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Structures of Membrane Proteins

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Summary. The possible conformations of integral membrane proteins are restricted by the nature of their environment. In order to satisfy the requirement of maximum hydrogen bonding, those portions of the polypeptide chain which are in contact with lipid hydrocarbon must be organized into regions of regular secondary structure. As possible models of the intramembranous regions of integral membrane proteins, three types of regular structures are discussed. Two, the alpha helix and the beta-pleated sheet, are regularly occurring structural features of soluble proteins. The third is a newly proposed class of conformations called beta helices. These helices have unique features which make them particularly well-suited to the lipid bilayer environment. The central segment of the membrane-spanning protein glycophorin can be arranged into a beta helix with a hydrophobic exterior and a polar interior containing charged amino-acid side chains. Such structures could function as transmembrane ion channels. A model of the activation process based on a hypothetical equilibrium between alpha and beta helical forms of a transmembrane protein is presented. The model can accurately reproduce the kinetics and voltage dependence of the channels in nerve.

The proteins of cellular membranes are thought to function variously as receptors for drugs and hormones [7], as mediators of metabolically coupled vectorial transport (i.e., active pumps) [24], and as electrically excitable passive diffusion pathways (ion channels) [11]. Our understanding of these crucial cell functions would be greatly enhanced if the structures of the proteins involved were known. The characteristic physical properties of membrane proteins make them difficult to isolate and crystallize; consequently the determination of their three-dimensional structure has lagged behind that of their water-soluble counterparts. To date, a three-dimensional structure is available for only one membrane protein. For the moment, membrane proteins must be viewed as black boxes whose functions are partially known but whose structures we may only guess at. Fortunately, this guesswork is simplified by the extreme regimentation required by the membrane environment. The possible conformations of membrane proteins are so restricted that it may be possible

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to predict their three-dimensional structures from a knowledge of amino acid sequence. It is the purpose of this article to examine the constraints imposed upon protein structure by the lipid bilayer environment and to discuss the ways that proteins might satisfy those constraints.

Protein Structure

Since the application of the techniques of X-ray crystallography to the determination of the structure of proteins, the three-dimensional structures of more than 70 polypeptide chains have been solved [18, 19]. Despite the seemingly endless variability observed in the structures of different proteins, it has become apparent that certain broad generalizations can be made about the way that polypeptide chains are folded to generate tertiary structure. Typically, a protein contains regions of regular secondary structure (α helices and β-pleated sheets) connected by regions of nonregular structure. The term nonregular structure encompasses extended chain types of conformation as well as hairpin (or beta) turns and is used to describe all areas of the polypeptide chain in which adjacent residues have dissimilar conformational parameters. The major difference between regular and nonregular types of structure is that regions of regular structure are entirely internally hydrogen bonded; each residue makes hydrogen bonds with similarly oriented residues in adjacent turns of the helix (or strands of the sheet). This is not true for nonregular regions, and they usually occur at the surface of the protein where their hydrogen bonding requirements may be satisfied by water molecules [6, 16].

Proteins have compact, globular structures with predominantly hydrophobic interiors and comparatively polar exteriors. Nonpolar residues are not located exclusively in the hydrophobic interior; about half the residues exposed on the surface of the protein have nonpolar side chains [17]. Charged residues *are* essentially excluded from the interior, however. Energy is required to bury a charged side chain in a hydrophobic environment, and charged residues are found in the interior of proteins only in a few exceptional instances. Uncharged, polar residues occur both in the interior and exterior regions of proteins; in a hydrophobic environment, the polar atoms of these side chains are almost invariably hydrogen-bonded, either to other polar groups or occasionally to buried water molecules [3, 6, 16]. When their hydrogen-bonding requirements are satisfied, uncharged polar side chains are nearly as hydrophobic as nonpolar side chains of equivalent size [5, 6, 26]. In a protein, nearly every atom

capable of participating in a hydrogen bond does so [3, 6, 16]. About half of these bonds are made to water molecules at the surface of the protein. Of the remainder, 80% occur in regions of regular secondary structure [6].

