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Minireview

Specificity and promiscuity in membrane helix interactions

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Abstract

Transmembrane α-helices can associate with one another in lipid bilayers. This association is important in the folding and oligomerization of many integral membrane proteins, and may also play a role in their function. The interactions between helices may be highly specific or relatively non-specific, and their roles may differ accordingly. These two cases are discussed.

Key words: Membrane protein; Transmembrane α-helix; Protein–protein interaction; Oligomerization; Folding

1. Helix–helix interactions in membrane folding and oligomerization

The membrane-spanning portions of many integral membrane proteins consist of one or a number of transmembrane α-helices, which are expected to be independently stabilized by hydrophobic interactions with the bilayer and by main chain hydrogen bonds. Side-by-side interactions between these transmembrane α-helices are important in the folding and assembly of such integral membrane proteins and their complexes [1–4]. The establishment of independently stable transmembrane α-helices, and their self-association without rearrangement of secondary structure comprise two stages in a conceptual model for the thermodynamics of integral membrane protein folding [2]. This concept is supported by a body of experimental evidence [5–11], so an understanding of the factors involved in helix–helix interactions within lipid bilayers could yield useful biological insights.

2. Specificity vs. non-specific membrane helix interactions

In considering the contribution of helix–helix interactions to membrane protein folding and oligomerization, a distinction between stability and specificity should be recognized. A number of contributions to the energetics of transmembrane helix association within the lipid bilayer will be relatively non-specific, including those resulting from charge–charge interactions [12,13] and lipid-packing effects [14,15]. Specificity (and part of the energy) in transmembrane α-helix association, however, appears to rely mainly upon a detailed stereochemical fit between sets of dynamically accessible states of particular helices [16]. In some cases, these interactions are mediated in part by prosthetic groups.

Portions of the protein outside the lipid bilayer also contribute to the folding and oligomerization energies of integral membrane proteins, and have the capacity to define specificity. Extramembraneous contributions may include the effects of helix-connecting polypeptide loops, the ligation of extramembraneous domains to other molecules, as well as interactions between extramembraneous domains of adjacent proteins in an oligomer.

It seems reasonable to propose that interactions between transmembrane α-helices themselves contribute to the energetics of folding and oligomerization in different integral membrane proteins in a manner that is highly specific in some cases, but relatively non-specific in others. The contribution would be expected to be specific in the folding of polytopic integral membrane proteins in which most of the protein is buried within the membrane. In these cases, the simultaneous interaction of several transmembrane α-helices with prosthetic groups would also contribute to the specificity, as is seen in the structures of bacteriorhodopsin [17], bacterial photosynthetic reaction centers [18,19], and a plant light-harvesting complex [20]. There are also several clear cases in which interactions between single transmembrane α-helices are highly specific in the absence of prosthetic groups. Examples include dimerization of the single transmembrane helix of human glycophorin A [7,11,16] and association of the transmembrane domains of the MHC Class II α- and β-chains [21]. In these cases, a precise pattern of amino-acid side chains on one face of the helix is required for helix–helix interactions. In the case of glycophorin A dimerization, a sequence pattern in the transmembrane α-helix has been identified (LIxxGVxx-GVxxT) that is responsible for driving helix association

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[22]. This motif also drives the specific dimerization of other transmembrane α-helices into which it is introduced [22].

3. Biological roles for specific interactions

Membrane-spanning α-helices, rather than serving merely as featureless hydrophobic anchors, may thus be responsible in part for stabilizing the assembly of membrane protein complexes through helix–helix interactions, and may be employed in creating highly specific structures by self-sorting in a complex membrane. Close packing between complementary faces of the two helices could define specificity, as seen in Fig. 1. A pattern of glycine residues, similar to that seen in the motif identified in glycophorin A is also seen in the transmembrane helices of class II MHC [21], as well as other helices that oligomerize [23,24]. This pattern would permit the close approach of the helices, reminiscent of the occurrence of residues with small side chains in helix–helix interfaces in soluble proteins [25]. The sequence motif that drives dimerization of the glycophorin transmembrane α-helix is exquisitely sensitive to even very conservative substitutions [16]. It can be estimated that up to 10^7 such motifs could exist, and even if only a relatively small proportion of these could mediate helix association, the potential for a large repertoire of specific interactions between transmembrane α-helices is present. Similar specific interactions between transmembrane α-helices, which may employ such motifs, appear to be important in the assembly of several receptor complexes such as the T-cell antigen receptor [12,13,26], an Fcγ receptor [27], as well as MHC class II [21]. They may also play a role in other sorting events. In particular, the determinants for subcellular localization of several integral membrane proteins appear to be contained in their transmembrane domains. Many reports suggest that Golgi-localized membrane proteins are retained in this compartment as a result of determinants in their putative transmembrane α-helices [28–30]. The mechanism of this retention may involve interactions between transmembrane α-helices [31], although the dependence upon specific sequences is not clear [32], and other proposals have been made [33,34]. In addition, a number of recent studies indicate that specific interactions between transmembrane helices may

