## **New and Notable**

## **Proton Wires Are Different**

Benoît Roux

Groupe de Recherche en Transport Membranaire, Départements de Physique et Chimie, Université de Montréal, Montréal, Québec H3C 3J7 Canada

In this issue of the Biophysical Journal, interesting experimental findings about proton fluxes through gramicidin A (gA) channels and related analogs are reported. Phillips et al. (1999) compared the conduction of alkali metal ions and protons for related analogs of the gA channel in which the dipole of the Trp indole side chains were augmented (by fluorination) or decreased (by substitution with Phe) in two type of membranes with different headgroups. They observed that the ratelimiting factor governing H<sup>+</sup> transport through the gA channel is modulated in an opposite direction from that governing alkali metal cations upon changes in the side chains or lipid headgroups. In particular, the conductance of K<sup>+</sup> through the gA channel in glyceryl monoolein (GMO) membranes decreases when all four tryptophans are substituted by phenylalanines, while the conductance of H<sup>+</sup> increases for the same substitution. When Trp-11 is fluorinated, the K<sup>+</sup> conductance increases, while the conductance of H+ decreases. Interestingly, variations of the lipid headgroups exhibit a similar trend: the conductance of H<sup>+</sup> in diphytanoyl phosphatidylcholine (DPhPC) membranes is larger than in GMO membranes, contrary to the K<sup>+</sup> conductance. These observations essentially demonstrate that transport of H<sup>+</sup> and alkali cations through the gA channel

are governed by qualitatively different mechanisms.

Phillips et al. (1999) show that the observed variations in the K<sup>+</sup> conductance are correlated with the modulations in the electrostatic field near the channel entrance. In particular, the larger K+ conductance in GMO relative to DPhPC is consistent with the interfacial dipole potential of GMO membranes, which is larger than that of PC membranes (Pickard and Benz. 1978). Furthermore, the observed variations in the K+ conductance are correlated with the modulations of the indole dipole of the tryptophan side chains. Since they are oriented with the positive end of the indole dipole pointing toward the bulk solution (Hu et al., 1993) the Trp side chains favor the passage of a positive charge through the pore. Obviously, since proton also carry a positive charge, the observed variations in the H<sup>+</sup> conductance cannot be explained with such a mechanism. What could be going on? The authors propose that part of the answer lies in a recent theoretical study of a proton wire by Pomes and Roux (1998).

The conduction of protons along a linear hydrogen bonded chain of water molecules, a "proton wire," can be decomposed in two complementary microscopic steps. The first step involves the rapid translocation of a proton along the water chain by way of a Grotthuss mechanism, whereas the second step involves the reorientation of the water chain. The second step is necessary to complete the transport cycle of a proton wire. After the translocation of the proton from entry to exit, the water chain is left with all dipoles oriented in the opposite direction. A preliminary reorientation of the water chain is thus required to accept the next incoming proton. Using molecular dynamics calculations based on atomic models, Pomes and Roux (1998) found that a free energy barrier of a few kcal/mol opposes the reorientation of the water chain, whereas the

translocation of the H<sup>+</sup> along the chain is essentially barrierless. Furthermore, the simulations showed that the ratelimiting water reorientation in a single file of hydrogen bonded water molecules proceeds sequentially, i.e., it is initiated at the end of one chain and the transition state occurs when the hydrogen bond defect reaches the center. The observed variations in the H<sup>+</sup> conductance reported by Phillips et al. (1999) may be the first evidence for the rate-limiting water reorientation step in the transport cycle of a proton wire

Transfer of H<sup>+</sup> mediated by chains of water molecules is an important mechanism in a wide range of proton transport phenomena involved in bioenergetics. For example, the functional importance of a 23-Å-long water chain observed in the photosynthetic reaction center from Rhodobacter sphaeroides (Ermler et al., 1994) was demonstrated by site-directed mutagenesis (Baciou and Michel, 1995). Similarly, perturbation of the internal water chain in cytochrome f shows a loss of the concerted reduction of cytochrome f and  $b_6$  (Ponamarev and Cramer, 1998). Nonetheless, the properties of proton fluxes in these complex biological systems are not easy to characterize, and one is seeking simpler model systems to investigate. The gA and its related analogs provide good prototypical systems for investigating proton transport along a hydrogen bonded water chain. Several aspects of proton transport through the gA channel are fascinating and generate continued interest (Cukierman, 1999; DeCoursey and Cherny, 1999). In another article in this issue, Quigley et al. (1999) investigate the proton fluxes through RR and SS dioxolanelinked gA analogs. Their measurements show that dioxolane-linked channels exhibit stereospecific modulation of H<sup>+</sup> conductance. The proton conductance in the SS-linked dimer is about 2 to 4 times larger than in the RR-linked dimer. The authors propose

Received for publication 14 September 1999 and in final form 14 September 1999.

Address reprint requests to Benoit Roux, Dept. Chemistry, Rm. D603, C.P. 6128, Succursale Centre-Ville, Montreal, Quebec H3C 3J7, Canada. Tel.: 514-343-7105; Fax: 514-343-7586; Email: rouxb@plgcn.umontreal.ca.

