they are available for binding and cause a further increase in conductance. In Fig. 2 *d*, under the influence of a *trans*-positive membrane potential, the protein goes to a new nonconducting state on the *trans* side. This can be observed by removing the HBP from the *cis* side with pronase.

It seems likely that protein-protein interactions are important in the translocation. They provide a way to overcome the energetic barriers for movement of hydrophilic charged residues through the low dielectric bilayer interior. Charge delocalization and neutralization by apposition of charged groups either within a molecule of HBP or by aggregation could drastically lower the barrier. A possible role for $Ca^{2+}$ in charge neutralization is suggested by the symmetrization seen in Fig. 1 *c*. The schematic representation of two protein molecules in Fig. 2 is meant to suggest possible protein-protein interaction, not necessarily dimeric.

The experiments described here indicate that the transmembrane potential can influence the disposition, and perhaps the orientation, of membrane proteins.

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# QUANTITATIVE APPLICATION OF THE HELICAL HAIRPIN HYPOTHESIS TO MEMBRANE PROTEINS

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We have proposed (1) that the initial event for either the secretion of proteins across or their insertion into membranes is the spontaneous penetration of the hydrophobic portion of the bilayer by a helical hairpin. The major proposals of this model are (*a*) energetic considerations of polypeptide structures in a nonpolar lipid environment as compared with an aqueous environment have led to the conclusion that only $\alpha$- and $3_{10}$ helices will be observed in the hydrophobic interior of membranes. (*b*) During protein synthesis, the nascent polypeptide chain folds in the aqueous environment to form an anti-parallel pair of helices, each of which is $\sim$ 20 residues long. (*c*) The helical hairpin partitions into the membranes if the free energy arising from burying hydrophobic helical surfaces exceeds the free energy cost of burying potentially charged and hydrogen bonding side chain groups. (*d*) Globular membrane proteins will be formed by the insertion of several pairs of helical hairpins which are expected to be the fundamental unit of membrane protein folding. (*e*) In secreted proteins, the hydrophobic leader peptide forms one of these two helices and functions to pull polar portions of the secreted protein into the membrane as the second helix of the hairpin. (*f*) Insertion of the helical hairpin into the bilayer initiates secretion if the second helix is polar and secretion of the newly synthesized protein continues until or unless a hydrophobic segment is encountered. (*g*) Alternatively, if both helices are hydrophobic the hairpin will simply remain inserted in the membrane. (See reference 1 for complete references.)

The course of cotranslational insertion and folding varies in defineable ways for anchored membrane proteins, globular membrane proteins, and secreted proteins. Thus, it should be possible to estimate whether a protein is a secreted, globular, or anchored membrane protein merely by analyzing the relative polarity or hydrophobicity of the amino acid sequence.

## RESULTS AND CONCLUSIONS

We have now written a computer program to analyze the amino acid sequence of secreted and membrane proteins in order to estimate quantitatively whether insertion of membrane proteins into lipid bilayers can be expected to be spontaneous on thermodynamic grounds and also to establish the probable topology of membrane proteins. We have assumed that the favorable energy contribution to partitioning into the membrane arises from hydrophobic forces and have assumed this to be equal to 60 kcal/mol of helical hairpin. A range of unfavorable energetic contributions arises from the burying of different polar and charged residues. The values that we think appropriate are +14 kcal/mol for Arg; +10 kcal/mol for Lys; +8 kcal/mol for Asp and Glu; +7 kcal/mol for Gln and Asn; +6 kcal/mol for His; +3 kcal/mol for Pro; and +3 kcal/mol


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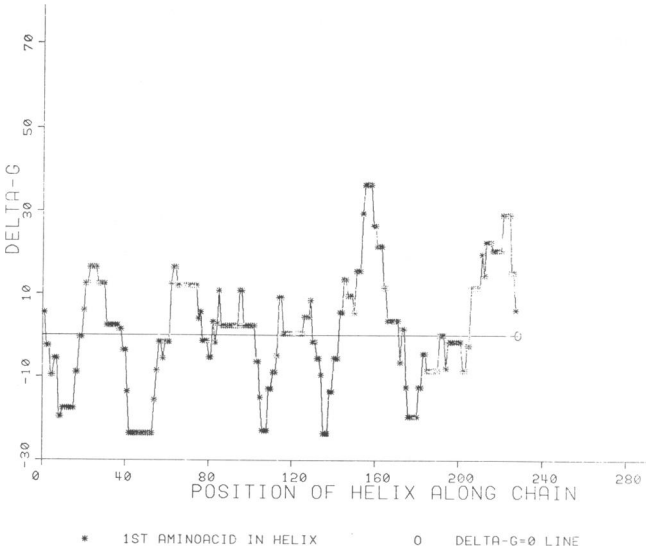


