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Molecular mechanisms for proton transport in membranes

(proton pump/molecular motor/proton wire/hydrogen-bonded chains)

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ABSTRACT **Likely mechanisms for proton transport through biomembranes are explored. The fundamental structural element is assumed to be continuous chains of hydrogen bonds formed from the protein side groups, and a molecular example is presented. From studies in ice, such chains are predicted to have low impedance and can function as proton wires. In addition, conformational changes in the protein may be linked to the proton conduction. If this possibility is allowed, a simple proton pump can be described that can be reversed into a molecular motor driven by an electrochemical potential across the membrane.**

The last decades of research in bioenergetics have increasingly focused attention on the transport of protons as an intermediate mechanism in energy transduction. Mitochondrial ATP formation (1–3), photophosphorylation in chloroplasts (4, 5), and anaerobic metabolism in *Halobacterium halobium* (6, 7) are all believed to involve energetic protons as intermediates. One of the outstanding problems in this area is to discover well-defined molecular mechanisms for the linkage of active protons to chemical reactions. In this paper we draw upon studies of proton transport in inorganic systems to suggest likely mechanisms for the conduction of protons primarily through membranes, although the mechanisms may also pertain to other biological systems.

Hydrogen-bonded chains

Our theory begins with a molecular concept that was noted by Onsager on many occasions (8–10) although it was only recently put into the context of energy transduction mechanisms by Morowitz (11). This idea is that proteins with a suitable fraction of hydrogen bonding side groups, such as the hydroxyls of serine, threonine, and tyrosine and the carboxyls of aspartic and glutamic acids, may fold in the hydrophobic lipid environment of the membrane in such a way as to form chains of about 20 or more hydrogen bonds that span the membrane and that conduct protons across it. A portion of such a chain is shown schematically in Fig. 1. Although the formation of such structures, given the many degrees of freedom available to form and fold a protein, may seem obvious, we have satisfied ourselves that one can build at least one specific molecular example, shown in Fig. 2. This structure is based upon two parallel beta pleated polypeptide sheets with hydrophilic serine side groups interdigitated. With extended beta sheets, such a structure would form ribbons in the membrane (12). In the structure shown in Fig. 2 the beta sheets are truncated and the loose ends between successive rows are joined by a connecting amino acid group so that the proteins form two polypeptides of about 40 residues per chain. The amino acids in each polypeptide chain are 75% beta pleated and 25% connecting. The CNCC axes of the pleated groups are in the plane of the membrane and the CNCC axes of the connecting groups are perpendicular to the membrane surface. All of the beta pleated groups are hydrogen bonded via the usual N—H···O=C hydrogen bond. Although none of the connecting groups are hydrogen bonded, the structure balances this loss of energy by forming an equal number of hydrogen bonds between the serine residues in the hydrophilic region between the two polypeptides. This structure is only an abstract model to demonstrate feasibility. Work is in progress to construct such bridges in alpha helical structures such as those found in the purple membrane (13). Also, Dunker and Marvin (14) and Dunker and Jones (15), using the knobs-into-holes packing of α-helices, have suggested the possibility of chains of hydrogen bonds running in the general direction of the helical axis. A model of murein-lipoprotein of *Escherichia coli* shows this feature. Another method of associating chains of hydrogen bonds with protein structures involves waters of hydration which may provide ice-like proton pathways (16).

A hydrogen-bonded chain in a protein environment such as the one shown schematically in Fig. 1 should be long-lived and more like a solid structure than like liquid water. To provide a liquid water pore would require a much larger channel area than is needed for a single chain of hydrogen bonds. Since proton mobilities are comparable in ice and in water (16), one does not gain in efficiency by going to the more cumbersome pore. In addition, a liquid water pore would be permeable to ions other than hydrogen and would lose the semipermeable feature associated with the hydrogen-bonded chain. Of course, a well-known difficulty with a long one-dimensional chain is that a single break in the chain destroys its connectivity and conductivity. However, to traverse a membrane, one needs only 20–25 successive hydrogen bonds, so questions of long-range order in one-dimensional systems do not arise here.

