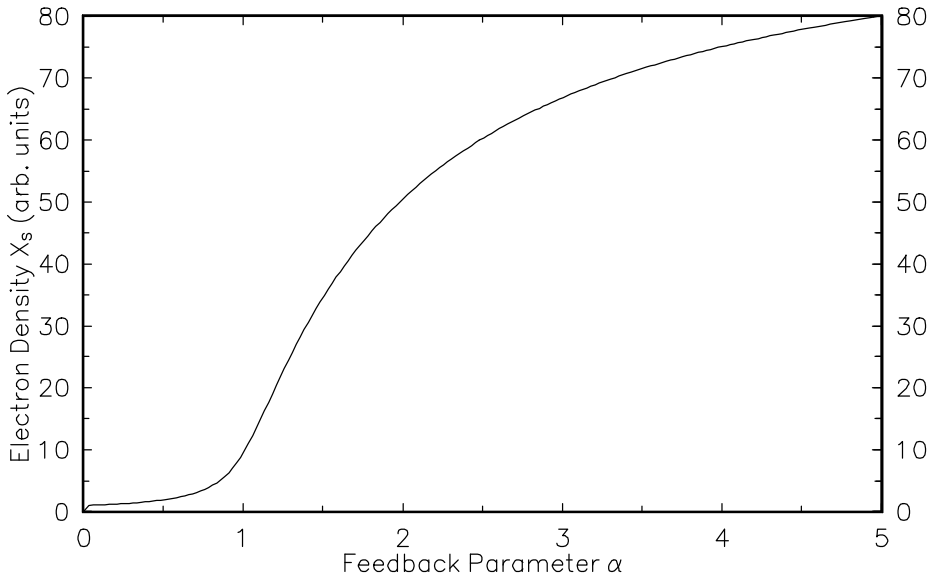


Fig. 2 Dependence of electron density X_S on the catalytic strength α (with K=100, κ_a= 1.0; F=0

It should be noted, that mathematically the concentration (or electron density) on the final electron acceptor (F) only insignificantly influences the tendency of the electron density to increase towards the final acceptor F: If the scaled value of B is increased from zero up to one, the changes in electron density X_S will be only a few percent (if the ratio of forward and backward rate constants K is much larger than one as to be assumed for irreversible processes). This fact is important if we want to introduce a measure of the effective irreversibility of the electron transfer chain relating the forward electron flux kX_S to the backward flux k'F:

$$\gamma = kX_S/k'F \qquad (10)$$

In order to evaluate the effect of feedback strength α on the transfer irreversibility γ one can use the limiting values of X_S obtained from equ. (8):

$$X_S \to (K+F)/(K+1), \quad \alpha \to 0 \quad \text{and} \quad X_S \to K, \quad \alpha \to \infty \qquad (11)$$

Consequently the transfer irreversibility will, depending on the magnitude of the feedback degree α, increase from (if we consider K>>1 and F=1):

$$\gamma \cong K \quad \text{to} \quad \gamma \cong K^2 \,. \qquad (12)$$

Moreover this effect increases exponentially with the number of intermediate sites in the transfer chain: If the chain has n intermediate steps, the transfer irreversibility will increase to $\gamma \cong K^{n+1}$ (because $X_S \rightarrow K^n$, see [14, 15]).
This clearly demonstrates why the forward reaction rate of electron transfer in the photosynthetic reaction center can become orders of magnitude larger than the back reaction rate. Simultaneously it explains why forward reaction rates can become very high.

4. Temperature dependence of stationary stimulated electron transfer. Negative effective Arrhenius activation energies

Due to the S-shaped dependence of the electron density X_S on the catalytic feedback α (Fig. 2) one can expect that at certain combinations of parameter values a decrease of the temperature does not necessarily lead to a decrease of X_S even though all reaction rates are decreasing according to the Arrhenius law. For exothermic reactions (1) - (3) the activation barriers may typically look like shown in Fig. 3.

