Hypothesis

Rendezvous in a membrane: close packing, hydrogen bonding, and the formation of transmembrane helix oligomers

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Abstract The interaction of transmembrane α-helices is promoted by a detailed fit between two helical surfaces, which results in close packing and van der Waals interactions of amino acid side chains between two helices. Recent studies additionally indicate an important role of hydrogen bonding for mediating and stabilizing transmembrane helix–helix interactions. The interplay between close packing and electrostatic interactions in influencing the specificity of helix–helix interactions on the one hand and the stability of an existing interaction on the other hand is still unknown. Here, we suggest that close packing mainly determines the specificity of a helix–helix interaction, whereas hydrogen bonding is important for stabilization of a preformed helix dimer.

Keywords: Helix interaction; Hydrogen bond; Membrane protein; Packing; Polar residue; Two-stage model

1. Rendzvous: forces that drive interactions of transmembrane helices

Folding and interaction of α-helical membrane proteins are still poorly understood, and more work is needed to develop an understanding of transmembrane helix interactions. Transmembrane α-helices form stable and rigid structures which can promote specific interactions within membranes. The hydrophobic effect facilitates the incorporation of an individual helix into a membrane and drives the formation of stable α-helices [1]: unfolding a 20 amino acid long helix in a membrane and disrupting all hydrogen bonds within a helix would cost around 80 kcal/mol [2]. This explains why an unstructured polypeptide chain is not stable in a membrane. But why do single helices interact in a membrane and how do individual helices find the right partner?

In vivo, proteins get incorporated into the membrane by membrane integral translocase complexes (for a recent review, see [3]) and some interactions between individual transmembrane helices most likely already occur either within the translocon or in the vicinity of the translocon. Although this in vivo process is quite complicated and involves many proteins, experiments with bacteriorhodopsin have shown that the folding of an α-helical membrane protein can conceptionally be reduced to two steps: first single transmembrane helices incorporate separately into a membrane, then individual helices interact and form higher order oligomeric structures [4]. This two-stage model has been supported by experiments with other α-helical membrane proteins. In many cases, a functional membrane protein can be regained after reconstitution from fragments of one membrane protein (examples are summarized in [5]). But what drives the association of transmembrane helices in a lipid bilayer?

Without considering the possible influences of extramembrane loops, or cofactor binding on membrane protein folding, at least three types of interactions must be considered when thinking about transmembrane helix–helix association [6]: protein–lipid interactions, protein–protein interactions, and lipid–lipid interactions are all involved in the process of forming an oligomeric helix bundle. It has been estimated that about 20 lipids are associated with a single transmembrane helix in a membrane. Formation of higher order helix structures requires the release of these lipids into the lipid-pool, which is coupled with an increase in entropy [7]. While just the formation of higher ordered-helix oligomers is not entropically favoured, at the same time the entropy of the lipids increases if the lipid–protein interface is kept as small as possible [8]. This increase of entropy could be one of the main driving forces for clustering of transmembrane helices in a lipid bilayer. Increased lipid entropy does not just result in unspecific clustering of transmembrane helices, but specific helix–helix interactions are favoured and individual helices can form tightly packed structures. As mentioned above, in addition to lipid effects other factors can also be involved in bringing two helices into a close distance, like the contribution of loops between helices, which bring two helices into close proximity by constraining their location. Moreover, cofactor binding could also provide a driving force for helix–helix interactions.

In recent years, the interaction of single transmembrane helices was extensively studied and the findings suggest that the helices pack in a way similar to soluble coiled coils: side chains from one helix (knobs) fit into holes on the other helix resulting in a coiled-coil like dense packing [9]. This packing allows the close contact of two helices, which is stabilized by van der Waals interactions between different amino acid side chains (Fig. 1). As a result, one can conclude that the association of two transmembrane helices is due to van der Waals interactions arising from poor packing of lipids next to a helix.