Can these general principles be expected to apply to the organization of integral membrane proteins? The answer is "only partially." Obviously, the inside/hydrophobic, outside/polar generalization cannot be valid for a protein intimately associated with the lipid bilayer. If we view soluble proteins as spheres with oily centers, we should perhaps view integral membrane proteins as cylinders with hydrophobic equators. It is to be expected that regions of the polypeptide chain in contact with the bilayer interior will be predominantly hydrophobic in character [28]. A more subtle conflict results from the fact that the hydrocarbon interior of a lipid bilayer can supply no hydrogen bonding potential. In soluble proteins, hydrogen bonds are formed within regions of regular structure or with solvent water molecules at the protein surface. For a polypeptide chain in contact with lipid hydrocarbon, hydrogen bonds must be made within regions of regular structure, or not at all. The energy of a hydrogen bond is on the order of 3 kcal/mole, and the failure to form these bonds would result in thermodynamically unstable structures. Because regions of nonregular structure require an external source of hydrogen bonds which the lipid hydrocarbon cannot supply, we should expect that membrane proteins will rarely, if ever, place regions of nonregular structure in contact with the bilayer interior. The exclusion of nonregular structure from the membrane interior has dramatic implications for the structure of membrane proteins. It means that all portions of the polypeptide chain which are in contact with lipid hydrocarbon must be organized into regions of regular secondary structure. Since there are only a few types of regular structure to choose from, the task of predicting structure from sequence is greatly simplified. Additionally, any membrane protein which interacts appreciably with the bilayer interior must completely span the hydrocarbon domain. This follows from the fact that all regular structures have exposed peptide bonds at their beginnings and ends. The hydrogen bonding requirements of these groups do not allow the termination of a region of regular structure in the interior of the bilayer.

These considerations emphasize the importance of regular secondary structure in the organization of membrane proteins. Certainly, we should expect that regions of regular structure will be found in those portions of membrane proteins exposed to lipid hydrocarbon, and that this will

occur to the exclusion of nonregular types of structure which must expose polar atoms to the hydrocarbon domain. The remainder of this article will discuss models of membrane proteins based on three types of regular structure: the α helix, the β-pleated sheet, and a newly proposed class of conformations called β helices.

Alpha Helical Architecture

The simplest way for a protein to span the lipid bilayer is in the form of an α helix. The helix must contain only about seventeen amino acid residues to traverse the 25-Å hydrophobic thickness of the membrane. It could, of course, be longer than this minimum, but the restriction against nonregular structures no longer holds in the aqueous (or polar head group) phases. This type of architecture is nicely illustrated by the structure of the purple membrane protein from *Halobacterium halobium* determined by Henderson and Unwin [10]. Their 5-Å resolution structure shows seven parallel α helices, each about 35 Å in length, packed together three against four in the shape of a rough cylinder. The axis of the cylinder is presumed to be perpendicular to the plane of the membrane, resulting in a transmembrane orientation for the helical segments, and for the protein as a whole. This arrangement is consistent with the protein's known function, that of a light-driven proton pump [9]. Since the protein consists of a single polypeptide chain, the seven α helices must be connected by loops of nonregular structure, although these cannot be seen at the present resolution. The amino acid sequence of the purple membrane protein has not been completely determined. However, a peptide segment containing the retinal chromophore has been sequenced. The amino-acid composition of this segment suggests that it forms one of the loops of nonregular structure connecting the ends of two helices, and that the retinal is therefore located at one of the membrane surfaces [4].