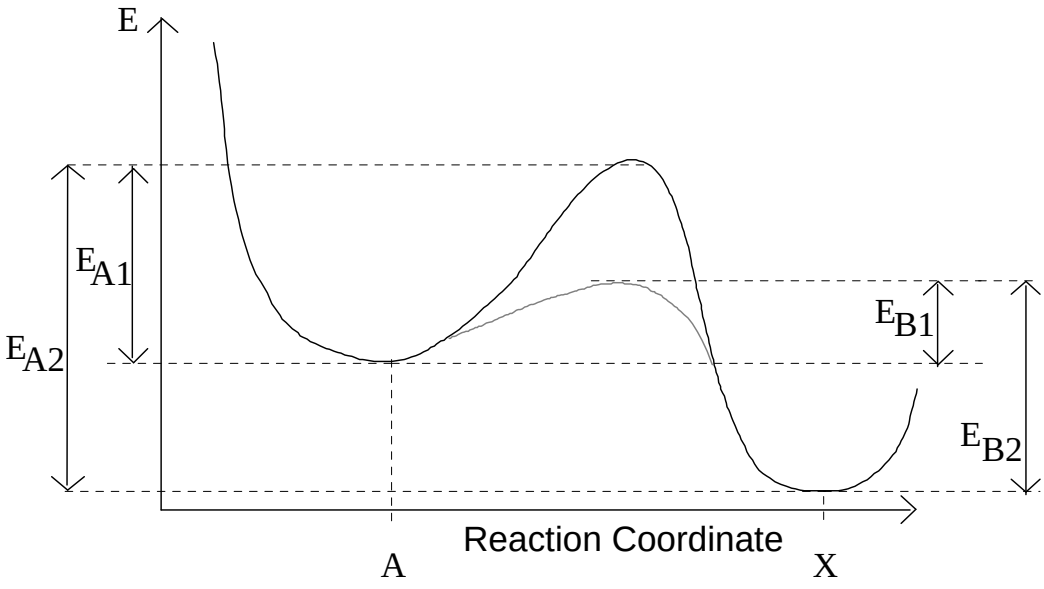


Fig. 3 Activation energy scheme of the reactions (1) and (2)

It should be noted that, according to equ. (8), the term describing the stationary electron transfer per time unit, $J=kX_S$, contains the functions of temperature $K(T)=k(T)/k'(T)$ and $\alpha(T)=b(T)/k(T)$, depending on the reaction enthalpies only, and the function k(T), which depends on the activation energy of reactions (1). If we assume as a first approximation the Arrhenius law for all reaction constants,

$$k_i=k_{i0}\exp(-E_i/RT), \qquad (13)$$

we obtain, according to Fig. 3, the following functional dependencies for K and α:

$$K(T)= K_0 \exp((E_{A2}-E_{A1})/RT) = K_0 \exp(\Delta H/RT)$$

$$\alpha(T)= \alpha_0 \exp((E_{A1}-E_{B1})/RT) = \alpha_0 \exp(\Delta E/RT) \qquad (14)$$

and

$$k(T)= k_0 \exp(-E_{A1}/RT) .$$

Since the exponents of the first two terms are always positive (if the reactions (1) are exothermic and reaction (3) as a catalytic one decreases the activation barrier), only the third one remains , which decreases with decreasing temperature. Therefore it is, for certain parameter values, possible that the temperature dependence of the electron flux J(T) in a finite interval of temperature formally shows a negative activation energy. Such a situation is depicted in Fig. 4 for a selected set of parameters (compare figure legend).

