Hypothesis

Proton currents and protein motion in membranes

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A mechanism is proposed whereby a proton gradient along a membrane-spanning α-helix is coupled to small changes in the torsional angles around the α-helix peptide bonds. Small concerted changes in the torsional angles are coupled to a change in the unit twist of the α-helix; a change in the unit twist is coupled to a change in the crossing angle between neighbouring α-helices; and a change in the crossing angle is coupled to a change in the size and shape of an assembly of α-helices. Following this logical linkage in one direction shows how a proton gradient could induce a pumping motion in an assembly of α-helices; following it in the other direction shows how motion in an assembly of α-helices could pump protons.

α-Helix *Chemiosmosis* *Protein conformation* *Protein transport* *Proton gradient* *Proton pump*

1. INTRODUCTION

Proton gradients drive the transport of ions and metabolites across membranes (reviewed in [1–3]). The molecular mechanisms that move protons across membranes in one direction to create gradients, or in the other direction to use the gradients to do molecular work are not understood. Models for proton conductance can be subdivided into models of passive wires and models of active wires. Passive wires just move protons to and from the proton pumps or proton motors that are postulated to create or use the proton gradient. Active wires are themselves intrinsic parts of pumps or motors, analogous to coils of wire in electric generators or electric motors [1–3].

Membrane proteins involved in these processes are rich in membrane-spanning α-helices arranged in bundles (reviewed in [2,4,5]). The α-helix can be described as 3 parallel chains of hydrogen-bonded peptides winding around the helix axis with a pitch of 27 Å [6–9], and this has led to proposals that the α-helix backbone itself might be a proton wire [8,10]. None of these proposals includes a detailed mechanism of coupling between protein motion and proton currents along an α-helical active wire.

2. THE LOGICAL LINKAGE

Several known facts about proteins are linked here in a new way to give a dynamic molecular model for the coupling between proton currents along an α-helix and protein motion in assemblies of α-helices:

(1) Change in protonation of the peptide group is coupled to change in the torsional angle ω around the peptide bond. The assumption that the peptide group is planar was an important simplifying factor in building the first model of the α-helix [11]. The peptide group is planar because delocalization of electrons into a π-like molecular orbital gives a partial double-bond character to the C–N peptide bond, but the constraint to planarity is not absolute. The energy required to rotate by $\Delta\omega$ around the C–N torsional angle is about 21 $\sin^2(\Delta\omega)$ kcal/mol, and in practice $\Delta\omega$ varies by up to $\pm$ 10° [12–15]. An external force that changes

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ω will change the pK_a of the peptide; and, conversely, changed protonation of the peptide (e.g., by forced local high proton concentration) will change ω [9,15]. Repeated protonation and deprotonation of peptides would be accompanied by oscillation in ω.

(2) Change in the torsional angle ω is coupled to change in the unit twist and unit rise of the α-helix [14]. Conformational energy calculations show that very small changes in ω are sufficient to induce substantial changes in the unit twist of the α-helix (table 1). The α-helix is not static, but shows characteristic internal vibrations (reviewed in [16]), and appropriate oscillations in ω would generate vibrational waves or solitons in the α-helix [6,17,18].

(3) Change in the helix parameters of two nearly parallel interlocking α-helices is coupled to change in the angle that the axes of the two helices make with one another. This relationship between helix parameters and crossing angle was first pointed out on the basis of model-building [19], and then generalized by analysis of known protein structures [20]. This relationship is illustrated in table 1.

(4) Change in the crossing angle between interlocking helices is coupled to change in the size and shape of a bundle of such helices. The relationship has been analyzed in detail only for perfectly cylindrical tubes whose walls are α-helices [21], but the size and shape of more general bundles of α-helices must also change with crossing angle.

