determine the role of these variables, we are preparing derivatives
of monomeric (LSLBLSL), with apolar, N-terminal blocking
groups, as well as tetraphilins with altered peptide sequences.

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and Jim Krywko for aid in the computer graphics modeling.

Supplementary Material Available: Listings of experimental
and spectral details for tetraphenylporphyrins and details of
channel measurements in planar bilayers (4 pages). Ordering
information is given on any current masthead page.

Observation of a Series of Degenerate Cyclic Double,
Triplet, and Quadruplet Proton Transfers in Solid
Pyrazoles

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The ability of proton donors to form different hydrogen-bonded
associates in the liquid state often makes it difficult to elucidate
their proton-transfer dynamics. For example, it has been postu-
lated that pyrazoles may exchange protons in cyclic dimers and/or
trimers.1-7 Such difficulties do not arise in solid-state studies
where structures can be studied by diffraction techniques
and proton-transfer dynamics by high-resolution NMR spectro-
scopy.8,12 Thus, it has been recently shown that 3,5-dimethylpyrazole

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Figure 2. Effect of voltage on the probability of forming the major
conductance state relative to the background conductance. The inset
shows a current vs time trace for tetraphilin 1. P on is the probability per
unit time that the major conductance is observed, and P off is the proba-
bility per unit time that this state is not observed. The slope is related
to the effective number of charges that are translocated across the
membrane in going from a closed ("off") to an open ("on") channel
state:12 2.4 for (LSLLLSS), and 0.5 for the tetraphilin. The voltage
dependence of single channel conductances for (LSSLSSL), has not been
measured because of its extremely short lifetimes in 1.0 M HCl.11 How-
ever, preliminary macroscopic conductance measurements indicate
that it has a gating charge of 1.2. The methods used to collect and analyze
the data are described in the supplementary material.

Figure 1, could readily be purified by reversed-phase HPLC.
Tetraphilin 1 forms proton channels in planar diphytanoyl
phosphatidylcholine bilayers in 1.0 M HCl with a major
conductance state of 470 pS and secondary, more variable conductance
states of 320 and 100 pS. As with the (LSSLSSL), channels, the tetraphilin channels are proton selective, as no
conductance was observed with LiCl as the electrolyte. The lifetime of the major conductance state (5 ms) is considerably
longer than that of (LSLLLSS), (<0.2 ms in 1 M HCl),12 indicating
that the attachment of the peptide to the template stabilizes the
conducting state of the peptide. Furthermore, the probability
of channel formation depends linearly on the bilayer concentration
of tetraphilin 1, suggesting that the channels are monomeric.

The formation of channels by tetraphilin 1 is nearly voltage
independent (Figure 2), in marked contrast to the behavior of
(LSLLLSS),. A mechanism to explain the voltage dependence
of the parent peptide has been postulated.3 In the absence of a
transmembrane potential, the peptide is oriented in planar lipid
bilayers with its a-helical axis parallel to the membrane surface.4
A transmembrane voltage stabilizes the channel-forming, vertically
inserted orientation of the peptide through favorable interactions
with the helical macrodipole. On the other hand, the small voltage
dependence for tetraphilin suggests that it forms helical bundles
that are predominantly vertically oriented in the membrane, even
in the absence of a transmembrane voltage. Other interpretations
of the voltage dependence are also possible, and we are attempting
to confirm this orientation through spectroscopic investigations
of tetraphilin 1 in planar multilayers.

These results show that the tetraphenylporphyrin template
exerts a major influence on the lifetime and voltage dependence
of (LSSLSSL), channels. These differences presumably arise
from changes in the overall hydrophobicity and geometric re-
strictions imposed on the peptide by the porphyrin template. To

(14) Datta-Gupta, N.; Jones, E.; Thomas, L. K.; Malakar, D. J. Indian
(15) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Mar-
molecules are present in the asymmetric units of both solids.

proton transfers with equilibrium constants of equal concentration.

positions.

double and quadruple proton transfers.