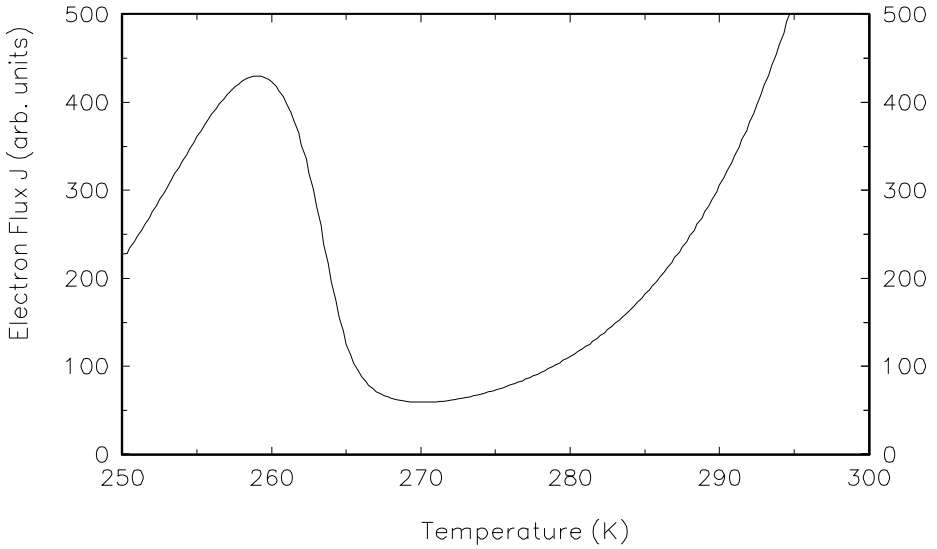
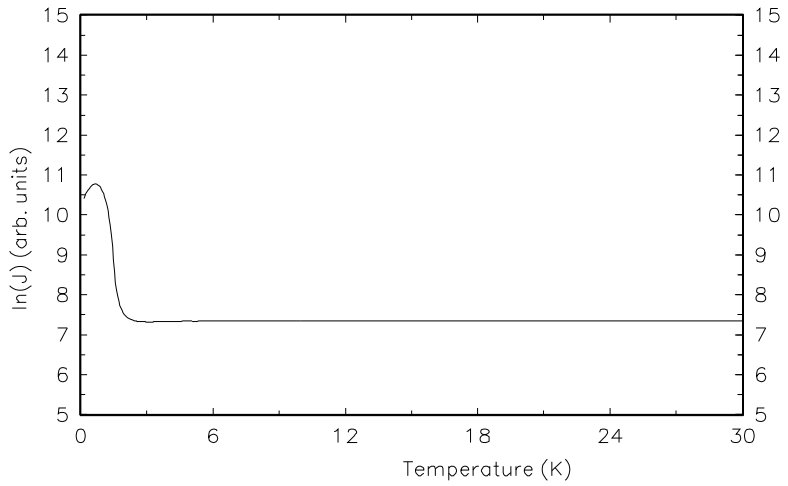


Fig. 4 Calculated temperature dependence of the electron transfer rate $J=kX_S$ with $K(0)=k_1(0)/k_{-1}(0)= 100$; $\alpha(0)= 6.18\ 10^{-9}$, $\kappa_a= 1.0$; $k_1(0)= 2.35\ 10^{17}$, $E_{a1}= 1.0\ 10^4$; $E_{a2}= 1.03\ 10^4$; $E_{b1}= 5.0\ 10^3$; $E_{b2}= 5.3\ 10^3$, F=0

Here, with decreasing temperature the rate constant k indeed decreases, but the simultaneous increase of the feedback parameter α(T) can exceed the decrease of k(T).

In Fig. 5 another set of parameters is used in order to simulate qualitatively the temperature dependency reported for the fast charge separation in purple bacteria at very low temperatures [7, 8].



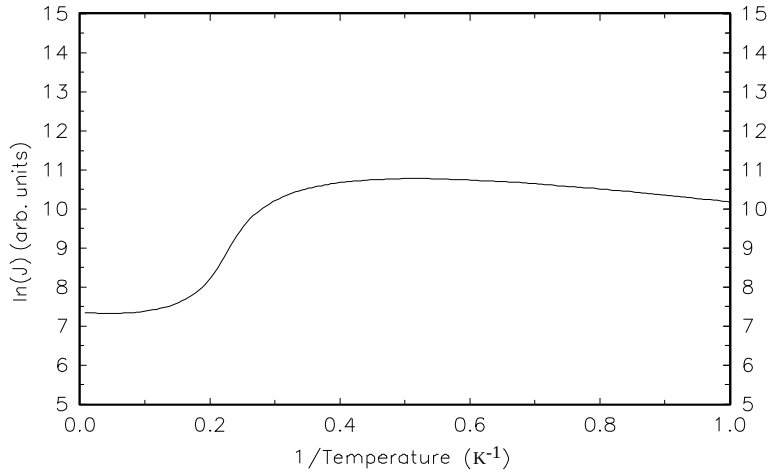


Fig. 5 The same as in Fig. 3 but with other parameter values (in Figures 5a and 5b different graphical presentations are choosen): $K(0)=k_1(0)/k_{-1}(0)= 300$; $\alpha(0)= 0.5$; $\kappa_a= 1.0$; $k_1(0)= 784.0$, $E_{a1}= 4.1$; $E_{a2}= 6.1$; $E_{b1}= 1.0$; $E_{b2}= 3.0$, $F=0$