Table 1

Relation between helix parameters and crossing angle

$\Delta\omega$ (°)	θ (°)	h (Å)	r (Å)	α_3 (°)	α_4 (°)	A_{34} (°)
	100	1.5	4.7	−47.6	28.7	18.9
	100	1.5	4.5	−46.3	27.6	18.7
	98.9	1.51	4.7	−48.9	25.8	23.1
−10[a]	105.3	1.41	4.7	−40.5	41.7	−1.2
−5[a]	102.9	1.45	4.7	−44.1	36.1	8.0
0[a]	100.8	1.48	4.7	−46.8	30.9	15.9
−10[b]	108.8	1.89	4.0	−22.5	34.8	−12.3
0[b]	112.5	1.85	4.0	−15.8	40.3	−24.5
0[b]	112.5	1.85	4.7	−18.4	44.9	−26.5

[a] Values for helices with i to $i + 4$ hydrogen bonding, from [14]
[b] Values for helices with i to $i + 3$ hydrogen bonding, from [14]

These entries show that small changes in $\Delta\omega$ give changes in θ that lead to large changes in the crossing angle A_{34}. Helices midway between the α-helix and the 3_{10} helix would be expected to have negative crossing angles

In a helix, each peptide is related to the next in the chain by the helix parameters: a unit rise h parallel to the helix axis and a unit twist θ about this axis. Two helices that touch one another do so at certain preferred angles, as the result of interlocking between ridges defined by side chains, and grooves between these ridges [20]. For helices that lie roughly parallel to one another, the ridges formed by the i and $i + 3n$ sidechains on one helix interlock with the ridges formed by the j and $j + 4n$ sidechains on the other helix. The pitch angle α of the helix defining the ridge is [19,20]:

$$\alpha_3 = \tan^{-1} [r(3\theta - 2\pi)/3h]$$

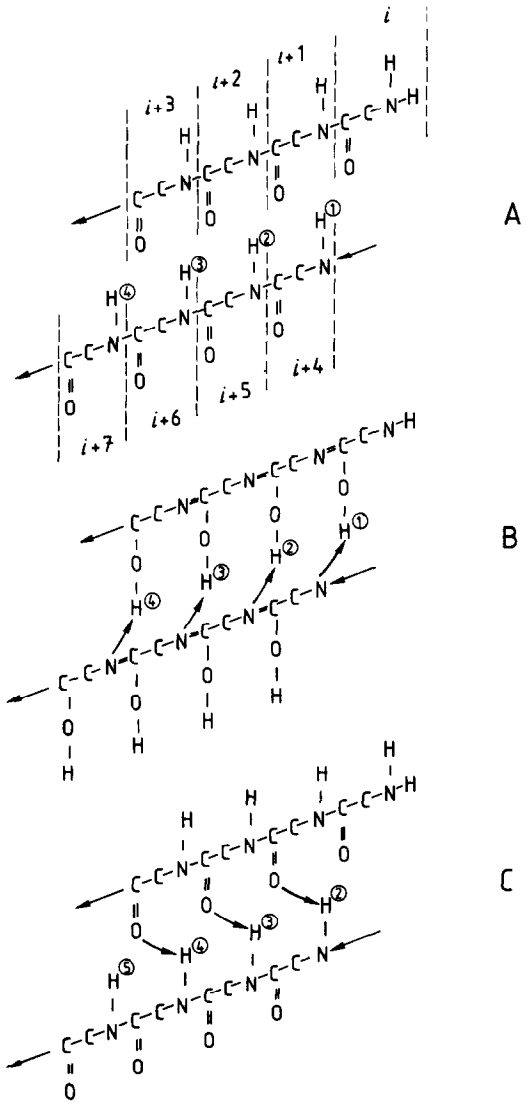
$$\alpha_4 = \tan^{-1} [r(4\theta - 2\pi)/4h]$$

where r is the contact radius (half the distance between the helix axes). The angle between the axes of two interlocked helices i and j is then:

$$A_{34} = -({}_i\alpha_3 + {}_j\alpha_4)$$

The first two entries and the last two entries in the table show that small changes in the contact radius have little effect on the crossing angle. The third entry is the average value found for a large number of helices in globular proteins [20]

These relationships together form a linkage of events that could couple oscillatory protonation of α-helices to oscillation in the size and shape of an assembly of α-helices. The primary transduction between the proton gradient and conformational change is suggested to be coupling between protonation of the peptide and slight changes in the torsional angle around the peptide bond. These slight changes are amplified by the geometry of α-helices. The hypothesis has the appeal that the general driving force, namely the protonmotive force, interacts with a general feature of the model, namely membrane-spanning α-helices. The specificity of each transport system would be determined by the sidechains. The oscillation in the size and shape of the assembly of α-helices could generate a travelling wave of structural alteration that could function in transport like peristalsis [6,17].



