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A Model for Proton Translocation in Biomembranes Based on Keto-Enol Shifts in Hydrogen Bonded Peptide Groups

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Summary. A mechanism is proposed for the translocation of protons through the hydrogen bonded peptide groups of a protein by means of keto-enol tautomerization. The model is discussed in relation to energy coupling in biomembranes.

According to Mitchell's chemiosmotic hypothesis, in oxidative phosphorylation and photophosphorylation the flow of electrons through an electron-transport chain is accompanied by translocation of protons across the coupling membrane (Mitchell, 1961). The proton-motive force generated by this proton translocation drives the synthesis of ATP catalyzed by the ATPase complex. Hydrolysis of ATP by the reverse reaction also gives rise to proton translocation across the membrane. During the last decade these basic predictions of the chemiosmotic hypothesis have received wide experimental support, but the precise mechanisms of proton translocation and ATP synthesis are still matters of debate (for reviews *see* Skulachev, 1975; Boyer *et al.,* 1977.)

The lipid bilayer of a biomembrane itself has very little proton permeability. Therefore, specific pathways or mechanisms have to exist to translate protons through the coupling membranes. In Mitchell's formulation, the proton translocation that takes place across the membrane during electron-transport is a consequence of loop-like arrangements of the electron transport chain components (Mitchell, 1966). Experimental findings on the stoichiometry of proton translocation and topology of the electron transport chain led investigators to consider other possibilities such as presence of proton pumps coupled to respiratory chain enzymes (Skulachev, 1975; Chance *et al.*, 1970; Papa, 1976). For the operation of such pumps, Papa (1976) suggested that during electron transport changes in pKa of protein groups in the proximity of iron or other oxidation-reduction components may initiate proton translocation through a membrane-spanning channel lined with rows of amino acid residues with appropriate pKa values.

In the last several years the proton-translocating function of the ATPase complex has been well established and its structural components have been better de-

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fined (Serrano, Kanner & Racker, 1976; Yoshida *et al.*, 1975). The hydrophobi component *Fo* is integral with the membrane. Its proton conducting functio across a membrane is demonstrated (Racker, 1972; Shchipakin *et al.*, 1976). Th hydrophilic component F_1 is attached at one end of *Fo* and has ATPase functio (Racker, 1967).

Some years ago Mitchell (1974) suggested that during ATP hydrolysis the O^2 group from a molecule of water is transferred to the γ-phosphate of ATP, whil the two protons cross the membrane through *Fo*, generating a proton-motiv force. Mitchell did not indicate a specific mechanism by which proton trans location takes place through *Fo*. Other suggestions on proton translocation mech anisms include models based on rotation of proteins within the membran (Skulachev, 1975; Papa, 1976), or shuttling of charged protein groups across th membrane (Boyer, 1975). Nagle and Morowitz (1978) have recently suggestec that proton translocation in biomembranes may take place along a chain of hy drogen bonds between the side groups of amino acids such as hydroxyls of serine threonine and tyrosine and the carboxyls of aspartic and glutamic acids. Stoe ckenius (1978) has applied this model to bacteriorhodopsin which he and his co workers had shown to function as a light-driven proton pump (Oesterhelt & Stoe ckenius, 1973; Lozier, Bogomolni & Stoeckenius, 1975).

The Model

I have recently devised a model for a proton pump based on keto-enol tautomerization of the peptide bond giving rise to proton translocation along a chain of hydrogen bonds. The proton-conducting pathway of a proton pump is assumed to be a chain of hydrogen bonds between the carbonyl and imino groups of peptide bonds in a membrane-spanning protein rather than a chain of hydrogen bonds between hydrophilic side groups as proposed by Nagle and Morowitz (1978). An α-helix structure readily provides such a pathway (Fig. 1). Other structures with hydrogen bonded peptide groups can also provide a similar pathway. The proposed mechanism of proton translocation is illustrated in Fig. 2. For reasons of simplicity the chain of hydrogen bonds is shown to consist of only three peptide groups. Actually about ten such peptide groups will be required to form a chain of hydrogen bonds that can span the membrane.

According to the model, proton translocation is initiated by the protonation of the carbonyl oxygen of the first peptide bond, on the side from which protons are translocated (Fig. 2, $I \rightarrow II$). This protonation induces a shift from the keto to the enol form of the peptide bond with concomitant transfer of the proton from the nitrogen to the carbonyl oxygen of the next peptide bond along the chain ($II \rightarrow III$). These keto-enol shifts are postulated to occur sequentially along the chain, resulting in a net translocation of a proton to the other end of the chain ($III \rightarrow VI$). The first peptide groups in the sequence after having shifted to the enol form will shift back to the more stable keto form as proton translocation proceeds along the chain. Therefore, at a given time only a fraction of the chain will be in a configuration different from the original one.