It is seen that the rate of electron transfer can be easily simulated to increase at very low temperature. In order to make reliable computer simulations of the temperature dependent electron transfer in real reaction centers, a more profound analysis of the kinetics and experimental temperature dependence will be required.

5. Differences between simple and co-operatively stimulated electron transfer: stability investigations

Despite of the identical stationary states of both models (1), (2), (3) and (1), (2a), (3) describing the stimulated electron transfer, their dynamic properties in phase space are very different: In the simple model only one nonnegative state exists which remains stable for all parameter values of κ_a. However, in the co-operative model there is another nonnegative state (describing the absence of any stimulation of electron transfer) crossing the first one at $\kappa_a=0$. This is a simple transcritical bifurcation [20], where only one real eigenvalue changes its sign. Consequently, in the vicinity of $\kappa_a=0$ the slaving principle applies enslaving the dynamics of the X-intermediate by the master variable M(t) [21, 22]. Accordingly, the dynamics of the system variables becomes highly co-ordinated, because the changes of all variables can be described in the first approximation by only one differential equation of first order (see [15])

This qualitative difference of the two models can be demonstrated by the application of Lyapunov's stability criterion: According to this criterion, a stationary state is asymptotically stable if the real parts of all eigenvalues of the corresponding linearized system are negative. Using the criterion of Hurwitz for a system of two differential equations we obtain the two inequalities as the condition for asymptotic stability:

$$L_1 = (J_{11} + J_{22}) < 0, \quad L_2 = (J_{11}J_{22} - J_{12}J_{21}) > 0 \; . \tag{15}$$

where the J_{ij} are the elements of the Jacobi matrix of the system. Here, the first inequality is always fulfilled, but the second one differs for the two models. In the case of the simple stimulated electron transfer it leads to the condition

$$\sqrt{(\alpha\kappa_a K - 1 - K)^2 + 4\alpha\kappa_a (K + F)} > 0 \tag{16}$$

which is fulfilled for all positive κ_a and cannot change its sign at $\kappa_a=0$. On the contrary, in the case of co-operative electron transfer we obtain the condition

$$-\eta' \kappa_a (K + 1) X_s = -\eta' \kappa_a K > 0 \tag{17}$$

for the new solution branch. This condition is not fulfilled for all positive parameter values of κ_a, i.e. the new stationary state is unstable. However at κ_a=0 the sign changes and the new state becomes stable. Accordingly the other state (8) becomes unstable. Therefore here, in difference to the simple model, the slaving principle applies (which mathematically bases on the center manifold theorem) and the variable M(t) becomes the master variable (the order parameter in the speech of Haken[21]) for the variable X(t). This corresponds to a maximum of dynamic co-operativity of the system.

6. Discussions.

The presented non-linear irreversible model on the primary electron transfer in photosynthetic reaction centers is able to explain qualitatively the initially mentioned characteristic electron transfer features observed in this system.The derived mechanism is based on the assumption that the negative activation energies measured in electron transfer systems are in fact only effective activation energies, i.e. the observed electron transfer process consists of several elementary processes, each of them with a positive activation energy. Nevertheless their combination can lead, as it is shown in the above model, to a temperature dependence of the overall process, which can be described formally by a negative activation energy (for a finite temperature interval). A necessary condition for that is a non-linear kinetic coupling of the elementary processes via positive feedback (autocatalysis). But it should be noted that also linear kinetics under suitable conditions can lead to negative activation energies, e.g. if one considers the formation of an intermediate bond complex, which can fastly dissociate back to the original reactants (forming a preceding equilibrium)(see [23]):

$$A + B \underset{\leftarrow}{\overset{\rightarrow}{}} C \rightarrow E + F.$$

But such a linear model would be only able to describe one change of monotonicity in the Arrhenius plot, in contrast to our model, which describes at least two changes. Therefore it should be possible to distinguish between these two model types by kinetic reasons (monotonicity of Arrhenius plot, possibility of preceding equilibria in the reaction considered). For example, the Arrhenius plot of the conductivity of reduced films of cytochrome c_3 shows one maximum and one minimum, which indicates the necessity of a non-linear kinetic description [24].