There are two integral membrane proteins whose amino-acid sequences are known: glycophorin, the major glycoprotein of human erythrocyte membranes, and cytochrome b_5 from liver microsomes. Glycophorin has a tripartite structure with a central hydrophobic segment separating two highly polar regions [29]. It has been suggested that the central segment, residues 73 to 95, spans the membrane as an α helix [27]. This helical segment would serve to anchor the protein to the cell membrane, leaving the N-terminal portion, which contains covalently-linked

oligosaccharide chains, exposed on the exterior surface of the cell where it functions as an antigenic determinant [29]. The central segment of glycophorin could also participate in a new type of secondary structure (the β helix), as will be discussed in the final section of this article.

The complete sequence of cytochrome b_5 has only recently been determined [20]. This protein consists of a large polar portion (90 amino-acid residues) which contains the heme and which can be cleaved from the membrane with trypsin. This treatment leaves behind a hydrophobic tail of 43 amino-acid residues. Once cleaved from its "anchor," the polar portion behaves like a typical water-soluble protein; it can be crystalized, and its three-dimensional structure has been determined. An analysis of the structure of the hydrophobic fragment according to the Chow-Fasman rules predicts a series of four overlapping β turns, only five residues of α helix, and flanking regions of β sheet [20]. This arrangement would be energetically unfavorable for the reasons presented earlier. In fact, the Chow-Fasman scheme will probably prove inapplicable to integral membrane proteins since their prediction rules were derived from a statistical analysis of the conformations of water-soluble proteins. A more energetically favorable prediction for the structure of the hydrophobic fragment is based on the α helix formed from residues 112 to 131. This portion of the sequence is predominantly hydrophobic but does contain two serine residues near the middle of the sequence. In the helix, the side chains of these residues are not located near enough to the aqueous interfaces to be hydrogen bonded to water molecules. A similar situation exists with threonine 87 in the glycophorin sequence. Whether these side chains can remain exposed to the lipid hydrocarbon or must be hydrogen bonded (perhaps through aggregation with other membrane proteins) remains to be determined.

The Beta-Pleated Sheet

As mentioned above, a β-pleated sheet must necessarily have exposed peptide groups at the edges of the sheet, and the hydrogen-bonding requirements of these groups cannot be satisfied by lipid hydrocarbon. However, maximum hydrogen bonding can be provided by the "beta-barrel," a recurring structural feature observed in immunoglobins and superoxide dismutase [19]. In these proteins, a cylindrical β sheet is formed from seven single strands arranged as the staves of a barrel, with the strands running roughly parallel to the axis of the cylinder.

Such a structure could form the intramembranous segment of an integral membrane protein since there would be no hydrogen bonding groups exposed to the membrane hydrocarbon. Regions of nonregular structure connecting adjacent strands of the sheet would be satisfactorily located in the aqueous phases. Generalized representations of the various types of secondary structure compatible with the membrane interior are shown in Fig. 1.