Conduction of protons along hydrogen-bonded chains

For purposes of discussion let us make the nonessential assumption that all the protein side groups that participate in the hydrogen-bonded chain are serines, so the chain is a simple OH···OH···OH··· chain formed from the hydroxyl groups. Fig. 3 shows how protons move along such a hydrogen-bonded chain under the influence of an electric field. We will not at this point deal with the mechanism of injection of protons into the chain but just with the transport along it. Two quite different processes are involved. In Fig. 3*a* an excess proton appears at the left end of the channel to form a positive $-OH_2^+$ *ion* which then moves to the right by a sequential hopping of the successive protons (shown by arrows in Fig. 3*a*) from the left-hand side of the double minimum potential well to the right-hand side, with the eventual emergence of a proton on the right end into solution. The proton configuration is now shown in Fig. 3*b* and clearly blocks passage of another proton by the same process. The next step involves the rotation of an OH dipolar group, such as the one on the right-hand end of the chain. This removes all

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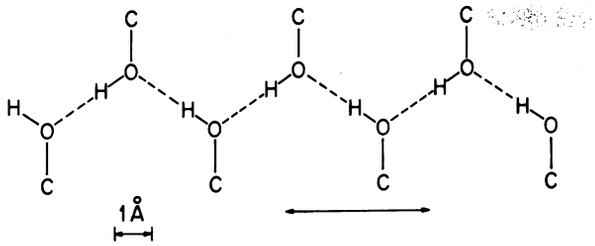

FIG. 1. A portion of a hydrogen-bonded chain formed by the hydroxyl groups attached to serine side groups of a polypeptide chain (not shown). This chain is assumed to be symmetrical so the protons could be all on the other side of the hydrogen bonds. The perpendicular to the membrane is shown by the arrow.

protons from one bond; in ice, its equivalent is known as a Bjerrum L (leer) *fault* (17–21). [A D (doppelt) fault puts two protons on the same bond.] Such a fault then propagates by successive rotation of OH groups, shown by the arrows in Fig. 3*b*. At the end of this process the proton configuration returns to its original state (Fig. 3*a*) and the chain is ready to transport another proton. Although such a two-process mechanism may appear awkward, it has a distinct advantage over a one-process mechanism. Each process carries only a fraction of the charge; in ice, e_I, the charge carried by the first (i.e., ionic) process is about $0.64e$, and e_B, the charge carried by the second (i.e., Bjerrum fault) process is about $0.36e$ (18, 20). Since the membrane has a dielectric constant much less than that of water, a partitioning of the charge lowers the electrostatic energy barrier for bringing a charge into the membrane from about 16 kT for a full charge to about 4 kT for a half charge, assuming a dielectric constant for the chain region of the membrane to be about 10 and an ionic radius of 3 Å.

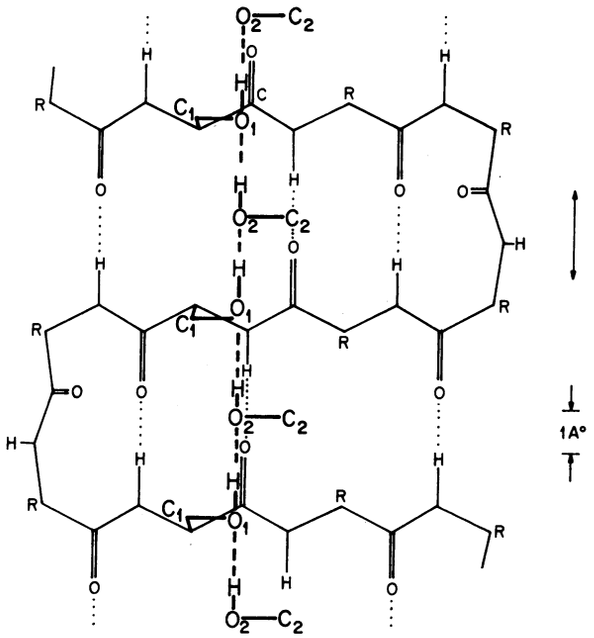

FIG. 2. The arrow shows the perpendicular to the membrane. Starting from the upper left a polypeptide chain is shown with three amino acids in the beta form followed by a vertical connecting residue followed by three more residues in the beta form, etc. All R side chains may be hydrophobic except for the serine groups explicitly shown by C_1O_1 lettering. The second polypeptide chain (not shown) has serine groups shown by C_2O_2. The hydrogen-bonded chain is shown by dashed lines. The $O_1O_2O_1$ angle is roughly tetrahedral, as shown in Fig. 1, so that the O_1O_2 distances are about 2.8 Å. The portion of the protein shown would be repeated until the membrane is spanned.