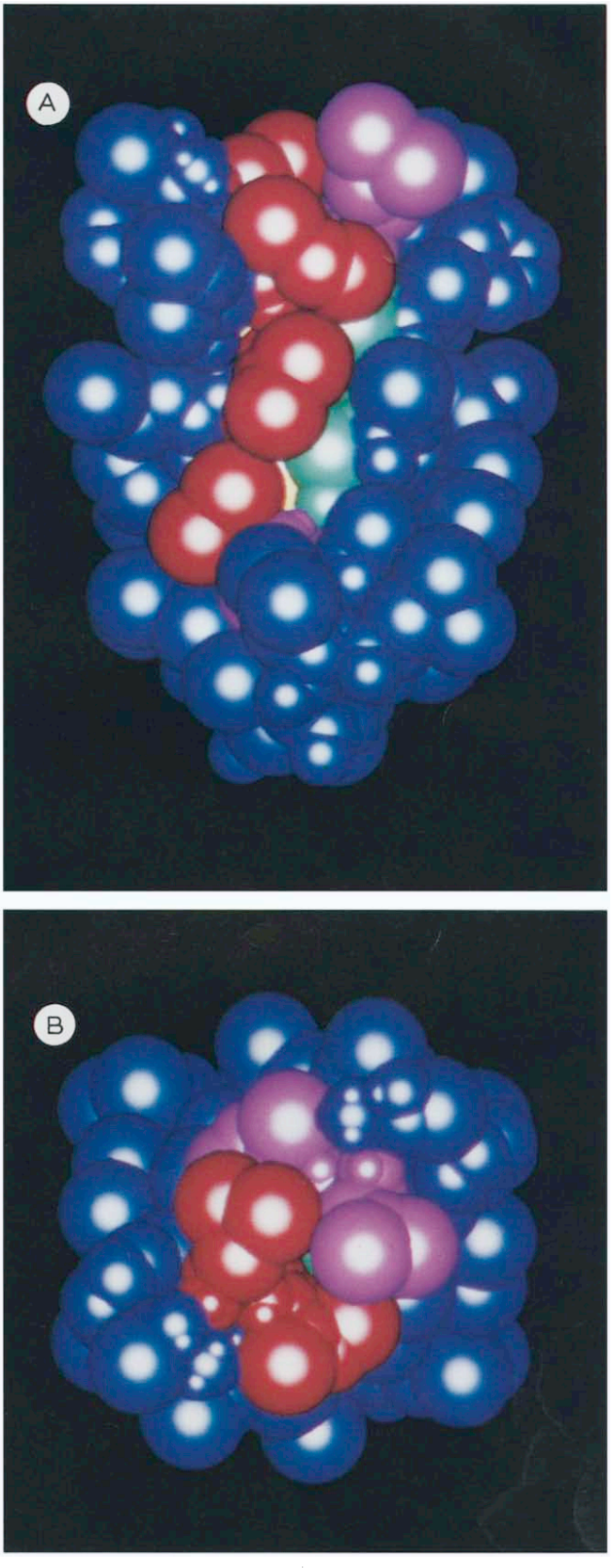

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Fig. 1. A model for the dimerization of the transmembrane α-helix of glycophorin A obtained from computational [22,40] and mutational studies [16]. The residues that comprise the LIxxGVxxGVxxT motif have been coloured differently from the rest of the helix. Residues that do not appear to be involved in helix-helix interactions are coloured blue. (A) A side-view of the model of the dimer, which is a parallel right-handed supercoil of α-helices, showing how the interfacial (LIxxGVxxGVxxT) residues are juxtaposed. (B) A view of the dimer model from the amino-terminal end of the helices, showing how aliphatic residues are proposed to pack with one another across the interface. Taken from [22], with permission.