In addition to van der Waals interactions, stronger polar attractions are also considered to be involved in helix–helix

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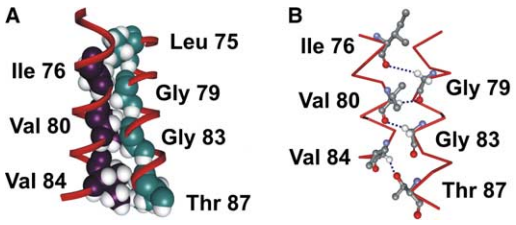

Fig. 1. Structure of the glycophorin A helix dimer determined using NMR methods [27]. The amino acids residues comprising the contact surface are indicated. In (A), the side chains involved in mediating/stabilizing the helix–helix interaction are shown as space-filling models. The two helices mesh in a ridge-into-groove arrangement with the ridge being formed by the valines and isoleucines and the groove by glycines and the threonine. In (B), a possible hydrogen network in the glycophorin A structure is indicated as suggested in [33]. The close distance between the β-hydroxyl group of Thr87 and the backbone carbonyl of Val84 on the opposing helix was shown by NMR methods and it was argued that these two groups form a hydrogen-bond [41]. Structures are constructed using the PBD file 1AFO.

interactions (Fig. 1). Early observations with bacteriorhodopsin have suggested that membrane proteins are ''inside-out'' soluble proteins, where the hydrophobic residues are exposed to the lipid bilayer while the core of the protein is more polar [10]. In this case, the folding of a membrane helix bundle would be driven by the positioning of polar residues in the core of a transmembrane helix bundle. Further studies have shown that many transmembrane proteins do not have a polar core and that the hydrophilic moment of the transmembrane helices does not generally point towards the centre of mass of a protein [11]. In contrast, the hydrophilic moment of a transmembrane helix frequently points between helix pairs rather than towards the centre of mass of a helix bundle [12].

If strong polar residues occur in transmembrane helices, the electrostatic attraction between polar side chains can be quite high in the membrane environment. It was calculated that if two charged groups are separated by 16 Å in a membrane, they would still have an interaction energy of about 10 kcal/mol [13]. Though electrostatic attraction between polar but uncharged residues will be lower, it still will result in strong interactions. In the context of model transmembrane helices, single polar residues were shown to drive the formation of helix oligomers in micelles as well as in a biological membrane [14–17]. The introduction of a single asparagine residue into a model transmembrane helix resulted in the formation of very stable transmembrane helix dimers or trimers. Interaction between the single polar side chains was considered to be the driving force for helix association, with the packing of the residual, hydrophobic side chains playing a less important energetic role [15]. One question these results bring up is why the presence of polar residues in a transmembrane helix does not generally result in unspecific aggregation of helices in a biological membrane?

2. Specificity vs. stability!?

Computational analysis of transmembrane domains and membrane protein structures has shown that though strong polar residues are rarely found in membrane proteins, they are frequently involved in hydrogen bonding between transmembrane helices [18].

Experiments with model transmembrane helices containing single polar residues indicate that polar attraction is sufficient to drive oligomerization of helices. Though polar residues can drive the formation of strong helix oligomers, they create the danger of non-specific aggregation and misfolding. In support of this, mutations causing the exchange of a non-polar to polar residues in membrane proteins often cause diseases [19,20]. In addition, it has been shown that aggregation of membrane proteins is quite common in vivo and it seems likely that non-specific attraction of polar side chains is involved in membrane protein misfolding [21,22]. Despite the fact that strong polar residues provide a strong free energy of association by forming hydrogen bonds in a hydrophobic environment with a low dielectric constant, it is more likely that these residues provide stability rather than oligomerization specificity.