3. PROTON TRANSLOCATION

A specific example of how a proton current might be coupled to oscillating changes in the unit twist of an α-helix is illustrated in fig.1. The C=O of one peptide in a helix is hydrogen-bonded to the N–H of a peptide further along the chain. Bonding of C=O of residue i to the N–H of residue $i + 4$ gives the α-helix; bonding to residue $i + 3$ gives the 3_{10} helix [14]. A wide distribution of unit twist and unit rise is found experimentally, almost spanning the range between the α-helix and the 3_{10} helix [20,22]. Bifurcated hydrogen bonds [23] are

Fig.1. Coupling between proton translocation and α-helix conformational change. (A) Two turns of an α-helix, joined by distorted hydrogen bonds, so the N–H of residue $i + 4$ is roughly midway between the O of residue i and the O of residue $i + 1$. The straight arrows indicate the lines of the α-helix. (B) Protonation causes a transient change in $\Delta\omega$ and thence in the α-helix unit twist θ, which brings the N–H of residue $i + 4$ closer to the O of residue i. The same protonation involves a transient keto to enol tautomerization, causing the H to move from N on one chain to O on its hydrogen-bonded partner (curved arrows). In practice the proton in a hydrogen bond can be fairly equally shared between the N and the O, and relatively small changes would be sufficient to associate the proton more closely with the O than the N. (C) The transient conformational change relaxes, bringing the N of residue $i + 4$ closer to the O of residue i + 1. Then the tautomerization relaxes, allowing the H to move from the O of residue $i + 1$ to the N of residue $i + 4$. The net effect of this cycle is to move all protons one step along the α-helix as the helix unit twist θ changes transiently. This is an example of how proton translocation could be coupled to change in α-helix parameters, but it is not the only model of this type. This example is given to clarify the ideas, and other related examples can be imagined. Flaws in this specific example need not imply flaws in the logical linkage. Migration of protons from the N-terminus to the C-terminus would be aided by the α-helix dipole [31,32], but this dipole would not preclude migration in the opposite direction.

sometimes found at the point of transition between an α-helix and a 3_{10} helix, but they are less stable than simple hydrogen bonds. The appropriate sidechain distribution and quaternary interactions in a hydrophobic environment might stabilize [24] a series of bifurcated hydrogen bonds along a helix. In such a helix, the C=O of residue i would be so placed that it could form hydrogen bonds with the N–H of either residue $i + 4$ or residue $i + 3$. Now, if a hydrogen were removed from one end of the chain, a proton hole [3,15,24,25] would be created, and protons could move along the chain as indicated in fig.1. As the protons move, the change in protonation of the peptides and thereby the torsional angle ω would be transiently altered. The effect would be rather like holding each end of the helix and twisting back and forth around the helix axis.

This is not a proposal of a classical proton transfer from N–H to C=O, which would be energetically unfavourable. Instead, it depends on the fact that a proton involved in a hydrogen bond has a quantum mechanical distribution of position between N and O [25], and will therefore sometimes be closer to O than to N. If the proton is involved in a bifurcated hydrogen bond, there will be uncertainty about which N it should return to after its excursion to O. If the other proton associated with that O has been removed by an external agent, then the first proton could take its place, as shown.

This mechanism is also valid if protons are injected at one end rather than removed from the other. It can easily be modified to explain a flow of protons towards the C-terminus. The mechanism can also be reversed to explain the pumping of protons by an external force on the α-helix that changes the unit twist and the peptide angle ω, thereby forcing a change in the electron distribution in the peptide, and a corresponding change in the relative affinity of the peptide N and O for protons.

4. DISCUSSION

Experiments to test these ideas are feasible. Hydrogen exchange experiments [26] could test for rapid exchange of the peptide protons in active membrane-spanning proteins. Fourier-transform infrared spectroscopy on the α-helical membrane-spanning protein bacteriorhodopsin shows unusual amide signals that have been attributed to altered conformation or orientation of the α-helices [10,27,28], but which might be consistent with the altered ω and unit twist suggested here. High resolution structural studies of membrane proteins would show any unusual conformation in the α-helices.