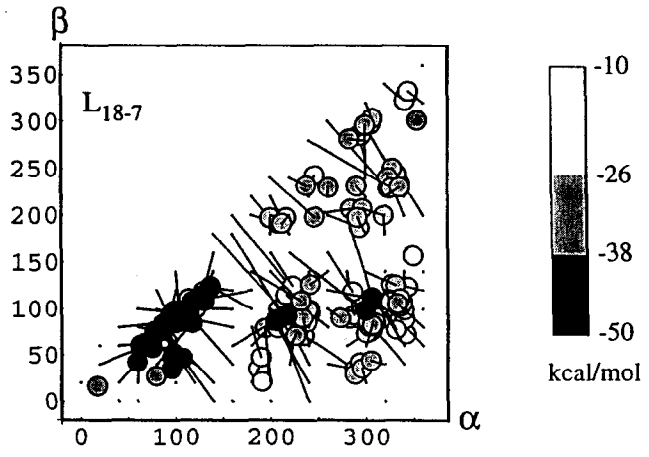

Fig. 2. A representation of the search for low interaction-energy dimeric conformations of polyleucine that contains the sequence motif LIxxGVxxGVxxT [22]. A number of starting structures (represented by small dots) were generated with different relative orientations (defined by α- and β-) of the two (parallel) helices in a right-handed supercoil dimer. During simulated annealing the configuration of a particular starting structure changes such that α and β are altered. This change is represented by a line connecting a small dot and a large circle. The large circle represents the configuration of the final structure and its shading the energy of that structure (dark circles represent the most stable structures). Details of the modeling procedures are given in [22,40]. Around α = 90°, β = 90°, a cluster of low-energy final structures is observed. This arrangement is in agreement with mutational analysis [16,22], and corresponds to the model presented in Fig. 1. In addition, other less well defined energy minima are seen at (α = 200°, β = 90°) and (α = 300°, β = 90°). These represent local energy minima. We suggest that isolated helices would be likely to adopt the global energy minimum (α = 90°, β = 90°) structure represented in Fig. 1. However, if the relationship between the helices were constrained by extramembraneous structures, this may be disfavoured. If the extramembraneous structures constrained the relationship between the two transmembrane α-helices such that β was around 90° and α was between 270° and 340°, for example, the helices would be likely to fall into the local energy minimum represented by (α = 300°, β = 90°). If a receptor molecule is considered, in the unliganded state the extramembraneous structure could constrain the helices such that α = 270° and β = 90°. The (α = 300°, β = 90°) local energy minimum would thus be favoured. If ligand binding induces a transition in the receptor's extramembraneous regions such that the transmembrane helices are constrained to around α = 240° and β = 90°, a different local energy minimum for membrane helix association would be favoured, that at α = 200° and β = 90°. In principle, therefore, it could be argued that a transition between modes (or local energy minima) of transmembrane helix association could be induced by ligand binding to a receptor. This could play an important part in transmembrane signalling. Reproduced from [22] with permission.

be exploited in mechanisms involved in the sorting of membrane proteins to the nuclear envelope [35,36].

4. Relatively non-specific membrane helix interactions

By contrast with these cases, the contribution of helix–helix interactions might be relatively non-specific in the folding and oligomerization of membrane proteins with large extramembraneous domains that are themselves involved in significant interactions. These extramembraneous interactions could constrain the spatial relationship between the ends of transmembrane α-helices, which may then interact with one another under this constraint via side-chain packing, lipid-packing effects and charge–charge interactions. Transmembrane α-helices have a tendency to pack with one another better than with lipid molecules. This has been treated theoretically [14,15], and the packing of the helices can be considered in a manner analogous to the packing of 'ridges' into 'grooves' [25] or 'knobs' into 'holes' [37] that has been applied to the packing of α-helices in water-soluble proteins. These packing modes are expected to result in stronger van der Waal's interaction between α-helices than between helices and lipid molecules for a wide range of helix amino-acid sequences. Moreover, certain rotational relationships of the helices will be favoured compared to others, and the mutual rotations will be well- defined, as local energy minima. But, these general interactions are not likely, on their own, to be sufficient to drive the oligomerization of helices, else general aggregation in membranes would ensue. If the ends of transmembrane α-helices are constrained to be close in space by an extramembraneous linkage, however, then their weak side-by-side interactions may be strong enough to stabilize a specific packing. Thus, in cases where the extra-membraneous linkage is large enough to adopt a specific structure, such as in the case of the dimer of the periplasmic domain of the bacterial aspartate chemoreceptor (Tar) [38], the orientational relationship of the transmembrane α-helices may be determined by this structure. The interfaces between the transmembrane α-helices themselves, such as those of the Tar receptor dimer that have been studied using disulfide cross-linking techniques [39], may therefore reflect relatively non-specific interactions. A similar situation may exist in polytopic integral membrane proteins, where the helix-connecting loops and chromophore molecules may define much of the specificity; and also in oligomers of bitopic membrane proteins in which there are extensive interactions between extramembraneous portions of the molecules. In each of these cases, the particular arrangement of the transmembrane α-helices would be defined outside the lipid bilayer, defining the relative rotations of the interacting helices as a local packing minimum. Molecular modeling studies of dimerization of the glycophorin A transmembrane domain [22,40] lend support to the suggestion that a variety of local energy minima, corresponding to different orientational relationships of the α-helices, can occur (Fig. 2).

5. A role for promiscuity

In cell-surface receptors, the constraint on helix-helix

interactions that results from extramembraneous structures may be created or altered upon ligand binding. Crystallographic studies of the bacterial aspartate receptor, for example, show an alteration in the relationship between the extracellular domains of the receptor upon binding of aspartate [38,41]. Such an alteration may result in a change to the array of local energy minima that are available for transmembrane helix interactions. Thus, the transmembrane helices may undergo a transition from one local energy minimum (in the unliganded state) to another (in the liganded state), resulting in an altered mode of helix association. The relationship between the intracellular domains of the receptor could be altered as a consequence of this transition, and this could be a mechanism of transmembrane signaling by such receptors. Thus, in such cases where there are significant extramembraneous influences, transmembrane helix association may be promiscuous.

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