However it should be mentioned that there are also other explanations possible applying at a more elementary level, e.g. it was shown by means of scattering theory that also genuine elementary chemical reactions can in principle have negative activation energies [25, 26, 27]. According to this theory, negative activation properties are expected especially for ion-molecule reactions. But to what extent these ideas can be applied also to intramolecular processes in large biomolecules is an open question.

According to our opinion it is thinkable that the presented possibilities of a non-linear theory with feedback processes are applicable to a more elementary level, at least for large biomolecules, which itself are embedded in a suitable matrix (i.e. the molecular environment inside of the living cell). Then a quasi-macroscopic description of the biomolecule as a dissipative system could allow dynamic pattern formation processes (leading to molecular arrangements and(or) limit-cycle oscillations), which may promote the electron transfer process. In this case part of the supplied energy would be consumed for the formation of the "microscopic dissipative structure", and the reaction probability would be enormously increased due to a significant increase of structuring. As a consequence the activation barrier would be decreased or totally vanishes.

The discussed mechanism is able to explain the efficient unidirectionality of electron transfer which has been elaborated in a preceding publication on stimulated and co-operative electron transfer [14, 15]. It simultaneously explains the high electron transfer rates obtained as a consequence of molecular-electronic autocatalysis (compare relation (12) and refs. [14, 15]). According to our model the L branch of the reaction center must contain molecular or electronic elements which are acting as feedback loops which may be considered to be the key to stimulated and co-operative electron transfer. This makes it understandable why homogenization of the branches attempted through genetic manipulation [9] makes electron transfer much slower.

The presence of a BChl pair in the reaction center has long been a matter of controversy. If the M - branch would not at all be needed, evolution would probably have eliminated or at least reduced it. Within our non-linear model of electron transfer the BChl pair may be needed to produce a time lag for molecular electronic feedback. The feedback must be triggered before the electron is passed on along

one branch. It may therefore shortly oscillate between the branches before the autocatalytic signal becomes active. This explains why picosecond fluorescence has been found to be emitted also from the M - branch [28]. The molecular electronic model imagined can qualitatively be compared with the behaviour of two capacitively coupled oscillating electric circuits. The electrical energy is oscillating between the two circuits until one circuit is interrupted. In the reaction center this happens, when molecular electronic feedback, triggered in the first portion of branch L provides the asymmetry to stimulate directional electron transfer.

Our model calculations have finally shown that negative activation energies can be obtained in agreement with experimental data. The key result is the derivation of an activated complex which is functioning as a dissipative structure. This means the energy needed for its formation is not derived from the thermal energy of the surrounding, but deduced from the reaction itself, this means from the photochemical energy. On the basis of a formal analogy with Marcus theory of electrochemical electron transfer we would have to postulate a "negative reorganization energy". This can be seen from Marcus' formula for the temperature dependence of the rate of electron transfer (see e.g. Marcus [29, 30])

$$k_{ET} \propto \exp\left[-\frac{\left(E_2{}^o - E_1{}^o + \lambda\right)^2}{4\lambda kT}\right],$$

(with $E_2{}^o - E_1{}^o$ - the difference between the minimum potential energy of the whole system in its initial state and that of its final state, and λ - the reorganization energy required to change the orientation of surrounding solvent dipoles) where a negative temperature dependence can be provided only by a change of sign of the reorganization energy λ which however leads to a distribution function without physial meaning. But what is the qualitative significance of a negative reorganization energy? The reorganization energy is the free energy needed to form the activated complex for electron transfer (Fig. 6a) and is supplied by thermal energy from the surrounding medium. If the vibrational activity of the surrounding medium is strongly restricted (e.g. as a consequence of adsorption to an interface), the reorganization energy may be significantly reduced (Fig. 6b). A formally negative reorganization energy (Fig. 6c) means then that the free energy for the formation of the activated complex is not provided by the thermal energy of the environment. It is rather provided by the reaction energy itself liberated during an exothermic reaction step at the beginning of the reaction chain. The relevant distribution function is not anymore

subject to thermal activation, but determined merely by the activated non-linear dissipative mechanism (the distribution chosen for Fig. 6c is arbitrary).

