# AN IMPLICATION OF THE STRUCTURE OF BACTERIORHODOPSIN

## GLOBULAR MEMBRANE PROTEINS ARE STABILIZED BY POLAR INTERACTIONS

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Bacteriorhodopsin captures incident photons and stores a portion of their energy by pumping protons across a membrane against the electrochemical gradient. The protein has been of great interest because it is a relatively simple example of an energy-transducing structure and occurs in ordered two-dimensional arrays as part of the microorganism *Halobacterium halobium*. In this note, the current status of structural information concerning the protein is described and its implications for globular membrane proteins examined.

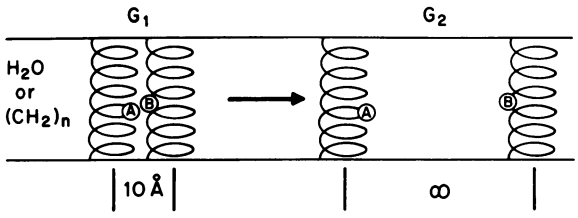
### RESULTS AND DISCUSSION

Two major structural findings form the basis of our work: the three-dimensional map obtained by Henderson and Unwin from electron microscopy (1), and the known amino acid sequence (2, 3). Using the known sequence and interpreting the structural map as indicating seven $\alpha$-helices traversing the membrane, we undertook a model-building study to predict the identity of the different helices in the sequence (4). This work was followed by experiments in which different amino acid types were specifically deuterated in the structure by biosynthetic incorporation. The success of such incorporation led to the possibility of using neutron scattering to study the distribution of each type of amino acid in projections of the structure (5). Model building shows that the helices have a rather amphipathic character, with a hydrophobic surface containing almost no polar groups and a hydrophilic surface containing abundant polar groups including Arg, Lys, Asp, Glu, Asn, Gln, Tyr, Ser, and Thr. Inspection of difference Fourier maps showing the locations of deuterated valine and deuterated phenylalanine led to the conclusion that, in the structure, the helices are oriented with their polar groups toward the inside of the molecule and their nonpolar surfaces toward the outside where they make contact with the nonpolar region of the lipid bilayer (5). It seems that this "inside-out" orientation of the protein groups may provide an important clue concerning the stabilization of the structures of globular membrane proteins.

Globular membrane proteins may be distinguished from anchored membrane proteins in having peptide chains with substantial tertiary structure within the nonpolar region of the lipid bilayer. Proteins may be anchored by a single polypeptide chain which traverses the bilayer, as appears to be the case for glycophorin (6, 7), or, in the cases involving transport across the bilayer, may be expected to have a polypeptide chain which traverses the bilayer many times. Consideration of the stability properties of a polypeptide in a nonaqueous environment together with considerations of the process of biosynthesis lead to the conclusion that most proteins of the globular type will have predominately $\alpha$-helical secondary structure (8). If we consider two $\alpha$-helices in a position spanning a membrane bilayer, we may examine the interactions which tend to hold them together or allow them to drift apart. Ignoring, for the moment, interactions outside the surfaces of the bilayer, the situation is essentially as shown in Fig. 1. Here two helices are considered as they would interact in an aqueous and a nonaqueous environment. Approximate free energies of interaction are shown based on the hydrophobic effect; the formation of an ion pair between groups *A* and *B*; the formation of a strong hydrogen bond (i.e., Lys-Tyr or Asp-Tyr) between groups *A* and *B*; and the formation of a conventional hydrogen bond between groups *A* and *B*. The energy change due to conformational entropy for the two processes may also be roughly estimated, based on the gain of one rotational and two translational degrees of freedom, and will be the same for the process in water or in hydrocarbon. Van der Waals interactions will contribute smaller energies and, again, will have comparable effects in both environments.

We therefore pose the following question: As most globular proteins in aqueous environments are stabilized in their folding by the hydrophobic effect, how can globular membrane proteins be stable in a nonaqueous environment lacking such a source of folding stability? The answer is suggested by inspection of the numbers in Fig. 1. For the association of the two helices to be stable, the free energy change for their separation must be positive. In an aqueous environment, the hydrophobic effect provides sufficient energy to oppose the entropy increase of separation, but in a nonpolar environment this influence is absent. However, polar interactions are much stronger in the nonpolar milieu and can provide the required stabilization. The substitution of polar interactions for the hydrophobic effect as the dominant stabilizing influence for membrane proteins is indicated.

A stable structure must be present if membrane proteins




| | ΔG = G1 − G2 (kcal/mol) | |
|---|---|---|
| | in $H_2O$ | in $(CH_2)_n$ |
| HYDROPHOBIC | 20 | 0 |
| AB ION PAIR | 2 | 20-30 |
| AB STRONG H BOND | 0 | 10-20 |
| AB H BOND | -1 | 5 |
| CONFORMATION ENTROPY | -5 | -5 |

FIGURE 1 Free energy effects in the separation of twenty residue $\alpha$ helices in aqueous and nonpolar environments.

are to be sufficiently specific in their transport properties to allow the unidirectional translocation of ions or small molecules while preventing reverse leakage through similar channels. Of course, it may be that those portions of the protein external to the lipid bilayer contribute to the stability of the overall structure. Bacteriorhodopsin shows that this need not be the case, since several of the polypeptide links between helices may be cleaved without substantially reducing the activity of the protein in proton translocation (2, 9). Furthermore, in this known example, the amount of protein external to the bilayer is rather small to provide important stabilization from hydrophobic interactions.

These considerations lead to the contention that all membrane proteins having extensive tertiary structure within the bilayer will be "inside-out" compared to the organization of soluble globular proteins, and that their structural integrity will rely on polar interactions among buried groups in the interior.

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