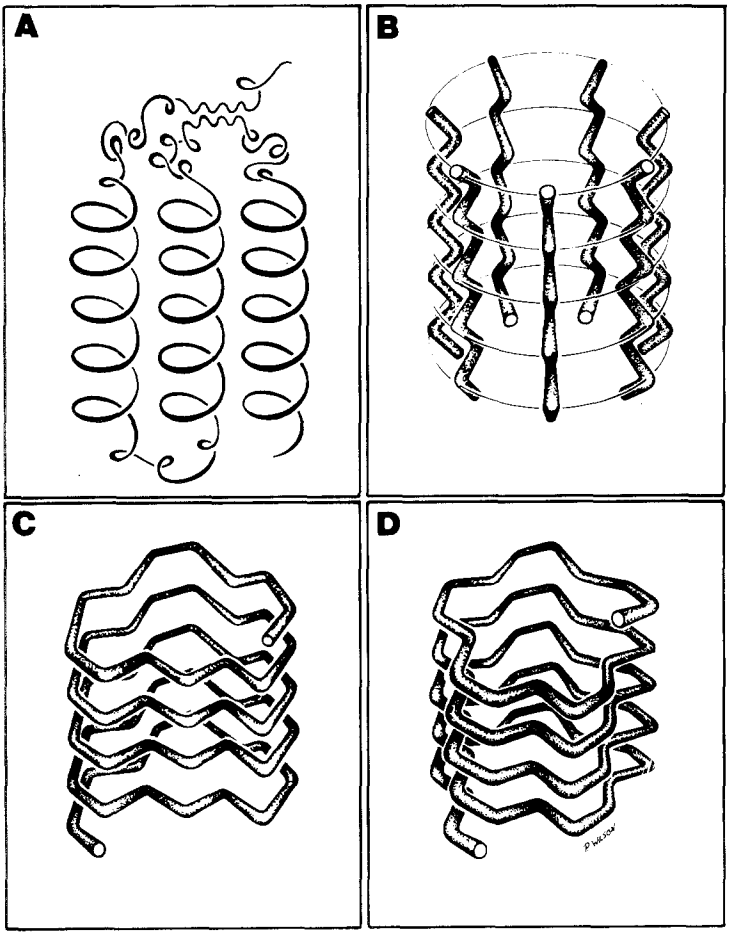

Fig. 1. Schematic representation of various types of regular secondary structure which can favorably interact with the bilayer interior. (*A*): Parallel alpha helices. (*B*): The beta barrel. (*C*) and (*D*): The β^{13} and β^{15} helices

Beta Helices

Beta helices are a recently proposed class of regular conformations that differ from previously described regular conformations in two ways. First, the individual amino-acid residues in a β helix may be nonequivalent; that is, they may exhibit different sets of conformational angles. Second, except for the smallest members of the class, β helices have amino-acid side chains in the interior of the helix. This unique capability has important implications for the structure of membrane proteins, especially those which function in the transport of ions. This section will describe the family of β helices and discuss models of membrane proteins based on this novel type of regular structure.

In the introduction to the first of their classic series of papers on the structure of polypeptide chains. Pauling *et al.* [23] stated: "The problem that we have set ourselves is that of finding all hydrogen bonded structures for a single polypeptide chain, in which the residues are equivalent..." They went on to describe the α helix and the β-pleated sheet, regular conformations that have since proved to be recurring structural features of proteins. Pauling and co-workers limited their search to structures with equivalent residues. The principle of equivalence stipulates that every residue in the structure has identical conformational angles and can be represented by a single point on the Ramachandran plot (Fig. 2). The atoms of a given residue can be superimposed on the atoms of the next residue in the chain by the appropriate symmetry operation.

Recently, a different type of helical conformation has been proposed for the structure of the channel-forming peptide gramicidin A by Urry *et al.* [31–33], and independently by Ramachandran *et al.* [25]. This helix differs from previously proposed conformations in that adjacent amino-acid residues are nonequivalent. Gramicidin A is composed of alternating L- and D-amino acids. In the proposed helix, both the L- and the D-amino acids have nearly the same local conformations they would have in a β-pleated sheet composed of the homoisomer. However, these two conformations are mirror images of each other and occupy separate regions of the Ramachandran plot. Stereo views of these two local conformations (β and β_D) are shown in Fig. 3, and they are plotted on the Ramachandran plot of Fig. 2. The helix proposed for gramicidin A has been referred to in various ways, but the term $\beta^6_{3,3}$ helix, introduced by Urry[1] is

1 In this notation, the superscript designates the number of residues per turn, and the subscripts refer to the number of peptide bonds in each turn whose carbonyl groups point, respectively, parallel and anti-parallel to the helix axis [32].