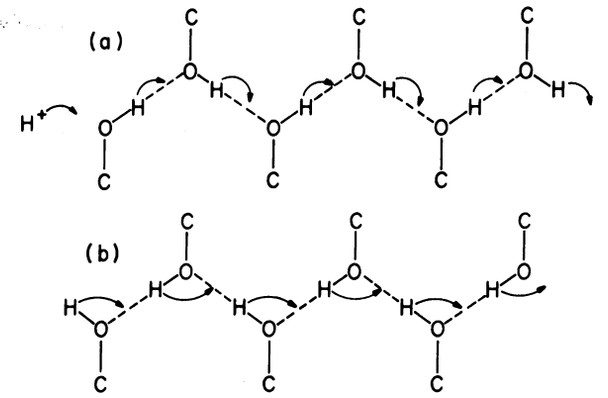

FIG. 3. (*a*) A proton enters from the left end to form an ionic defect that propagates to the right by sequential jumping of successive protons (shown by arrows), with the emergence of a proton on the right end to give the state shown in (*b*). (*b*) A negative Bjerrum L fault forms on the right end by rotation around the CO axis and the defect propagates to the left via sequential rotations of successive hydroxyl groups to return the chain to the state shown in (*a*).

Studies on ice show that the passage of protons via this two-process, ion-and-fault mechanism is fast and efficient. Typically, in biomembranes one can assume electrochemical potentials of 100 mV or more (22). Let us suppose that 99% of this is transduced into useful energy and only 1%, or 1 mV, is dissipated in driving the protons across the membrane. This, in effect, assumes that the impedance of the hydrogen-bonded chain is small compared to that of the energy transducing element. The mobility of ions in ice is about $\mu_I \simeq 10^{-3}$ cm^2/V·sec (18, 21) and the mobility of faults is $\mu_B \simeq 10^{-4}$ cm^2/V·sec (21). Since for the drift velocity $v = \mu V/d$ and for the transit time $\tau = d/v = d^2/V\mu$, for a $d = 50$ Å thick membrane the ion transit time is $\tau_I = 2.5 \times 10^{-7}$ sec and the Bjerrum fault transit time is $\tau_B = 2.5 \times 10^{-6}$ sec. Of course, one expects these mobilities in hydrogen-bonded chains to be different in membranes from those in ice. If the structure is less rigid and subject to more thermal agitation, then μ_I will decrease but μ_B will increase. In ice the ions and faults have more directions in which to move, but also more protonic configurational disorder to impede their orderly progress in a field. Thus, the mobilities of protons in membranes are likely to have the same orders of magnitude as the mobilities in ice.

From the preceding discussion it can be seen how a hydrogen-bonded chain can quickly transport protons from a region of high electrochemical potential across a membrane with little loss in energy. Before being released into a solution with a low electrochemical potential, such high energy protons could be made to drive a chemical reaction. Morowitz has discussed such a possibility in connection with ATP synthesis (11). In such a picture the hydrogen-bonded chain is simply a proton wire with low resistance or, equivalently, a semipermeable membrane for protons. This allows a cell to perform "protochemistry" with "protodes" in a way comparable to that in which one performs electrochemistry with electrodes.

New mechanisms using asymmetric hydrogen-bonded chains

In addition to the simple proton wire, which can be called a "passive" device, we now propose an "active" hydrogen-bonded chain device. In this active device the passages of the ions and the faults along the chain are intimately coupled to conformational changes in the protein containing the chain. It will be shown how this active device can act as a motor to transduce electrochemical energy into cyclic mechanical energy. The

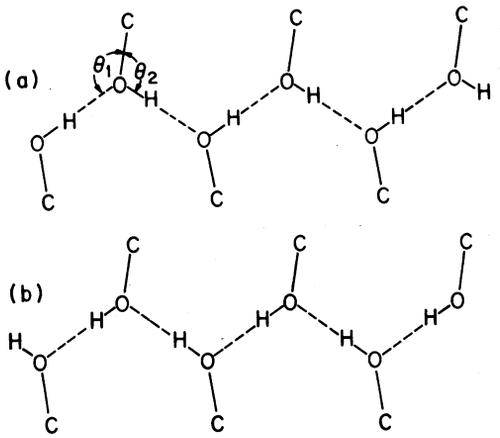

FIG. 4. An asymmetric chain that has different COO angles, $\theta_1 \neq \theta_2$. For such a chain the energies of the states in (*a*) and (*b*) will be different.

device can also be reversed to use conformational energy changes, caused perhaps by absorption of light or a chemical reaction, to pump protons up an electrochemical gradient. The next two paragraphs provide the background needed for the description of these devices.