FIGURE 1 The free energy of transfer of 21-residue stretches of bacteriorhodopsin sequences from water to nonpolar medium assuming an $\alpha$-helical conformation in both states. The minima in this plot indicate the probable starts of the seven $\alpha$-helices.

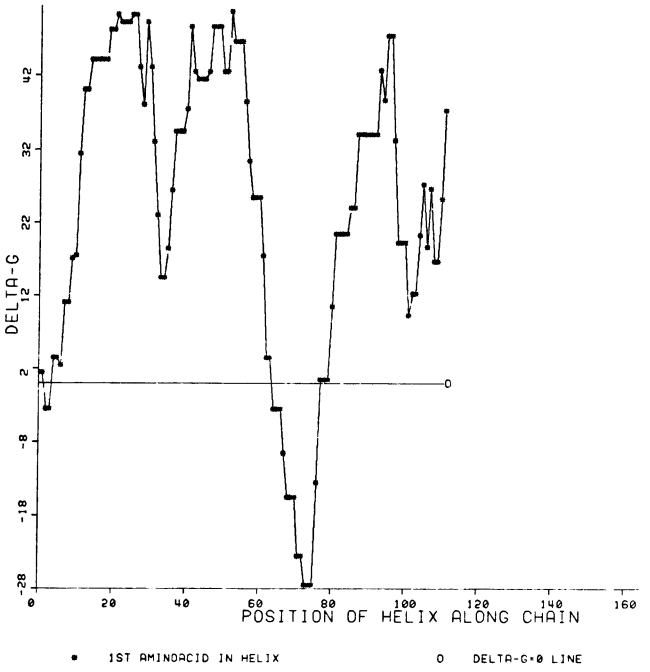


FIGURE 2 A free energy of transfer plot for glycophorin similar to Fig. 1. The large negative excursion represents the portion of the protein that traverses the membrane.

for Trp. The positive free energy of burying pairs of acidic and basic side chains was reduced to +5 kcal/mol if the acidic and basic groups were plus or minus three or four residues from each other in sequence. In that instance the interaction of acidic and basic side chains could allow them to be buried at much lower energetic cost.

Amino acid sequences were analyzed for the free energy of burying an $\alpha$-helix of definable length, usually 21 amino acid residues long. A 21-residue probe helix was removed down the sequence, and the free energy of burying each successive 21-residue helix was calculated. In Fig. 1 is plotted the free energy of burying such $\alpha$-helices for the protein bacteriorhodopsin. The ordinate is the $NH_2$-terminal amio acid of a given 21 residue helix for which the free energy of insertion is calculated. In this plot, regions of sequence that show positive bilayer insertion free energy would not be expected to insert spontaneously and remain in the membrane by themselves, whereas regions with substantial negative free energy of insertion could be stable in the lipid bilayer. For bacteriorhodopsin there are, as anticipated, approximately seven negative excursions along the amino acid sequence. These minima presumably correspond to the positions in amino acid sequence of the seven known $\alpha$-helices that traverse the lipid bilayer. The positions of the $\alpha$-helices traversing the membrane bilayer are exactly the same for those predicted by Engelman et al. (2) in all cases except the last two helices. According to the analysis presented here, the last two helices would be predicted to initiate a few residues later in the sequence.

We have also analyzed the polarity of the sequence of glycophorin. This protein has its amino terminus on the outside and COOH-terminus on the inside of the membrane of the red blood cell. Fig. 2 shows that both its amino and COOH-termini are very polar and are connected by a nonpolar region which would be stable in the lipid bilayer.

In conclusion, it appears that analysis of the amino acid sequence of integral membrane proteins allows one to make predictions concerning both the topology and disposition of various portions of membrane proteins. Similar calculations have been done on the sequences of three secreted proteins, bovine trypsinogen, penicillinase, and alkaline phosphatase. The variation of polarity with sequence is less easily interpreted in these cases.

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