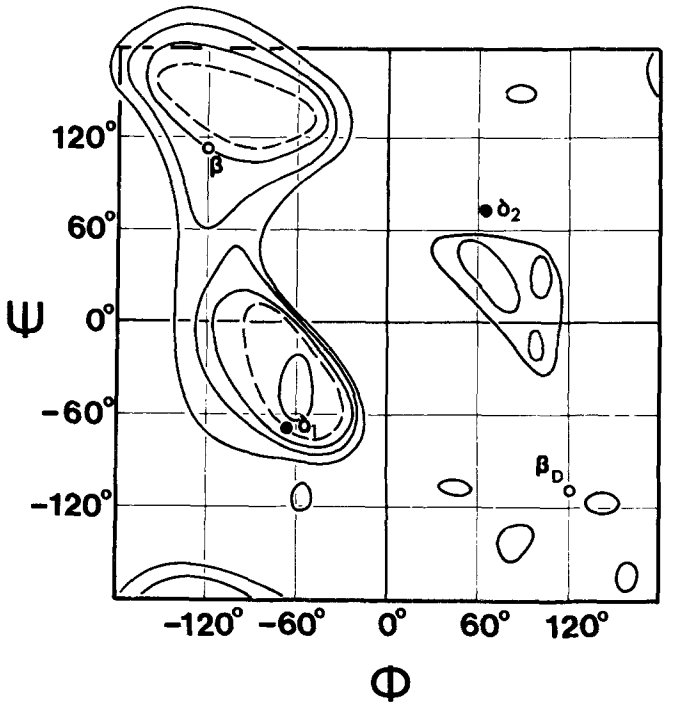

Fig. 2. Ramachandran plot of the four residue conformations which form the basis for the family of β helices. (Reproduced from [14] with permission of the publisher)

most descriptive in view of the similarity between this helix and the β-pleated sheet conformation. The relationship between these two types of structure is illustrated in Fig. 4. Urry also described a modification of the gramicidin helix called the $\beta^6_{2,4}$ helix which contains a third type of local conformation and which would be appropriate for polypeptides having a D-amino acid in every third position along the chain. This local conformation and its mirror image are labeled δ_1 and δ_2 in Figs. 2 and 3. It is interesting to note that the first β-pleated sheet proposed by Pauling and Corey consisted of alternating δ_1 and δ_2 residues [21]. The correct structure, which contains only β residues, was put forth in a later paper [22]. The two β^6 helices might be viewed as special cases since they contain D-amino-acid residues which do not normally occur in proteins. They do, however, illustrate the potential utility of regular conformations with nonequivalent residues. There is strong evidence that the channel conformation of gramicidin A is, in fact, the $\beta^6_{3,3}$ helix [1], and a synthetic peptide capable of forming the $\beta^6_{2,4}$ helix has been shown to function similarly to gramicidin A in lipid bilayers [8].