There is one example of a structural alteration in an assembly of α-helices that is coupled to a change in the unit twist of the α-helix. The protein coat of the filamentous bacterial virus Pf1 is an assembly of interlocking α-helical subunits. The virus undergoes a temperature-induced structural transition in the helix parameters of the virion that can be traced to a change in the crossing angle and thence to a change in the helix parameters of the α-helices themselves [29]. This kind of transition may also be involved in the assembly of filamentous bacterial viruses from membrane-spanning precursors of the α-helical coat proteins [30].

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REFERENCES

[1] Nicholls, D.G. (1982) Bioenergetics, Academic Press, New York.
[2] Ovchinnikov, Yu.A., Abdulaev, N.G. and Modyanov, N.N. (1982) Annu. Rev. Biophys. Bioeng. 11, 445–463.
[3] Nagle, J.F. and Tristram-Nagle, S. (1983) J. Membr. Biol., in press.
[4] Williams, R.J.P. (1980) Chem. Soc. Rev. 9, 281–364.
[5] Capaldi, R.A. (1982) Trends Biochem. Sci. 7, 292–295.
[6] Davydov, A.S. (1973) J. Theor. Biol. 38, 559–569.
[7] Ramasarma, T. and Vijayan, M. (1974) FEBS Lett. 41, 307–309.
[8] Kayalar, C. (1979) J. Membr. Biol. 45, 37–42.
[9] Metzler, D.E. (1979) Adv. Enzymol. 50, 1–40.
[10] Krimm, S. and Dwivedi, A.M. (1982) Science 216, 407–408.
[11] Pauling, L., Corey, R.B. and Branson, H.R. (1951) Proc. Natl. Acad. Sci. USA 37, 205–211.
[12] Dunitz, J.D. and Winkler, F.K. (1975) Acta Cryst. B31, 251–263.

[13] Winkler, F.K. and Seiler, P. (1979) Acta Cryst. B35, 1920–1922.
[14] Nambudripad, R., Bansal, M. and Sasisekharan, V. (1981) Int. J. Peptide Protein Res. 18, 374–382.
[15] Dunker, A.K. (1982) J. Theor. Biol. 97, 95–127.
[16] Peticolas, W.L. (1978) in: Methods in Enzymology (Hirs, C.H.W. and Timasheff, S.N. eds) vol. 61, pp.425–458, Academic Press, New York.
[17] Davydov, A.S. (1977) J. Theor. Biol. 66, 379–387.
[18] Straub, K.D. (1974) J. Theor. Biol. 44, 191–206.
[19] Crick, F.H.C. (1953) Acta Cryst. 6, 689–697.
[29] Chothia, C., Levitt, M. and Richardson, D. (1981) J. Mol. Biol. 145, 215–250.
[21] Dunker, A.K. and Zaleske, D.J. (1977) Biochem. J. 163, 45–57.
[22] Fraser, R.D.B. and MacRae, T.P. (1973) Conformation in Fibrous Proteins, Academic Press, New York.
[23] Baur, W.H. (1965) Acta Cryst. 19, 909–916.
[24] Warshel, A. (1979) Photochem. Photobiol. 30, 285–290.
[25] Freund, F. (1981) Trends Biochem. Sci. 6, 142–145.
[26] Richards, F.M. (1979) Carlsberg Res. Commun. 44, 47–63.
[27] Rothschild, K.J. and Marrero, H. (1982) Proc. Natl. Acad. Sci. USA 79, 4045–4049.
[28] Bagley, K., Dollinger, G., Eisenstein, L., Singh, A.K. and Zimányi, L. (1982) Proc. Natl. Acad. Sci. USA 79, 4972–4976.
[29] Marvin, D.A., Nave, C., Ladner, J.E., Fowler, A.G., Brown, R.S. and Wachtel, E.J. (1981) in: Structural Aspects of Recognition and Assembly in Biological Macromolecules (Balaban, M. et al. eds) pp.891–910, Balaban ISS, Rehovot, Philadelphia PA.
[30] Marvin, D.A. and Wachtel, E.J. (1976) Phil. Trans. Roy. Soc. Lond. B276, 81–98.
[31] Hol, W.G.J., Van Duijnen, P.T. and Berendsen, H.J.C. (1978) Nature 275, 443–446.
[32] Sheridan, R.P. and Allen, L.C. (1980) Biophys. Chem. 11, 133–136.