So how is the strong polar attraction in the membrane coupled to the specificity of interaction? Investigations on the transmembrane domain of the receptor tyrosine kinase ErbB2 have indicated that in transmembrane helices the residues surrounding a polar residue highly influence association of the helices [23,24]. A mutation that causes the replacement of a valine with glutamic acid activates the receptor only in the presence of a GxxxG-motif. This motif of two glycine residues was shown to mediate helix–helix interactions by allowing a close contact between two adjacent helices [25–27]. Another study, using the M13 major coat protein as a model, also suggested that a sequence context can strongly modulate association of polar residues in a membrane [28]. To form a specific hydrogen bond, the residues involved have to be in close contact, and the sequence context of a helix can mediate or prohibit close packing thereby enhancing or interfering with the interaction of polar residues.

Statistical analysis of membrane protein structures has shown that almost all helices are involved in hydrogen bonding [18] and polar and ionizable residues constitute more than 20% of all residues in transmembrane domains [26,29]. Nevertheless, serine and threonine are the most common polar residues in transmembrane helices, they are highly involved in all identified interhelical hydrogen bonds, and have a high propensity for occurring in helical interfaces [12]. The advantage of recruiting these less polar residues to transmembrane domains is that the danger of non-specific aggregation is lowered and their amino acid side chains are small, allowing close contact of two helices. Alanine and glycine also occur frequently in helical interfaces [12], and statistical and experimental studies have shown that motifs of two small residues often mediate interaction of transmembrane helices [25,26,30,31]. While all small residues allow two helices to come into close contact, the side chains of serine and threonine can also form hydrogen bonds [32]. Recent analyses also indicate that weaker electrostatic interactions can contribute to the stabilization of a transmembrane helix dimer. Structural analysis of membrane proteins as well as experimental work has revealed that Cα-hydrogen bonds can be involved in stabilizing helix–helix interactions [33,34]; though in general interactions to backbone atoms are rare in membrane proteins [18].

So if the context of a transmembrane helix is important for the formation of a hydrogen bond, what is then the interplay between close packing and hydrogen bonding during the formation of transmembrane helix oligomers?

3. Engagement: close packing mediates specific interactions

The observation that strongly polar residues tend to mediate formation of oligomeric structures in a membrane indicates that simply the formation of a possible hydrogen bond or a salt bridge cannot explain the specificity of a given interaction. Without specificity, polar residues would interact with the next closest potential binding partner. As a consequence, polar residues could form a very strong hydrogen bond resulting in an irrevocable association. Although an existing hydrogen bond has a direction and therefore does provide specificity, the attraction between two polar residues, which are not yet in a close enough contact and in the right orientation to each other to form a hydrogen bond, is unspecific, and the directionality of an existing hydrogen bond cannot be used to explain specificity during the formation of a helix oligomer. Also, many polar residues are not directly involved in helix–helix interactions but bind cofactors or are essential for enzymatic activity. These residues must be positioned in a way so that they are not involved in helix–helix contacts. Electrostatic interactions could bring two helices into a close proximity first and then close packing could "lock" the transmembrane protein into the native conformation. But how would this explain that specific electrostatic interactions take place between two helices? Electrostatic interactions would occur between any transmembrane helices, which contain polar residues, without specificity. These considerations make it more likely that other factors besides electrostatic interactions are important for determining the specificity of a given helix–helix interaction. One factor defining specificity of helix–helix interactions is the surface geometry of an α-helix as the surface of one helix is designed to fit ideally into the surface of the second helix [35], resulting in a close knob-into-hole or ridge-into-groove packing of two helices [9]. If two helices do not show complementary surfaces, a close contact of two helices could also be mediated by the extramembrane regions of the proteins [30,36]: specific interactions in the soluble domains could bring two helices in a very close contact. After bringing two helices in close contact, additional electrostatic interactions, like hydrogen bonds, can form.