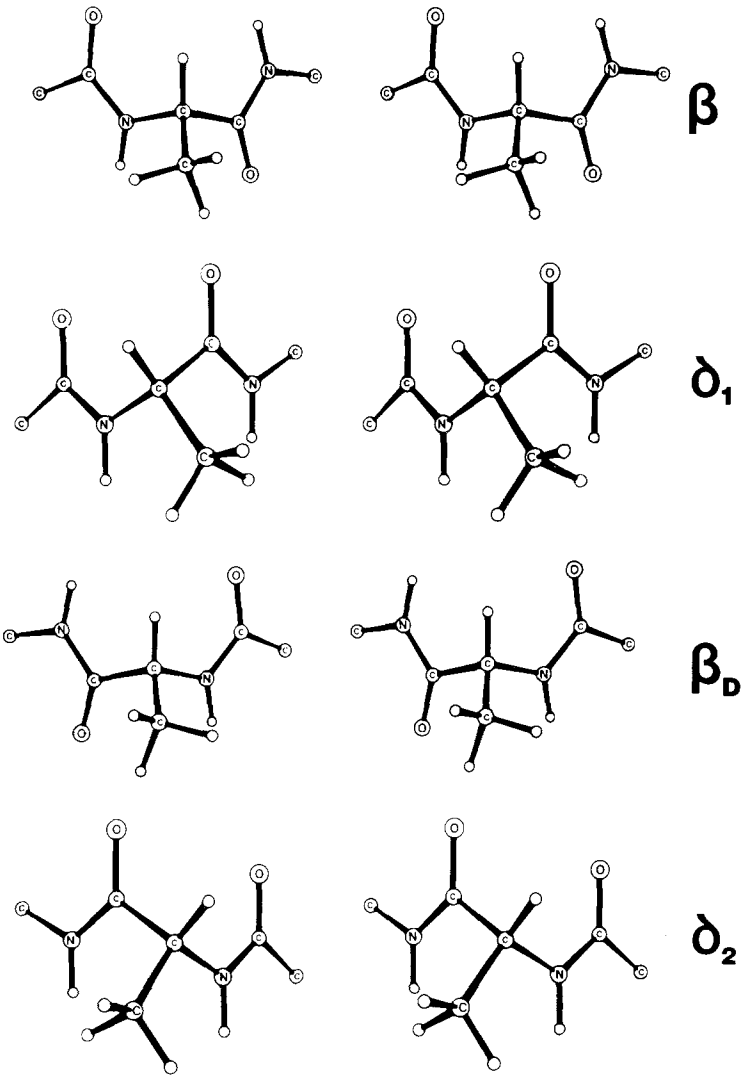

Fig. 3. Stereo views of the four residue conformations. β and δ_1 are formed from L-amino-acid residues. β_D and δ_2 are formed from D-amino acid residues, and are the mirror images of β and δ_1

The conformational principles underlying the β^6 helices have been generalized and extended by Kennedy *et al.* [12–15], who described several larger β helices including two which do not require the presence of D-amino acid residues. The four nonequivalent residue conformations in Fig. 3 form the structural basis for an entire family of related helical conformations. Of the four residue conformations, β and δ_1, are fully allowed for L-amino-acid residues while β_D and δ_2 are allowed for D-amino acid residues. To generate a β helix, the four local conformations

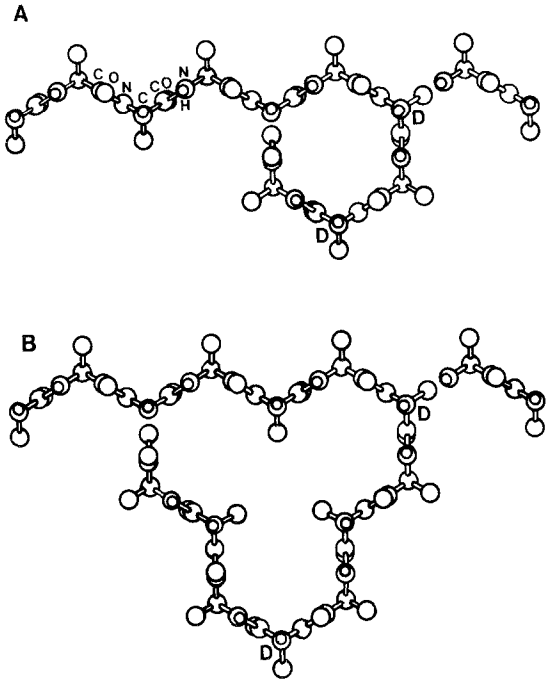

Fig. 4. The relationship between β helices and the β-pleated sheet. (*A*): Changing a sequence of L-amino acids to alternating L- and D-amino acids causes the β sheet to fold into a hexagon. (*B*): A D-amino acid in every fourth position generates the shape shown

are combined in the number and order required to produce a closed irregular polygon (the first turn of the helix) and then this pattern is repeated. Each residue makes hydrogen bonds to similar residues in adjacent turns of the helix. Like other regular structures, β helices are maximally hydrogen bonded, and the local conformations fall in low-energy regions of the Ramachandran plot. Single turns of some representative β helices are shown in Fig. 5. The β^{13} and β^{15} helices contain only the residue conformations β and δ_1, and consequently are expected to be allowed conformations of proteins. It might be supposed that proteins could adopt any of the β-helical conformations by substituting glycine at the positions requiring D-amino acids. Such structures would be unstable from an evolutionary standpoint since conservative mutations of the glycine residues are impossible. Any mutation at a position occupied by glycine would cause a change to an L-amino acid and would necessarily be lethal. Probably the only reasonable candidates for β-helical structure in proteins are the β^{13} and β^{15} helices.

