FEBS Letters 555 (2003) 134–138 FEBS 27772

Minireview

Synthetic peptides as models for intrinsic membrane proteins

J. Antoinette Killian*

Department of Biochemistry of Membranes, Center for Biomembranes and Lipid Enzymology, Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Received 25 August 2003; accepted 1 September 2003

First published online 15 October 2003

Edited by Gunnar von Heijne, Jan Rydström and Peter Brzezinski

Abstract There are many ways in which lipids can modulate the activity of membrane proteins. Simply a change in hydrophobic thickness of the lipid bilayer, for example, already can have various consequences for membrane protein organization and hence for activity. By using synthetic transmembrane peptides, it could be established that these consequences include peptide oligomerization, tilt of transmembrane segments, and reorientation of side chains, depending on the specific properties of the peptides and lipids used. The results illustrate the potential of the use of synthetic model peptides to establish general principles that govern interactions between membrane proteins and surrounding lipids.

Key words: Transmembrane peptide; α-Helix; Hydrophobic mismatch; Interfacial anchoring; Snorkeling; Tryptophan

1. Introduction

Most membrane proteins have one or more hydrophobic segments that span the membrane in an α-helical configuration. The proteins can interact with surrounding lipids in the membrane via a range of interactions, including hydrophobic interactions with the hydrophobic membrane interior and electrostatic interactions, hydrogen bonding and dipolar interactions in the lipid/water interfacial region. Not surprisingly, the activity of many membrane proteins has been shown to depend on the lipid environment and/or to require specific lipids (reviewed in ref. [1]). To understand how lipids modulate protein activity, knowledge is required of how lipids can influence structural properties of membrane proteins. This minireview will focus on the influence of bilayer thickness on membrane protein structure and organization and the insights that have been obtained by using synthetic peptides as models for membrane proteins.

Effects of variation of bilayer thickness have been observed on the functional activity of various membrane proteins upon reconstitution in lipid bilayers (reviewed in ref. [2] and have been attributed to a mismatch between the hydrophobic length of the membrane spanning segments of the protein and the hydrophobic thickness of the bilayer. How can a hydrophobic mismatch influence properties of membrane proteins? When the transmembrane segments are too long to span the hydrophobic bilayer thickness (positive mismatch), hydrophobic side chains may stick out and thus become exposed to a polar environment, which will be energetically unfavorable. To reduce this effective mismatch, the system may respond in several ways, as illustrated in Fig. 1. The proteins may adapt (i) by forming oligomers, thus shielding the exposed groups from the more polar environment, (ii) by changing their backbone conformation, (iii) by tilting away from the bilayer normal, thereby reducing their effective length, or (iv) by changing the orientation of the side chains near the lipid/water interface region. Similar responses, except tilting, may occur in the case of a negative mismatch, when the transmembrane segments are relatively short. Also lipids may adapt to reduce the effective mismatch, either by stretching or disordering their acyl chains, or by adapting their macroscopic organization. Finally, in mixtures of lipids, the proteins might preferentially surround themselves by best-matching lipids, which might lead to biologically important processes such as sorting.

Although the situation of hydrophobic mismatch clearly is unfavorable, also the various mismatch responses may be energetically costly. To gain insight into the energy costs of individual mismatch responses, one should investigate under what conditions they occur, and to what extent they occur. This can only be accomplished by using systematic approaches. Synthetic model peptides are ideally suited for this type of research, because they allow systematic variation of both the hydrophobic length and the composition of membrane spanning segments. Typical peptide families that have been used consist of a hydrophobic region of polyLeu or alternating Leu and Ala with variable length (reviewed in ref. [3]. Most peptides are either flanked with lysine residues to inhibit peptide aggregation and to better secure a stable transmembrane orientation [4–7], or with Trp, as a residue that frequently flanks transmembrane domains in intrinsic membrane proteins [8,9]. Examples of these peptide families are Trp-flanked WALP peptides and Lys-flanked KALP peptides (Fig. 2). The results obtained with these peptides illustrate both the huge variety in mismatch responses and the important role of flanking residues.

Most of the mismatch responses have been investigated in single-lipid systems. More complex mixtures of lipids are required only to investigate preferential interactions between peptides and lipids, for example to determine whether and

*Fax: (31)-30-253 39 69.
E-mail address: j.a.kilian@chem.uu.nl (J.A. Killian).

Abbreviations: *P/L*, peptide/lipid molar ratio

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)01154-2

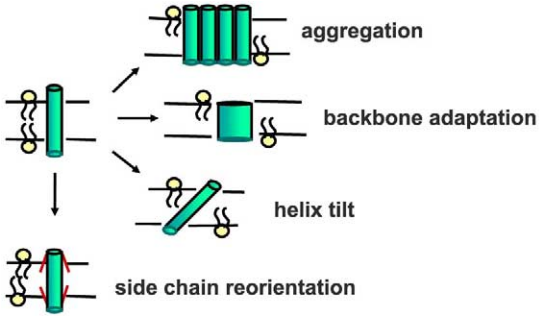

Fig. 1. Schematic illustration of various mismatch responses that can occur under conditions of positive mismatch. For details see the text.

to what extent molecular sorting occurs. It is important here to realize that in biological membranes sorting is not per se the energetically most favorable response to mismatch, because, just like other mismatch responses, it has an energy cost to it, in this case related to a loss of entropy.