The four sharp lines stem from a small quantity of the labeled solid 3,5-diphenyl-4-bromopyrazole (DPBrP), DMP, and 3,5-diphenylpyrazole (DPP) as a function of temperature are shown. As described previously for DMP, we also observe for DPBrP and DPP two sharp lines at low temperature, indicating the presence of protonated and nonprotonated nitrogen atoms of equal concentration. As the temperature is raised, the two lines broaden and coalesce into one sharp line, indicating degenerate proton transfers with equilibrium constants of $k = 1$.

(DMP) forms cyclic trimers in the solid state in which a degenerate triple proton transfer takes place. We now present evidence that pyrazoles may also form cyclic dimers and tautomers subject to double and quadruple proton transfers.

In Figure 1 the 30.41-MHz $^{15}$N CP MAS NMR spectra (CP = cross polarization, MAS = magic angle spinning) of $^{15}$N-labeled solid 3,5-diphenyl-4-bromopyrazole (DPBrP), DMP, and 3,5-diphenylpyrazole (DPP) as a function of temperature are shown. As described previously for DMP, we also observe for DPBrP and DPP two sharp lines at low temperature, indicating the presence of protonated and nonprotonated nitrogen atoms of equal concentration. As the temperature is raised, the two lines broaden and coalesce into one sharp line, indicating degenerate proton transfers with equilibrium constants of $k = 1$.

Two crystallographic independent molecules are present in the asymmetric units of both solids.

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Reactivity of Tunichromes: Reduction of Vanadium(V) and Vanadium(IV) to Vanadium(III) at Neutral pH

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Ascidians accumulate vanadium ions to extraordinarily high concentrations (up to 1 M) from sea water, where vanadium is present in the +5 oxidation state. In ascidian blood cells, however, the vanadium was found to be in the +3 and/or +4 states—in *Ascidia nigra*, at least 90% of the total vanadium is in the oxygen-sensitive +3 state. Organic ligands are thought to maintain the solubility of concentrated vanadium at biological pH values. These ligands may belong to a class of oxygen-sensitive pigments 1 and 2, called tunichromes, whose polyphenolic moieties suggest a role in vanadium accumulation by complexation and/or reduction. It is known that tunichromes and catechols can reduce V(V) to V(III) in vitro, however, proof of any relationship between tunichrome and vanadium in vivo has been elusive.

In studies of the general reactivity of tunichrome in vitro, we have obtained the first evidence (using EPR spectroscopy) that tunichrome can reduce V(V), and also V(IV), to the +3 oxidation state, V(III). These reactions were conducted in neutral aqueous media. Similar redox reactivity was previously observed under anhydrous conditions: V(IV) was reduced by pyrogallol in THF at pH 7 and by 3,5-di-tert-butylcatechol in toluene or methanol. The present results corroborate the hypotheses that tunichrome could generate V(III) in vivo and that an oxidized form of tunichrome could sequester native V(III).

The reaction between the simplest tunichrome 2a (synthetic Mm-1) in methanol/phosphate buffer pH 7 and V(V) (from V2O5) or V(IV) (from VOSO4) was studied by EPR spectroscopy to determine the oxidation state(s) of product vanadium. EPR measures the V(IV) oxidation state selectively, but the levels of V(III)

Table I. Ratio of V(IV) versus V(III) Found by EPR Analysis of Mm-1 Treated with V(V) or V(IV) (1, 2, and 4 mol equiv) at pH 7

<table>
<thead>
<tr>
<th>entry</th>
<th>starting metal</th>
<th>V(IV) found vs V(III) found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 mol equiv of V(V)</td>
<td>85:15</td>
</tr>
<tr>
<td>2</td>
<td>2 mol equiv of V(V)</td>
<td>90:10</td>
</tr>
<tr>
<td>3</td>
<td>3 mol equiv of V(V)</td>
<td>60:40</td>
</tr>
<tr>
<td>4</td>
<td>1 mol equiv of V(IV)</td>
<td>trace&lt;100</td>
</tr>
<tr>
<td>5</td>
<td>2 mol equiv of V(IV)</td>
<td>60:40</td>
</tr>
<tr>
<td>6</td>
<td>4 mol equiv of V(IV)</td>
<td>93:7</td>
</tr>
</tbody>
</table>

*Adjusted to pH 2 before EPR measurement at room temperature.

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