biochemical studies have showed 50–80% inhibition of glycosylation by prior trypsin treatment of Golgi membranes. Preliminary EM studies have revealed that trypsin-treated Golgi membranes were morphologically indistinguishable from control membranes.

The results implicate transfer of UDP-gal to dol-P-gal on the cytosolic surface of Golgi membranes. Membrane fusion then allows translocation of dol-P-gal across the lipid bilayer by inverted micelles (4) at points of fusion (Fig. 2). The model, although speculative, satisfactorily resolves the topological questions of glycosylation in vivo.

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VOLTAGE-DEPENDENT CHANGES OF A MEMBRANE PROTEIN IN LIPID MODEL MEMBRANES

STUDIES WITH THE HEPATIC ASIALOGLYCOPROTEIN RECEPTOR

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A major question about the interaction of membrane proteins with lipids is whether the biosynthetic process determines once and for all the orientation of the protein with respect to the bilayer, or whether later processes can affect the protein's disposition. We show here that a membrane receptor protein for asialoglycoproteins (the hepatic binding protein, or HBP [1]) can undergo voltage-dependent changes in its disposition with respect to the lipid bilayer.

RESULTS AND DISCUSSION

This protein mediates endocytosis of desialylated glycoproteins by hepatocytes. It has the great advantages of being obtainable in relatively pure form and of being soluble in aqueous solution free of detectable lipid and detergent (2). In its water-soluble, detergent-free form it does not bind ligands (galactose-terminal glycoproteins such as asialo-orosomucoid, ASOR). When we mixed HBP with phosphatidyl choline vesicles, the protein associated nonelectrostatically with the lipid, and specific ligand binding activity indistinguishable from that of the native receptor was restored (3).

Because of this spontaneous reconstitution, we thought that HBP might insert into preformed black lipid membranes (BLMs); it did (4). An increase in conductance was observed when a *trans*-positive voltage was imposed across a BLM (Fig. 1 *a*). The nature of this conductance increase pointed strongly to lipid perturbation in that: (*a*) no discrete step size of conductance was observed; (*b*) the conductance tracing fluctuated irregularly, in a way not suggesting true channels; (*c*) the conductance was not linearly proportional to the first, or any higher, power of the HBP concentration; and (*d*) the conductance displayed little ion selectivity. Also consistent with a lipid perturbation was the observation that HBP lowered and broadened the phase transition of dipalmitoyl phosphatidyl choline vesicles (3).

For a signal to be transduced by a receptor after ligand binding, a conformational change in the receptor is usually assumed to take place. Given the possibility that such a change could alter lipid-protein interactions, we examined the effect of ligand binding on the conductance in an HBP-doped BLM. When specific ligand was used, we noted a dramatic voltage-dependent increase in conductance (Fig. 1 *b*). In contrast to the observation with HBP alone, both a *trans*-positive and a *trans*-negative potential induced a conductance increase. The monovalent uncharged ligand *N*-acetyl galactosamine also produced this effect. Thus, ligand binding results in an alteration of the receptor which can be monitored by the conductance measurements.

It is well known that calcium is specifically required for the binding activity of this receptor. Calcium (and not other divalent cations) had an interesting effect on HBP induced conductance. Although the magnitude of conductance was not increased, the voltage-dependent conductance was again made symmetrical as in the presence of ligand (Fig. 1 *c*).

Our model for the stages of interaction between HBP and lipid bilayers is shown in Fig. 2. In Fig. 2 *a*, the protein (added to the *cis* side) associates with the lipid bilayer in