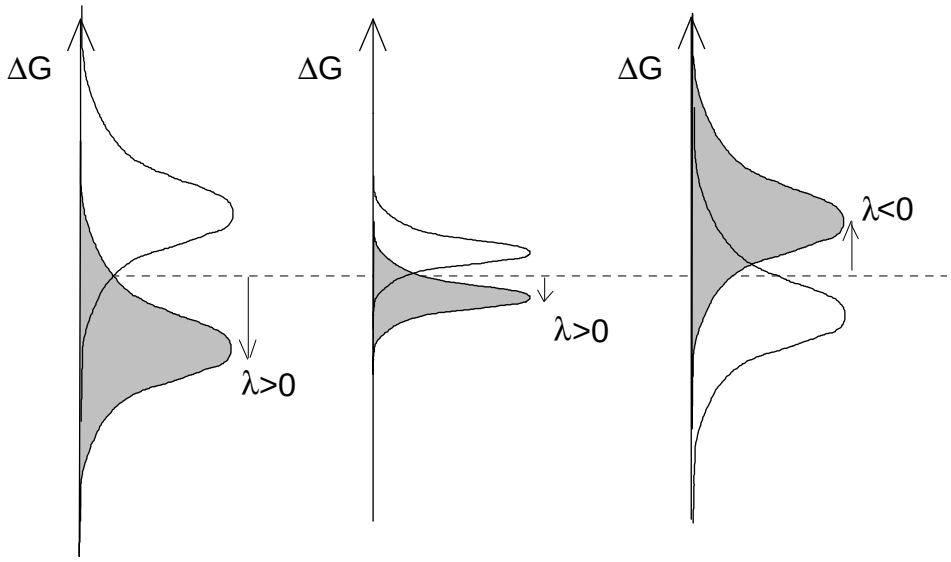


Fig. 6 Schematic diagram of the state densities (gray: filled states) in the Marcus picture for different reorganization energies: (a) - high reorganization energy, (b) - small reorganization energy for adsorbed species, and (c) formally negative reorganization energy in the case of negative Arrhenius activation energy (distribution function was choosen arbitrarily)

This energy supplied for the activated complex would generate an "energized medium" around the molecular electron transfer chain (in analogy to the active medium in a Laser crystal due to the population inversion) providing the formation of a dissipative conformational structure before it is dissipated. The consequence would be a strongly irreversible overall reaction with an activated complex the formation of which is controlled by free energy from the reaction and not from the thermal environment.
Qualitatively the situation is depicted in Fig. 7. While thermal energy from the environment is responsible for the formation of the activated complex in the classical case (Fig. 7a), the reactants must liberate some energy which is transduced by the medium to form the activated complex (Fig. 7b).