We propose an asymmetric hydrogen-bonded chain as shown in Fig. 4. In Fig. 4*a* all the protons are on the left side of the hydrogen bonds, whereas in Fig. 4*b* all the protons are on the right side. These two states need not have the same energy. There are many possible molecular interactions that can cause such a difference in potential. For example, the COH bond angle is more favorable in one case than in the other; this will be the possibility assumed in subsequent discussion. Another possibility is the presence of other positively charged groups closer to one of the two proton positions than the other. The magnitude of the energy difference can be as small as zero for a symmetrical chain and has a gross upper bound given by the total hydrogen bond energy of about 6 kcal/mol for each bond. More refined estimates come from studies of ferroelectric and antiferroelectric phase transitions in hydrogen-bonded crystals, where the transitions are caused by local energy differences in proton configurations similar to the ones proposed here (23–25). Per bond these local energy differences are roughly kT_c, where the transition temperature T_c is usually in the range 100–300 Kelvin. Thus, the energy difference in Fig. 4 could be as high as 0.6 kcal/bond mol or 12 kcal for a chain with 20 bonds. This energy difference is equivalent to membrane potentials as high as 500 mV for protons.

The proposed proton pump and motor require control over the presence of ions and faults in the chain which will be discussed now so as to expedite the description of the mechanism. In ice the intrinsic concentration of ions, $c_I \simeq 10^{-12}/H_2O$, and the concentration of faults, $c_B \simeq 5 \times 10^{-7}/H_2O$, are very low (18, 21), so the probability of having an intrinsic fault or ion in the hydrogen-bonded chain at any one time is only about 10^{-10} or 10^{-5}, respectively. The dissociation constant for creation of an intrinsic ion is $k_D \simeq 3 \times 10^{-9}$ sec^{-1} (26), which is 3×10^8 sec per bond or about 10^7 sec for our chain of 20 bonds! Although faults will dissociate more frequently, it is clear that the *intrinsic* recovery time for generation of a new fault or ion after passage of an old one is very long. Thus, the recovery time for chains will be governed by introduction of ions and faults from the ends in solution, and this can be controlled by various gating mechanisms at the ends of the chains. (Because the membrane system is undoubtedly less rigid than ice the dissociation constants will go up, but there seems to be ample latitude for this without affecting the conclusion that faults and ions in the chain will be overwhelmingly introduced extrinsically from solution.) These injection processes can arise either from electrochemical activity unbalance between the solution and chain phases or from enzymatic reactions at the termini of the chains that give rise to proton-generating or -utilizing reactions.

In this paper a very simple gating mechanism is used based on the following fact. It is easier to form Bjerrum faults and to transport them along long hydrogen bonds and easier to transport ions along short hydrogen bonds. To illustrate this, consider the limit of very long bonds where the barrier to hopping of a proton from one oxygen to another is obviously very high, whereas, because the bond energy is small, molecules can turn easily, thereby forming or transporting faults. In contrast, as the bond shortens the bond energy becomes higher, making it harder to break a bond by turning a molecule, but the barrier to hopping goes to zero as the proton potential changes from a double minimum to a single minimum for bonds of about 2.4 Å (29). This fact will be used to vary the relative transferal rates of ions versus faults by the simple expedient of changing the length of the bonds connecting the chain to the aqueous phase. These two connecting bond lengths can be adjusted by recessing the chain from the surface of the membrane or by binding the first water to other membrane groups.

Proton pump

Fig. 5 shows a mechanism, consisting of four processes, that transports protons from the right side of the membrane to the left side. In the resting configuration shown in Fig. 5*a*, the protons are in their low energy configuration on the left side of each bond. The α and ω gating bonds that connect the chain to water are both short. In Fig. 5*a* a conformational change in the protein, induced by light or a chemical reaction, causes the sign of the energy difference of the two proton configurations to reverse. As shown in Fig. 5*a* by arrows, this may be due to a change in the COH angles. Accompanying this change, a lengthening of the α and ω connecting bonds is postulated. The protons can now lower their configurational energy by moving to the other side of their bonds. Since the α and ω connecting bonds are long, this will be accomplished most often by the entry of an L fault from the left or a D fault from the right, either of which propagates along the chain as shown in Fig. 5*b*, transporting a charge e_B from right to left in about a μsec. (Of course, with thermally activated gates there will be a small percentage of times when an ion will enter the chain and transport a charge e_I from left to right. This current is equivalent to a leakage current that lowers the efficiency of the pump but does not impair its subsequent operation.) The chain now has the proton configuration shown in Fig. 5*c*. Next, the protein, which is assumed to be in an intermediate excited state, now relaxes back to its original conformation, as shown by the arrows in Fig. 5*c*. This step transfers the remaining energy from the protein into proton configurational energy of the state shown in Fig. 5*d*. The connecting α and ω bonds are also shortened so that, in the overwhelming majority of cases, an ion will enter and propagate a charge e_I from right to left across the chain as shown by the arrows in Fig. 5*d*, leading back to Fig. 5*a*. Thus, the cycle is complete, and a net charge of $e_B + e_I = e$ has been transported from right to left across the membrane.