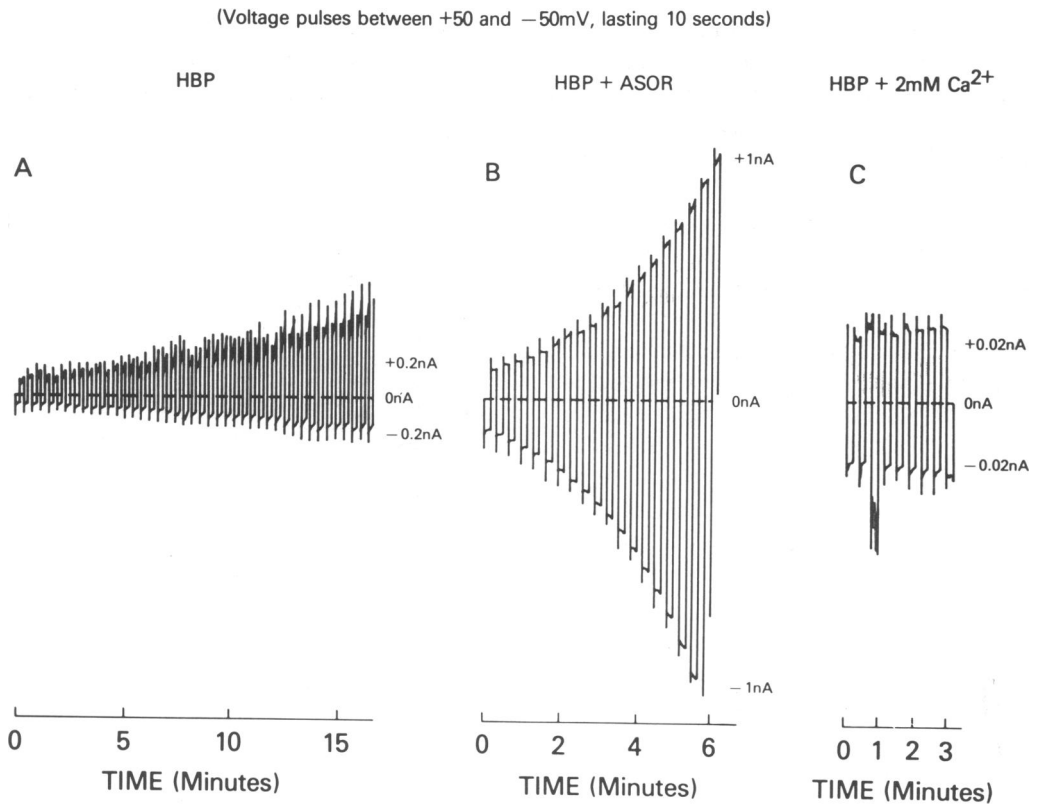

FIGURE 1 Conductance change induced by HBP. *A*, (10 μg/ml); *B*, in the presence of ligand (10 μg/ml ASOR); *C*, or Ca^{2+} (2 mM). Oxidized cholesterol/decane BLM; 0.15 M NaCl; 25 C°; HBP and ASOR added to the *cis* compartments; Ca^{2+} to both. Membrane potential defined as $\Psi_{trans} - \Psi_{cis}$ (from reference 4, with permission).

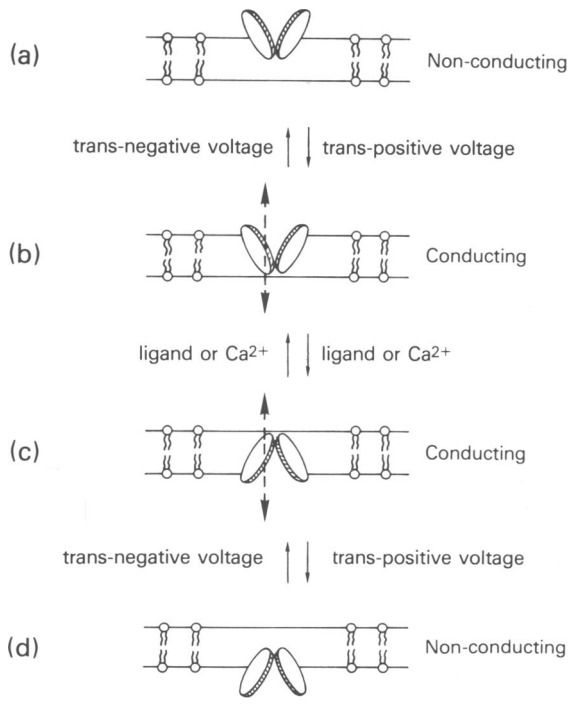

FIGURE 2 Model for the interaction of HBP with a lipid model membrane (see text for explanation). The HBP is added to the *cis* side (top).

the presence or absence of a membrane potential. This association is hydrophobic, involving a perturbation of the lipid and a conformation change in the HBP (3). In Fig. 2 *b*, under the influence of a *trans*-positive membrane potential the protein can be promoted reversibly into a conducting state in which it perturbs the bilayer sufficiently to permit passage of ions, that is, to increase the conductance of the membrane. The negative charge on the protein at neutral pH (isoelectric at pH = 4.7) is consistent with the direction of the voltage effect, and we can titrate this voltage effect reversibly by lowering the pH on the *cis* side of the membrane. Voltage-controlled penetration of the lipid bilayer is also suggested by studies with aqueous collisional quenchers of tryptophan fluorescence on HBP in lipid vesicles. When an inside-positive membrane potential is established by valinomycin in the presence of a K^+ gradient, the quenching decreases. Thus, an inside-positive potential tends to "bury" the tryptophan. In Fig. 2 *c*, in the presence of a galactose-terminal ligand or 2 mM Ca^{2+} the protein apparently crosses from the *cis* to the *trans* side of the membrane when a *trans*-positive electric field is applied. Observations supporting translocation are that the membrane conductance becomes symmetrical (Fig. 1 *b* and 1 *c*); that part of HBP becomes exposed on the *trans* side and is destroyed by pronase there; and that ligand binding sites also become exposed on the *trans* side, where

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 α- 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 ~ 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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