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PROTON CONDUCTION IN BACTERIORHODOPSIN VIA A HYDROGEN-BONDED CHAIN WITH LARGE PROTON POLARIZABILITY.

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SUMMARY:
A Corey-Pauling-Kolthun molecular model of bacteriorhodopsin was built. This model shows that a largely structurally symmetrical hydrogen bonded chain asp, 6 tyr, glu may be formed. With regard to the total proton potential this chain shows very large proton polarizability and thus via this chain the positive charge can be conducted to the outside of the membrane via a Grotthus mechanism.

I. INTRODUCTION

Halobacteria are able to use light energy for ATP synthesis (1). This is performed - according to Mitchell's chemiomotic theory (2) - by a proton gradient, caused by outward proton pumping due to the so-called purple membrane (1). The only protein constituent of this membrane is bacteriorhodopsin, a pigment containing a retinal Schiff base as chromophoric group (for a recent review see ref.(3)). Upon light absorption, the chromophore undergoes a photocycle (4), during which protons are transported across the membrane. Herewith, firstly, light energy is used by the retinal Schiff base and its protein environment to increase the electrochemical potential of a proton. This proceeds by a deuteration-dependent (5) psec step, whereby the initially protonated Schiff base, however, is not deprotonated (6). Secondly, this potential causes the transport of the positive charge to the outside of the membrane. A chain of hydrogen bonds between polar amino acid side groups (like serine, threonine, tyrosine, aspartic or glutamic acid) was proposed by Nagle and Morowitz (7),

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by Knapp, Schulten and Schulten (8), and by Dunker and Marvin (9) to explain this proton transport. In building a CPK-model of bacterio rhodopsin we tried to determine, if such a linear hydrogen-bonded system may really be present in this protein.

Such a system may conduct positive charge via a Grotthus mechanism. As shown in previous publications (10) - (13) a necessary prerequisite of this mechanism is the presence of hydrogen bonds with great proton polarizability. Hydrogen bonds show great proton polarizabilities when double minimum proton potentials or proton potentials with a broad flat well are present in these hydrogen bonds (10)(12)(14). Such potentials occur, of course, with all structurally symmetrical hydrogen bonds, as, for instance, $B^+H\cdots B \rightleftharpoons B\cdots H^+B$. Great proton polarizabilities are, however, also found with heteroconjugated $B_1H\cdots B_2 \rightleftharpoons B_1^-\cdots H^+B_2$ bonds (15). Furthermore, large proton polarizabilities occur with hydrogen bonded chains having largely symmetrical total proton potentials (11)(12)(16). Recently it was shown that various types of hydrogen bonds formed between polar side chains of proteins show great proton polarizability (13)(17). Thus, all these hydrogen bonds may participate in proton conducting systems in proteins.

II. RESULTS FROM THE COREY-PAULING-KOLTUN MODEL OF BACTERIORHODOPSIN

We built a CPK molecular model (The Ealing Corp., South Natick, Mass., U.S.A. 01760) on the basis of the following:

1. The primary structures given by Ovchinnikov et al.(18), by Walker et al.(19) and by Khorana et al.(20).
2. The primary structure is arranged in seven α-helical segments (21) according to the structure proposed by Engelman et al.(22) which is only slightly different from that of Ovchinnikov et al.(18).

Our model leads to the following results illustrated in fig.1:

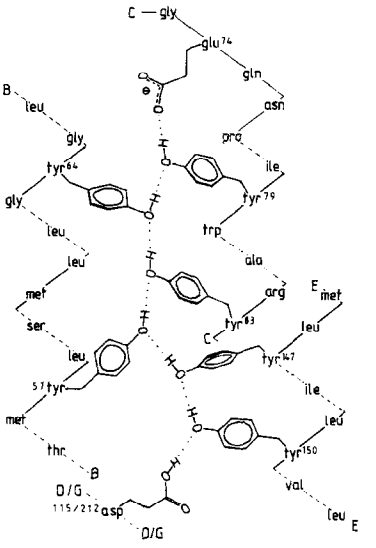


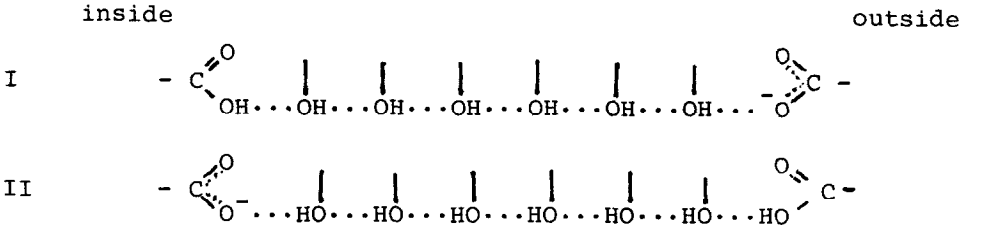
Fig. 1 Schematic representation of the proton channel in bacteriorhodopsin suggested by the CPK-model.