Such a pump will continue to transport protons when energy is supplied to the protein until the opposing electrochemical potential V is large enough so that either e_BV equals the proton configurational energy difference U_{bc} between Fig. 5*b* and *c* or e_IV equals the proton configurational energy difference U_{da}

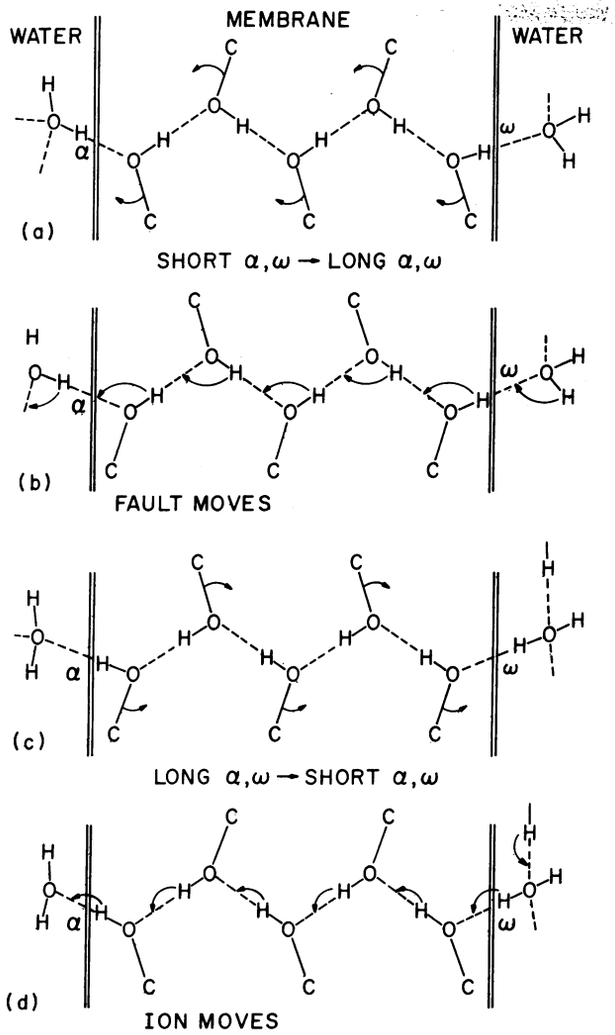

FIG. 5. The four processes of the proton pump are shown in (*a–d*). An asymmetric chain is shown spanning a thinned membrane and connected by the α and ω bonds to water molecules at the surfaces. The arrows in each lettered figure transform the state to the state shown by the next letter. (*a*) A conformational change in the protein changes the COO angles from those shown in (*a*) to those in (*b*) and the short α and ω bonds are lengthened. (*b*) A Bjerrum fault is driven along the chain by the decrease in proton configurational energy to give the state in (*c*). (*c*) The protein relaxes back to its original conformation and shortens the α and ω bonds to give (*d*), where an ion is driven across the bridge by the decrease in proton configurational energy to return the state to (*a*). The reversal of the four processes gives the motor.

between Fig. 5*d* and *a*. When the potential exceeds these limits, then the propagation of the faults in Fig. 5*b* and the ions in Fig. 5*d* will be stopped because the opposing electrochemical gradient exceeds the configurational energy driving force. Since e_B is not necessarily equal to e_I, this raises the possibility that U_{bc} need not equal U_{da} but that U_{bc}/U_{da} may be equal to e_B/e_I. This is indeed a possibility because the chains in Fig. 5*a* and *c* need not be mirror images of each other. Stated somewhat differently, the magnitude of the angle of the undirected CO bonds to the plane of the membrane may be different in Fig. 5*a* than in Fig. 5*c* and this makes the magnitude of the energy differences different for these two protein confirmations. Also, the amount of energy exchanged with the gating bonds α and ω decouples the energy ratio U_a/U_c given up by the protein in Fig. 5*a* and *c*, respectively, from the ratio U_{bc}/U_{da}.

