Communications to the Editor

Tetraphilin: A Four-Helix Proton Channel Built on a Tetraphenylporphyrin Framework

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Ion channel proteins provide efficient and selective conduits for the transmission of ions across biological membranes. Previously, we prepared a number of α-helical peptide models for channel proteins[1-4] including the proton-selective ion channel H_2N-(Leu-Ser-Leu-Leu-Leu-Ser-Leu)$_3$-$CONH_2$ ((LSLLLSL)$_3$), which forms channels in response to a transmembrane voltage. Molecular modeling suggests that the channel formed by (LSLLLSL)$_3$ consists of a parallel four-helix bundle, although other aggregation states are also possible.[2] We therefore sought to control the aggregation state of the peptide by attaching four copies of it to a C_4-symmetric template. Tetraphenylporphyrins appeared to be attractive templates for this purpose and would represent a logical extension of our previous work with membrane-spanning tetraphenylporphyrin derivatives containing rigid steroidal appendages.[5]

In previous work, bundles of parallel α-helices of defined aggregation numbers have been prepared by covalently binding peptides to linear or cyclic lysine-rich peptide templates.[6,7] Other workers have designed α-helical bundles that assemble around metal ions.[8] Sasaki and Kaiser[9] introduced coproporphyrin as an alternative, more rigid template for stabilizing a four-helix bundle. Tetraphenylporphyrins offer additional advantages as they are more rigid than coproporphyrin and also are protected from oxidation at their meso positions. We therefore attempted to attach four copies of (LSLLLSL)$_3$ to *meso*-tetrakis(3-carboxyphenyl)porphyrin via *m*-carboxamido linkages (Figure 1). A meta attachment provides a favorable interhelical spacing as well as a degree of conformational flexibility about the interannular C–C bond. However, problems associated with the solubility of the product prompted us to replace (LSLLLSL)$_3$ with the more soluble proton channel forming peptide (LSLBLSL)$_3$[3] (B = α-aminoisobutyric acid). The resulting conjugate,[10] tetraphilin **1**

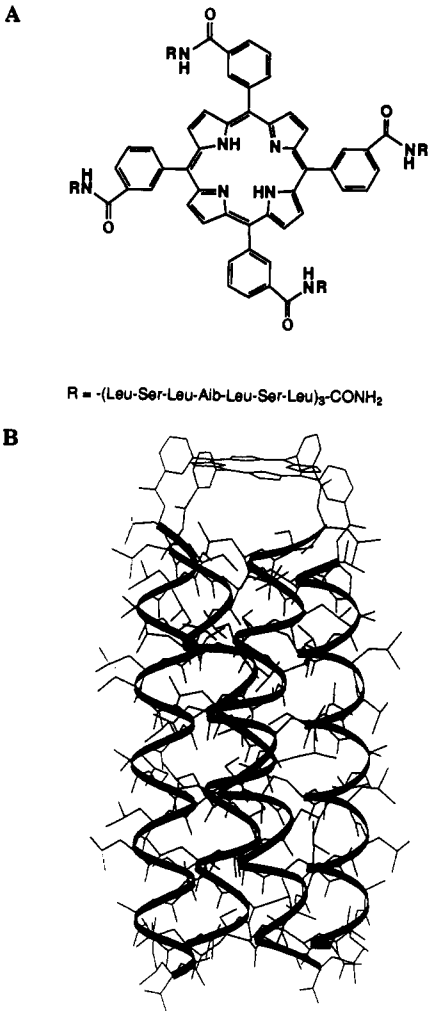

Figure 1. Chemical structure (A) and energy-minimized computer graphics model (B) of the putative proton-conducting state of tetraphilin **1**. The model for tetrameric (LSLLLSL)$_3$[1] was docked against the crystal structure of an *o*-amidophenyl tetraphenylporphyrin.[11] A geometrically reasonable amide bond could be formed between the N-terminus of the helices and the phenylporphyrin if a carboxyl group was introduced at the 3-position of the phenyl ring. Finally, one Leu per heptad was changed to Aib to give (LSLBLSL)$_3$.

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(10) (LSLBLSL)$_3$ was prepared as described.[13] *meso*-Tetrakis(3-carboxyphenyl)porphyrin[14] was synthesized by condensation of pyrrole and methyl 3-formylbenzoate using the method of Lindsey.[15] Purified, unprotected (LSLBLSL)$_3$ (800 mol %) was coupled to *meso*-tetrakis(3-carboxylphenyl)-porphyrin (100 mol %) using benzotriazole, tetramethyluronium tetrafluoroborate (1000 mol %), and *N*-methylmorpholine (2000 mol %) in dimethyl sulfoxide, as described in the supplementary material.
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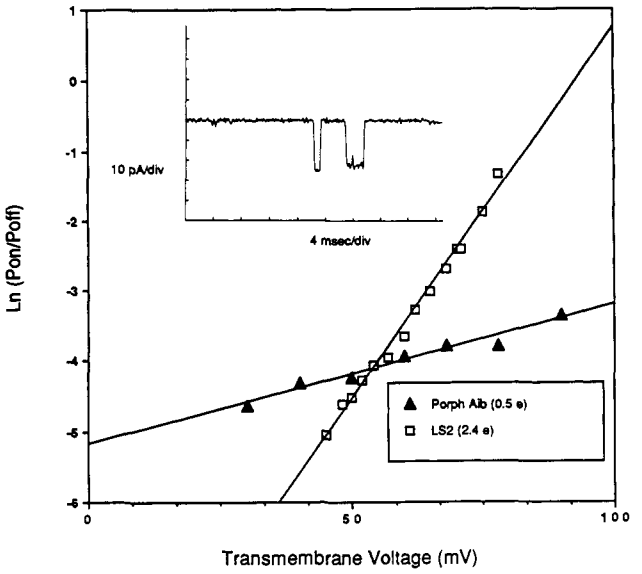

Figure 2. Effect of voltage on the probability of forming the major conductance state relative to the background conductance. The inset shows a current vs time trace for tetraphilin **1**. P_{on} is the probability per unit time that the major conductance is observed, and P_{off} is the probability per 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 $(LSLLLSL)_3$ and 0.5 for the tetraphilin. The voltage dependence of single channel conductances for $(LSLBLSL)_3$ has not been measured because of its extremely short lifetimes in 1.0 M HCl.[3] However, 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 $(LSLBLSL)_3$ 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 $(LSLBLSL)_3$ (<0.2 ms in 1 M HCl),[3] 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 monomolecular.

The formation of channels by tetraphilin **1** is nearly voltage independent (Figure 2), in marked contrast to the behavior of $(LSLLLSL)_3$. 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 α-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 inserted 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 $(LSLBLSL)_3$ channels. These differences presumably arise from changes in the overall hydrophobicity and geometric restrictions imposed on the peptide by the porphyrin template. To determine the role of these variables, we are preparing derivatives of monomeric $(LSLBLSL)_3$ with apolar, N-terminal blocking groups, as well as tetraphilins with altered peptide sequences.

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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.

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Observation of a Series of Degenerate Cyclic Double, Triple, and Quadruple 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 postulated that pyrazoles may exchange protons in cyclic dimers and/or trimers.[2-7] 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 spectroscopy.[8-12] Thus, it has been recently shown that 3,5-dimethylpyrazole

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