4. Wedding: electrostatic interactions stabilize a preformed dimer

Besides binding of cofactors and specific lipid interactions, analysis of the glycophorin A transmembrane helix dimerization has indicated that the dominant contribution towards dimerization is the way that transmembrane helices fit together, guided by van der Waals interactions and side-chain rotamers [5,37]. As we have already seen, the formation of hydrogen bonds most likely does not directly mediate specific interactions. It is more likely that after a specific interaction is formed by matching helical surfaces, hydrogen bonds form and stabilize a preformed dimer. Close packing allows the polar residues to come into a close enough contact to form a stable hydrogen bond. This idea is supported by the observation that helical pairs with hydrogen bonds are packed tighter and have more atomic contacts than non-polar helix–helix interactions [38]. Also, a loss in close packing interactions can be compensated by the introduction of a new hydrogen bond, which stabilizes the helix–helix interaction in the context of the surrounding residues [39]. While single serine residues were found not to mediate or stabilize a helix–helix interaction alone in a model transmembrane helix [16], a single serine residue was found to be capable of supporting dimerization of a helix if other factors promote formation of a helix dimer [39].

In addition to the formation of hydrogen bonds between two amino acids, binding of a cofactor could also account for stabilization of a given helix dimer. In the case of bacteriorhodopsin, it was shown that loss of the cofactor destabilizes the helix bundle by around 60 kcal/mol [40]. Thus, cofactor binding can contribute significantly to the stabilization of transmembrane helix oligomers.

The interplay between close packing and electrostatic interactions during the formation of a helix dimer is summarized in Fig. 2.

5. Additional considerations

It should be noted that the stability of helix–helix interactions can be explained solely by considering effects on packing [37]. Since in some cases no (potential) hydrogen bonds between two helices are found, hydrogen bonds may play a role in the structure and function of specific proteins. For example, in cases where too many hydrogen bonds between adjacent helices occur, interactions could be locked in a certain conformational state resulting in a very stable protein, while less hydrogen bonds would allow more movements of the helices and conformational changes in the protein. Such re-organization of a helix–helix interaction could be important for transferring signals across biological membranes [35]. If such a re-organization of a helix pair results in a reduction of the contact area between two helices and subsequently in a loss of stabilizing van der Waals interactions, a strong hydrogen bond between two helices could serve as a basis for structural changes. Breaking and reforming of a hydrogen bond could also be involved in conformational changes, and since several

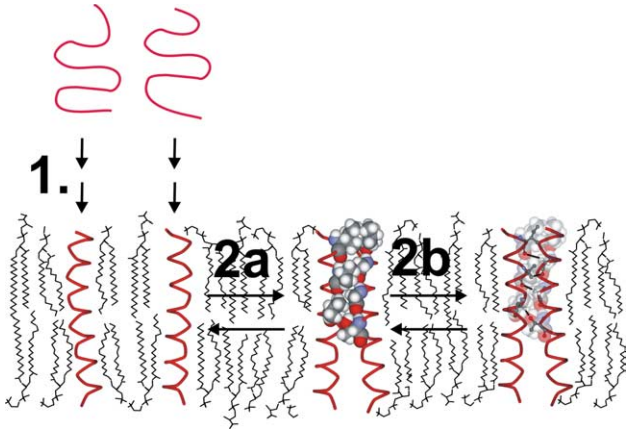

Fig. 2. Interactions that determine specificity and stability of transmembrane helix oligomers. After incorporation of individual helices into the lipid bilayer (stage 1), specific interactions between helices are mainly mediated by a perfect match between the surfaces of two transmembrane α-helices (stage 2a). After two helices have come into a close contact, hydrogen bonds can stabilize the helix dimer in addition to van der Waals forces (stage 2b).

polar residues – like asparagine, aspartate, glutamine, and glutamate – can form strong hydrogen bonds, there is no need for dense packing [14]. A single, strong hydrogen bond could allow more flexibility along the length of the helices than would be allowed in the case of closely packed helices.

It is obvious that these two forces, packing and electrostatic attraction, cannot be separated in vivo, but work in concert to determine stability and specificity of helix–helix interactions. Separating electrostatic forces from packing is just a way to simplify the model of how helix dimers and higher order structures form and to determine the roles of each type of force in this process.

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