Motor

The proton pump can be reversed to produce cyclic conformational changes in the protein which, via a molecular lever, could produce cyclic mechanical action. The condition for operation is that the electrochemical energy e_BV be greater than U_{bc} and e_IV be greater than U_{da}. Let the sequence of processes start at Fig. 5*a*. Since the α and ω gating bonds are short, an ion enters and propagates across the chain, transporting a charge e_I from left to right across the membrane. The high energy proton configuration is now shown in Fig. 5*d*. (In going from Fig. 5*a* to Fig. 5*d* the protons move in the reverse direction of the arrows in Fig. 5*d*). At this point passage of a fault would restore the low energy proton configuration and result in a net dissipation of $(e_I + e_B)V$ in energy. However, the α and ω gating bonds are short, so the entry of Bjerrum faults is discouraged and the protein conformational change, the reverse of which is shown in Fig. 5*c*, can take place. This also lengthens the α and ω gating bonds, so the next process is the passage of a Bjerrum fault, which changes the state in Fig. 5*c* to the one in Fig. 5*b* by reversing the arrows in Fig. 5*b*. Since the gating bonds discourage the entry of ions into the high energy chain configuration shown in Fig. 5*b*, there is time for the protein conformational change shown by the reverse of the arrows in Fig. 5*a*. This completes the cycle, the output of which is a cyclic conformational change in the embedding protein. As drawn in Fig. 5, this motion is like a molecular rowing machine, although one can easily envisage torsional motions in alpha helix arrays that could more easily be leveraged to larger dimensions. These conformational changes could also be used to forcibly change molecular distances in tightly bound substrates, thereby cleaving or forming chemical bonds with energies in the 0–12 kcal range.

Discussion

The preceding models are quite specific as regards proton motion, which is presumed to be a universal feature. The postulated protein conformational changes have not been specified because it is unlikely that they are similar for all proteins of biological interest. Structural and sequencing information for specific proteins is required before the details of the coupling between hydrogen-bonded chain configurations and protein conformations can be described. However, such a coupling seems plausible and has also been postulated by Hui in connection with the gating current in nerve axons (30).

It is promising biologically that the energy per cycle in these devices is tunable by the protein within the range 0–12 kcal, corresponding to membrane potentials of 0–500 mV. In addition, one can envisage a protein with several hydrogen-bonded chains, all of which are linked to the protein conformational change and all of which must conduct to activate the protein change. The net energy would be several times as large as for the cases discussed with only one hydrogen-bonded chain. From studies in ice the cycle times of operation should be of the order of 10^{-5} sec, although more intricate gating mechanisms might slow this down. As with most thermodynamic cycles these require several processes, at least two for the passive proton wire and four for the active proton pump and for its reversible cycle, the motor. One feature of these mechanisms that should be emphasized is that the individual steps taken by the protons in each of the ionic and fault processes only involves about kT in energy, so these thermally activated steps are fast, yet the cumulative energy transduced upon passage of a proton through the membrane is about pkT, where $p = 20$ is the number of hydrogen bonds in the chain. As presented, the hydrogen bonds

in the chain are identical. This is not a necessary restriction, although introduction of irregularities can be expected to lengthen the cycle times.

Although our examples have been in terms of transmembrane proteins, this is not a necessary feature of the general theory. Compartment separation will be required if the model is to operate from macroscopic differences of electrochemical potential. However, protons may be injected or withdrawn from a chain of hydrogen bonds by a chemical reaction at a terminus of such a chain. This is thermodynamically similar to the models we have just been discussing and can lead to proton migration, chemomechanical conversion, and organic synthesis through linked acid–base reactions (6). However, this kind of device is different enough mechanistically that its exposition is best deferred to a later communication.

The proposed hydrogen-bonded chains, the proton pump and the motor mechanisms, have been stripped to bare essentials, especially in connection with the gating mechanism. If such devices exist in biomembranes, they are probably different in detail and greatly embellished by evolution. However, we think that there is a very good possibility that the basic proton conduction mechanisms that we have adapted from solid state chemistry will play a central role in proton transport and bioenergetics.

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