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that this reflects differences in waterwater and water-channel hydrogen bonding in the two stereoisomers affecting the rate of the reorientation step. Although this explanation is consistent with the analysis of Phillips et al. (1999), a definitive conclusion will require a comparison of the transport properties of H<sup>+</sup> with those of alkali cations in the two stereoisomers.

Understanding proton wires from a theoretical and computational point of view is very challenging. In the long term, studies with analogs of the gA channel such as reported by Phillips et al. (1999) and Quigley et al. (1999) provide important information about H<sup>+</sup> conduction along proton wires which will be essential for guiding the construction of theoretical models. Nonetheless, the conclusions from computer simulations of proton wires will remain speculative unless further information is obtained at the atomic level from experiments. A key guestion is, how can we learn more about proton wires at the microscopic level? For example, x-ray scattering (Olah et al., 1991) and nuclear magnetic resonance (Smith et al., 1990; Jing et al., 1995; Tian et al., 1996) have provided a wealth of information about the interaction of alkali cations with the gA. Perhaps those powerful approaches could be used to investigate the gA proton wire. Lastly, other experimental

techniques such as Fourier transform infrared spectroscopy offer promising avenues. Recently, Bartl et al. (1998) detected large collective proton motions in the interior of the gA channel using this technique. Meanwhile, the recent and interesting results of Phillips et al. (1999) and Quigley et al. (1999) contribute to the current reflection about proton transport. This is a story to follow.

Discussions with David Busath, Sam Cukierman, and Régis Pomes are gratefully acknowledged.

## REFERENCES

- Baciou, L., and H. Michel. 1995. Interruption of the water chain in the reaction center from Rhodobacter sphaeroides reduces the rates of the proton uptake and of the second electron transfer to QB. *Biochemistry*. 34:7967–7972.
- Bartl, F., B. Brzezinski, B. Rozalski, and G. Zundel. 1998. FT-IR study of the nature of proton and Li<sup>+</sup> motions in gramicidin A and C. J. Phys. Chem. B. 102:5234–5238.
- Cukierman, S. 1999. Flying proton in linked gramicidin A channels: ion channels and other proton channels. *Israel J. Chem.* In press.
- DeCoursey, T. E., and V. V. Cherny. 1999. An electrophysiological comparison of voltage-gated proton channels, other ion channels and other proton channels. *Israel J. Chem.* In press.
- Ermler, U., G. Fritzsch, S. K. Buchanan, and H. Michel. 1994. Structure of the photosynthetic reaction centre from Rhodobacter sphaeroides at 2.65 Å resolution: cofactors and protein-cofactor interactions. *Structure*. 2:925–936.
- Hu, W., K. C. Lee, and T. A. Cross. 1993. Tryptophans in membrane proteins: indoles

- ring orientations and functional implications in the gramicidin channel. *Biochemistry*. 32: 7035–7047.
- Jing, N., K. U. Prasad, and D. W. Urry. 1995. The determination of binding constants of micellar-packaged gramicidin A by <sup>13</sup>C-and <sup>23</sup>Na-NMR. *Biochim. Biophys. Acta.* 1238: 1–11.
- Olah, G. A., H. W. Huang, W. Liu, and Y. Wu. 1991. Location of ion-binding sites in the gramicidin channel by x-ray diffraction. J. Mol. Biol. 218:847–858.
- Phillips, L. R., C. D. Cole, R. J. Hendershot, M. Cotten, T. A. Cross, and D. D. Busath. 1999. Noncontact dipole effects on channel permeation. III. Anomalous proton conductance effects in gramicidin. *Biophys. J.* 77: 2492–2501.
- Pickar, A. D., and R. Benz. 1978. Transport of oppositely charged lipophilic probe ions in lipid bilayer membranes having various structures. *J. Membr. Biol.* 44:353–376.
- Pomes, R., and B. Roux. 1998. Free energy profiles for H<sup>+</sup> conduction along hydrogenbonded chains of water molecules. *Biophys. J.* 75:33–40.
- Ponamarev, M. V., and W. A. Cramer. 1998. Perturbation of the internal water chain in cytochrome f of oxygenic photosynthesis: loss of the concerted reduction of cytochromes f and b6. *Biochemistry*. 37:17199–17208.
- Quigley, E. P., P. Quigley, D. S. Crumrine, and S. Cukierman. 1999. The conduction of protons in different stereoisomers of dioxolanelinked gramicidin A channels. *Biophys J.* 77: 2479–2491.
- Smith, R., D. E. Thomas, A. R. Atkins, F. Separovic, and B. A. Cornell. 1990. Solid-state <sup>13</sup>C-NMR studies of the effects of sodium ions on the gramicidin a ion channel. *Biochim. Biophys. Acta.* 1026:161–166.
- Tian, F., K. C. Lee, W. Hu, and T. A. Cross. 1996. Monovalent cation transport: lack of structural deformation upon cation binding. *Biochemistry*. 35:11959–11966.