Keto-enol tautomerism is a known property of amide bonds. The keto form is

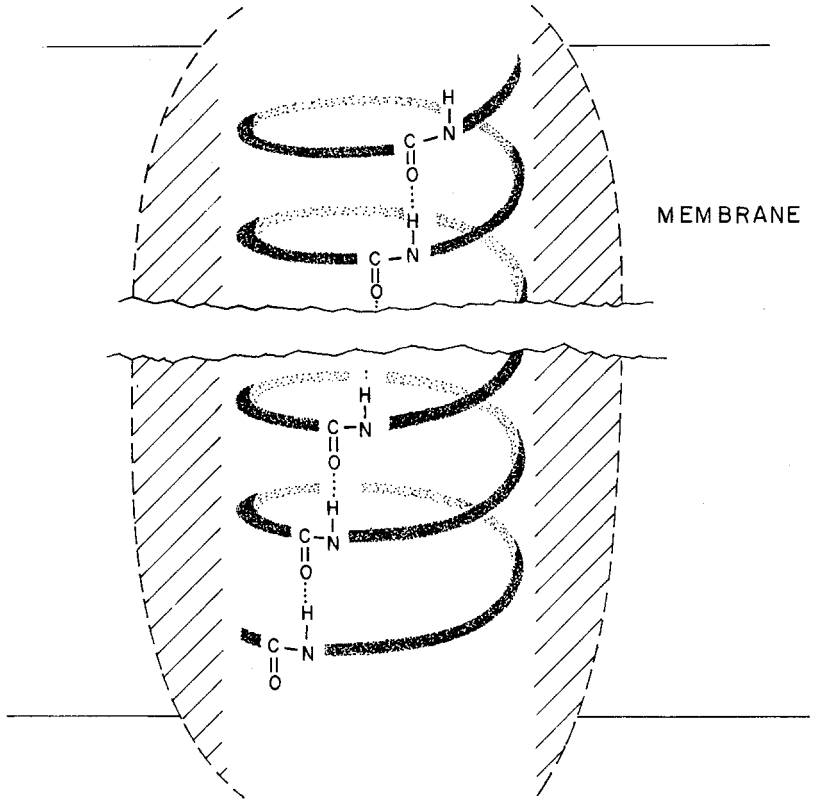

Fig. 1. A chain of hydrogen bonded peptide groups in an α-helix structure of a membrane-spanning protein

energetically more stable and therefore is the dominant configuration. The suggested spontaneous conversion of the enol form back to the original keto form therefore provides a simple mechanism to re-generate the pathway for the translocation of the next proton.

In Fig. 2, *X* and *Y* that are shown at the ends of the chain represent specific groups that catalyze the donation or acceptance of the protons to and from the chain. They may be certain amino acid side groups with the appropriate pKa values, such as the imidazole moiety of histidine, or they may be specific protein-bound co-factors. These groups can provide the regulation or gating mechanism needed during proton translocation. They can also be involved in the mechanism of energy input into the system. In bacteriorhodopsin, the Schiff base formed between the protein and the chromophore retinal may play such a role (Stoeckenius, 1978) since it has been shown to go through a light-driven protonation, de-protonation cycle (Lewis *et al.*, 1974).

For the ATPase complex, Kayalar, Rosing and Boyer (1977) have presented evidence for an alternating site model in which protein conformational changes accompanying substrate and product binding and release steps are coupled to the energization of the enzyme. It is conceivable that in the course of ATP hydrolysis,

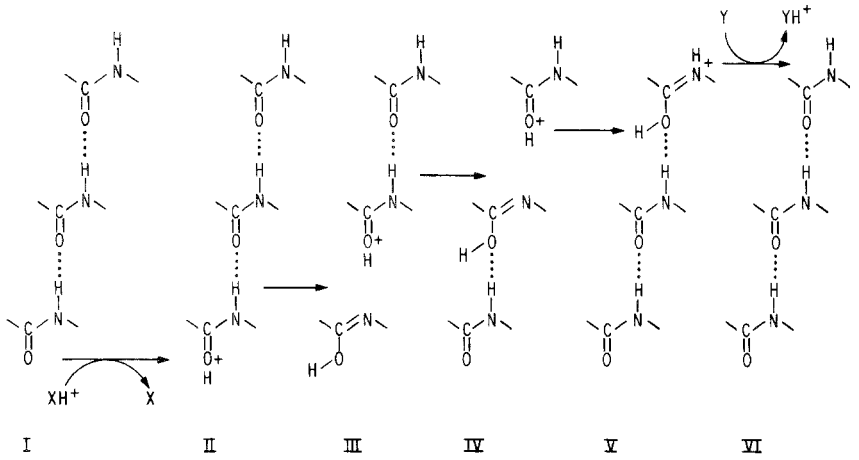

Fig. 2. Proton translocation is initiated by the protonation of the carbonyl oxygen (*I - II*). This induces a shift from the keto to the enol form of the peptide bonds, sequentiall along the chain, resulting in a net translocation of a proton to the other end of the chai (*II* → *VI*). As proton translocation proceeds, peptide bonds shift back to the more stab keto form and the original configuration of the chain is re-established (*III* → *VI*)

conformational changes in the enzyme may induce a cyclic pKa change in grou *X* which is not necessarily located at the catalytic site. During this cycle *X* ma accept protons from the aqueous phase and donate them to the proton-tran locating chain. Presumably the chain is included in the structure of *Fo* con ponent. If one end of the proposed proton-translocating chain opens directly t the catalytic site of the ATPase (Mitchell, 1974), *X* could be a group that also pa ticipates in the catalysis. The protons released at the catalytic site during ATP h drolysis could first be accepted by *X* and then donated to the proton-translocatin chain.

A question that is frequently discussed in the literature is the mode of energ transmission between different energy transducing units in coupling membran (Boyer *et al.*, 1977). The possibility exists that a proton-translocating chain suc as the one proposed here may be established within the membrane by appropr ate protein-protein interactions, for example, between the ATPase complex an the electron-transport proteins. Energy transmission could then take place witl out equilibration of the protons with the extra-membrane space (Williams, 1961 Data have been presented that are consistent with this possibility (Ernster, Junt & Asami, 1973; Ferguson, Lloyd & Radda, 1976; Yaguzhinskii *et al.*, 1976).

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