Beta helices would seem to be ideal conformations for the intramembranous segments of integral membrane proteins. They are regular struc-

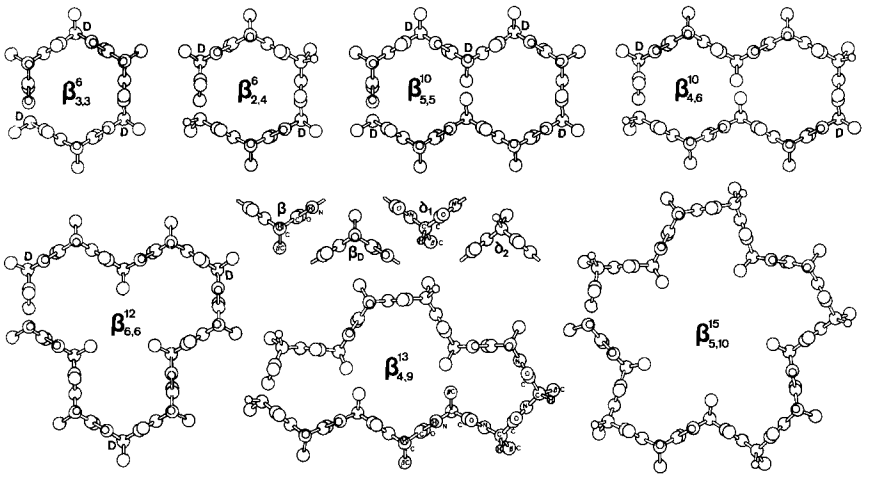

Fig. 5. The family of β helices. A single turn of each type of helix is shown. The four residue conformations which produce the various helices are shown in the center of the diagram. (Reproduced from [14] with permission of the publisher)

tures, maximally hydrogen bonded, with distinct interior and exterior regions. This provides the potential for the generation of amphipathic structures by amino acid sequences having the appropriate pattern of hydrophobic and polar residues. Polar side chains in the interior of a β helix could supply ligands for ion coordination and form the selectivity filter [11] for an ion channel. Because of the very direct relationship between amino-acid sequence and β helical structure, it is likely that the three-dimensional structure of a β helical membrane protein could be predicted from a knowledge of its amino-acid sequence. Conversely, for a given β helix, amino-acid sequences can be chosen which should form the helix in a lipid bilayer environment. To test this possibility, a series of peptides having the sequence $(\text{Leu-Ser-Leu-Gly})_n$ were synthesized with the expectation that they would form β^{12}-helical ion channels [12, 15]. Experiments with these peptides in an artificial lipid membrane system showed that they do function as ion channels and that the properties of the channel are consistent with the β^{12}-helical model in which the inside of the channel is lined with serine hydroxyl groups. These studies also showed that four turns of β helix are of sufficient length to span the membrane interior, but that three turns are not. Such studies of synthetic, membrane-active peptides can be expected to increase our understanding of the interactions between polypeptides and membranes and perhaps to provide us with realistic models of the hydrophobic segments of integral membrane proteins.

A Beta Helical Model of Glycophorin

Examination of the amino-acid sequences of integral membrane proteins could provide examples of β-helical structure. The pattern of amino acids in the central (membrane spanning) segment of glycophorin suggests the β^{13}-helical model shown in Fig. 6. The portion of the sequence comprising residues 55–107 can be arranged into four turns of β^{13} helix with a hydrophobic exterior [12–14]. Charged residues are located only at the ends of the helix or in the interior where their solvent requirements can be satisfied by water molecules. The segment involved is bounded by regions rich in proline, so the length of the helix is