As can already be seen from the sequence data, the tyrosines 57 and 64 in helix B are seven amino acid residues apart and are therefore on the same side of this helix (the helical segments into which the primary structure can be divided are named with capital letters according to Engelmann et al.(22)). The tyrosines 79 and 83 in helix C are four residues apart and therefore on the same side of this helix. If one brings the helices B and C into contact with one another - which is possible with the CPK-model in a nearly spacefilling manner - then a chain of hydrogen bonds tyr^{57}-tyr^{83}-tyr^{64}-tyr^{79} fits very well. The outside end of this chain - tyr^{79} - can be connected to the carboxylate group of glu^{74} which serves as hydrogen bond acceptor (see fig.1).

In the interior of the molecule two additional tyrosine side groups can be connected to this chain: tyr^{147} and tyr^{150} in helix E

(numbering according to ref.(20)) which are three residues apart and so, on the same side of this helix. They can form a hydrogen bond between one another and tyr^{147} can form a hydrogen bond to tyr^{57}, if helix E is brought into contact with the helices B and C, which is readily possible, too.

The molecular model shows further that asp^{115} in helix D or asp^{212} in helix G, respectively, may form a hydrogen bond to tyr^{150} if they are protonated. Both arrangements fit very well in our CPK-model. Thus, our model shows that at least four helices take part in the proton transport mechanism. Thus, a largely structurally symmetrical hydrogen-bonded system may be present in bacteriorhodopsin:



III. DISCUSSION

A hydrogen-bonded chain between the imidazole side groups of histidine residues may be protonated under physiological conditions. As discussed in ref.(11) and ref.(12) p.761 in this way a system with large proton polarizability arises, which is very suitable for proton conduction and thus for shifts of positive charge through biological membranes. A linear protonated chain of water molecules was recently treated theoretically by Scheiner (23) regarding their proton conduction.

It is, however, not possible to protonate a tyrosine residue under physiological conditions. Not even perchloric acid is able

to add a proton to phenol (24). Thus, a conduction mechanism via only a chain of tyrosine residues, analogous to the mechanism using histidine residues, is impossible, since no single protonated tyrosine may exist.

The molecular model shows that at both ends of the chain of tyrosines, carboxylate groups may be present. When one of these groups is protonated, the whole system is largely structurally symmetrical. The total proton potential of this system is then a double minimum with high barrier and thus shows very great proton polarizability (10)(12)(14)(16). Thus - when the aspartate residue 115 or 212, respectively, is protonated by the chromophoric mechanism, the positive charge may easily be shifted via this hydrogen bonded system with great proton polarizability within the purple membrane. The charge may be shifted in this way since the transfer steps occur strongly correlated.

The transmembrane potential would, however, favor the proton limiting structure I in which the proton is present near the chromophoric center. Two influences work against this and shift the positive charge to the outside of the membrane. Firstly, there is a number of positively charged groups (lys^{30}, lys^{172}, arg^{175}, and lys^{216}) in the neighborhood of asp^{115} or asp^{212}, respectively. These positive charges are not completely compensated by negative charges. Secondly, the hydrogen-bonded system shows a weak structural asymmetry of the chain favoring the flux of the positive charge to the outside since at the one end of the chain in the membrane an aspartate and at the outside a glutamate residue is present.

From the glutamic acid residue 74 the proton may transfer to the medium at the outside. After every transport process of a positive charge - as with the Grotthus conductivity in ice (25) - all OH

groups must be turned - which may proceed cooperatively - to regenerate the hydrogen bonded system. A possible mechanism for the protonation of the aspartate residue will be discussed in a following paper.

IV. CONCLUSIONS

Our CPK model suggests that in bacteriorhodopsin the residues asp^{115} (or asp^{212}), tyr^{150}, tyr^{147}, tyr^{57}, tyr^{83}, tyr^{64}, tyr^{79}, glu^{74} may form a hydrogen bonded system. This system is largely structurally symmetrical and thus, the total proton potential of this system is a double minimum. Thus, this system shows very great proton polarizability and hence, the positive charge may be conducted by a Grotthus mechanism along this system to the outside of the purple membrane.

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