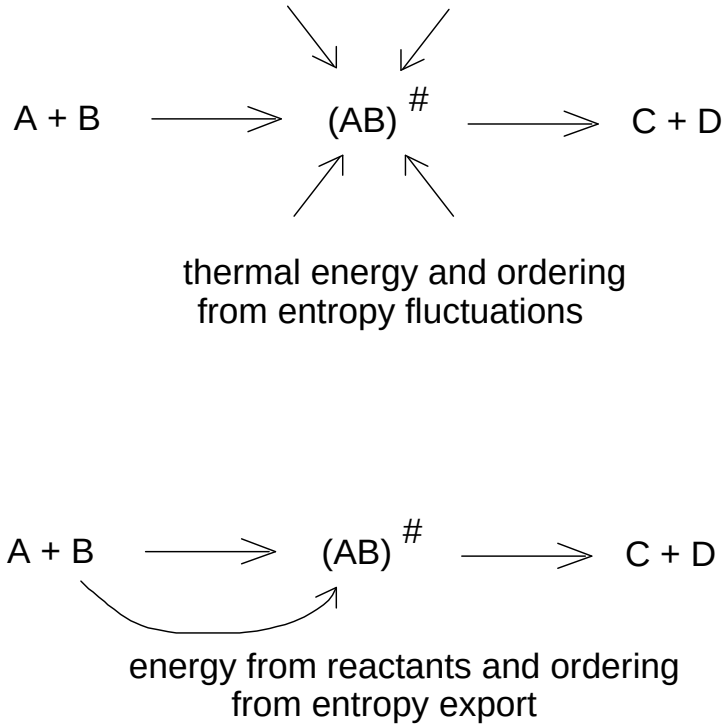


Fig. 7 Schematic drawing showing the energy and entropy flows in (a) thermally and (b) via feedback processes activated electron transfer

The molecular order needed is supplied by export of entropy, which is possible through autocatalytic steps. In this way the activated complex may be considered a dissipative structure.
We are convinced that the proposed electron transfer model can further be elaborated and extended to other irreversible biological mechanisms.

Necessary experimental verification and possible support from recently published data

In order to experimentally explore the proposed molecular feedback mechanisms, adequate experimental techniques would have to be applied:
The Raman scattering technique like the infrared absorption technique is a vibrational spectroscopy, which sensitively detects small perturbations of molecular vibrations thus giving structural information concerning changes in conformation and protein interactions. Resonance Raman spectroscopy in addition yields electronic information since the vibrational modes observed are enhanced via the resonant electronic transition of the chromophore. Therefore femto and pico-second time resolved infrared and infrared resonance Raman experiments will be needed to

identify and understand the transient molecular feedback mechanisms postulated in this publication. Ongoing research based on mutants with respect to the protein environment will be especially instructive and should include the above mentioned spectroscopic studies.

After submission of the manuscript experiments demonstrating long-lasting coherent nuclear motion in the picosecond region during primary photosynthetic electron transfer have been published[31]. They cannot be understood in terms of classical electron transfer models and have tentatively been interpreted referring to a multilevel Redfield theory for electron transfer dynamics in a dissipative environment[32]. This theory however only explains how electrons can be transferred in presence of coherent vibrational nuclear motion, but does not provide a mechanism for the surprisingly long duration of coherence.
In the frame of our model the observed long duration of phase coherence of nuclear oscillations after photoexcitation indicates once again the possibility of a dissipative structuring phenomenon (selfsustained oscillations) during the electron transfer process:
In linear systems with many degrees of freedom the fluctuations of system parameters fastly destroy any coherent motion of the particles and only at very low temperatures dephasing times can be become longer.
In contrast, in dissipative systems beyond the limit of linear irreversible thermodynamics (i.e. if appropriate non-linear feedback processes between particle and bath variables can occur) the system state can become unstable. Here the fluctuations of the system will initiate the formation of a new system state representing an ordered dynamically structure, e.g. a limit cycle oscillation ("order through fluctuations" in the sense of Prigogine). This process can occur at any temperature, depending on the system parameters only. Consequently, in the (hypothetical) case of dissipative structuring (in contrast to the mostly linear dissipation without feedback processes in the usual electron transfer theories) the dephasing time of nuclear motion would not be essentially affected by fluctuations and therefore relatively insensitive to an increase of temperature - as it was observed by Vos et al[31].
For this reason we cannot believe, that a full quantum mechanical treatment alone, as suggested by Vos et al.[31], is able to explain the new observed results of anomalously long dephasing times because all the classical and quantum mechanical approaches treat the dissipation as an interaction of the system with an passive acting bath, which can be described as a collection of non interacting

oscillators. We are convinced that sustained coherent motion requires feedback in analogy to the model presented in this publication. We therefore conclude, that the observation of coherent nuclear motion supports our interpretation of the primary photosynthetic charge separation as an event facilitated by co-operative mechanisms: A coherent motion may be induced through energy liberated into a feedback loop during femto-second relaxation of the excited electron. We therefore suggest to study the role of the protein environment, especially the L181Phe-M208Tyr pair[18], which we suggest ed as an element of molecular electronic feedback with repect to the generation of coherent vibrations of reaction center molecules.
As a consequence electron transfer can become coherent taking advantage of an especially favourable activation complex, which we have described as a dissipative structure.

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