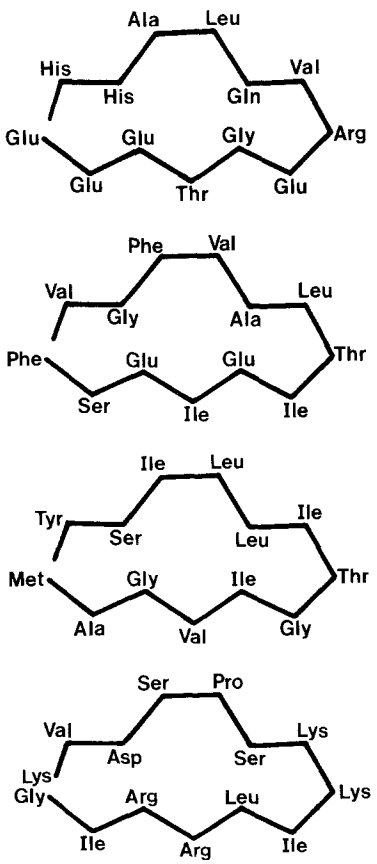

Fig. 6. The glycophorin β^{13} helix. Glu 55 is at upper left. Residues 55 to 107 generate a β helix long enough to span the membrane with a hydrophobic exterior. Charged residues appear only at the ends or in the interior of the helix. (Reproduced from [14] with permission of the publisher)

strictly defined. This length is one residue in excess of four turns, a length sufficient to span the membrane interior.

The size of the glycophorin helix and the negative charges in its interior strongly suggest that glycophorin could function as a cation channel. The low cationic permeability of the erythrocyte membrane, however, argues against this possibility *in vivo*. Tosteson *et al.* have demonstrated that purified glycophorin does form ion channels in artificial membranes and that the induced conductance is reduced by the addition of spectrin to the opposite side of the membrane [30]. Spectrin is a peripheral membrane protein that is thought to be associated with glycophorin at the inner surface of the membrane. It may be that in the red cell membrane under normal conditions, glycophorin is prevented from functioning as a channel by its association with spectrin. Whatever the real role of glycophorin, the β^{13} helix provides a stimulating conceptual model for the organization of an integral membrane protein.

A β-helical membrane protein can contain within its amino-acid sequence a segment of hydrophobic residues long enough to span the membrane as an α helix. In such a case the α and β-helical forms of the protein could exist in equilibrium with each other in the membrane. Because of the differing dipole moments and charge distributions of the two types of helix, such an equilibrium would, in general, be a function of the transmembrane potential. If the α and β-helical forms are viewed as the closed and open states of an ion channel, such an equilibrium provides a mechanistic model for the activation process in excitable membranes. Calculation of the voltage dependence of the equilibrium for a hypothetical amino-acid sequence similar to glycophorin gives an e-fold change in the equilibrium for a 6.8-mV change in potential [13]. Thus the voltage dependence of a model β-helical channel can be nearly as steep as that observed in squid axon, for instance. The sigmoidal kinetics of natural channels are also well described by a β-helical model. Because conformational changes in helical proteins occur by means of two consecutive processes (nucleation and elongation), the $\alpha \rightleftarrows \beta^{13}$ equilibrium provides a physical explanation of kinetic models in which the channel passes through a sequence of closed states before opening [2, 12, 13]. Thus, the lag in the rise of sodium or potassium conductance after a depolarization would reflect the time required for the formation of the β-helical nucleus. Furthermore, because relatively little charge movement occurs during the nucleation step, this model predicts a rising phase for the gating current at the beginning of a depolarizing voltage clamp pulse. Measured gating currents in squid axon display a rising phase [2].

The preceding discussion is intended to illustrate the potential of the family of β helices in the structure and organization of membrane proteins. Physical studies of integral membrane proteins may soon provide examples of β-helical structure. Studies of synthetic peptides in membrane systems provide a separate and extremely powerful technique to aid our understanding of the structure, function, and architecture of membrane proteins.

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