This review will be focussed on the various mismatch responses that have been studied for model peptides under conditions of positive and negative mismatch in single-lipid systems.

2. Consequences of positive mismatch for protein and lipid organization

2.1. Aggregation

When the hydrophobic length of a peptide exceeds the bilayer thickness, this may result in a tendency of the peptides to aggregate. This may be expressed as an increase in peptide–peptide interactions upon increasing the extent of mismatch. Such a response indeed has been observed for Lys-flanked peptides by fluorescence measurements [6,10]. Upon increasing the peptide/lipid molar ratio (*P*/*L*) and/or the extent of mismatch, the tendency to aggregate may ultimately lead to the formation of macroscopic aggregates. For WALP peptides it was shown that under such conditions peptide-enriched aggregates are formed, that can be separated from a peptide-depleted bilayer by sucrose density gradient centrifugation [8]. In line with these results, Fourier transform infrared spectroscopy (FTIR) measurements [9] indicated that the amount of peptide that can be stably incorporated in a lipid bilayer depends on the extent of mismatch: the larger the mismatch, the less peptide can be incorporated. Interestingly, it was found that KALP peptides can more easily be incorporated in lipid bilayers at a large extent of positive mismatch than WALP peptides [11], providing a first indication that the flanking residues may play an important role in determining consequences of mismatch.

2.2. Backbone conformation

Theoretically also the peptide backbone could adapt to a positive mismatch, by decreasing its pitch and forming, e.g., a π-helix. However, this does not seem to be a favorable response, because WALP peptides show a stable α-helical conformation that is independent of the extent of mismatch, as observed by FTIR measurements [9] and by solid state ^{2}H nuclear magnetic resonance (NMR) experiments [12]. Even under conditions of a considerable positive mismatch, when only a small amount of peptide can be stably incorporated, FTIR measurements show that the peaks in the amide I region remain at the same position and do not change their lineshape, demonstrating that the peptides indeed form very stable α-helices [9].

2.3. Tilt

By tilting away from the bilayer normal peptides could reduce their effective length, thereby compensating for a positive mismatch. Such tilting can indeed occur, as was observed by using solid state ^{15}N NMR for Lys-flanked peptides [13]. Although in these experiments the absolute values of the tilt angle could not be quantified, the data indicate that the increase in tilt angle with increasing mismatch is rather small and cannot be sufficient to compensate for the mismatch. Interestingly, it is possible that also the extent of tilting may be dependent on the flanking residues, because FTIR and solid state ^{2}H NMR experiments indicated no or only a small tilt for WALP peptides [9,12], even under conditions of considerable mismatch [9]. Clearly, there must be an energy cost of tilting, which may be due to a disturbance of the packing of lipids adjacent to a tilted helix, and/or to an unfavorable orientation of the side chains of a tilted helix near the lipid/water interface.

2.4. Side chain reorientation and interaction of flanking residues with interfacial region

Also a change in the orientation of side chains near the lipid/water interface may affect the effective hydrophobic length of a peptide. For example, positioning of the backbone Cα of a Leu in the more polar region, while pointing the side chain towards the membrane interior, may result in a decrease of the effective hydrophobic length. For Lys, with its aliphatic chain and positively charged end, it is difficult to visualize how in the case of positive mismatch reorientation of this side chain could result in a decrease of the hydrophobic length, and this is considered unlikely. In contrast to Leu and Lys, Trp is relatively rigid. However, it is also rather large, and therefore the precise orientation of this side chain, in particular the position of the indole NH, can be expected to significantly influence the effective peptide length.

Additional 'mismatch' parameters could result from preferential interactions of specific side chains in the membrane/water interface region. The effective length of the peptide would then also be dependent on the exact position at the

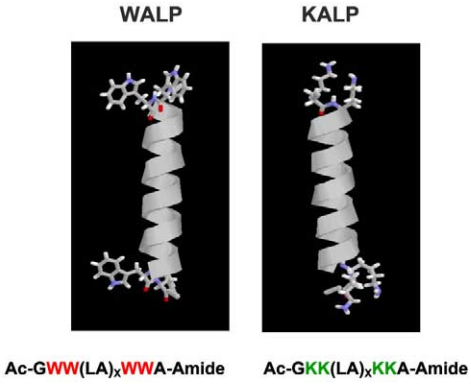

Fig. 2. General structure of WALP peptides and KALP peptides.

interface where this preferred 'anchoring' interaction takes place, and the energy cost of mismatch would be dependent on the strength of this interaction. Trp has been shown to have a preferred interaction with the interfacial region [14–16]. This results in a resistance of Trp against displacement from the interface towards the aqueous phase upon inducing a positive mismatch, as shown for WALP peptides by mass spectrometry in combination with ^{1}H–^{2}H exchange [17] and by ^{2}H NMR on the lipids [18]. Using WALP analogs with Trp at different positions in the peptide, it was shown that interfacial anchoring interactions can even dominate over hydrophobic mismatch effects in determining mismatch responses [11]. In contrast, Lys does not have a specific preference for the interface [16] and indeed does not appear to resist displacement from the interface towards the aqueous phase upon introducing a positive mismatch. Mass spectrometry measurements on relatively long KALP peptides suggested that a significant part of the hydrophobic transmembrane $(Leu\text{-}Ala)_n$ sequence is exposed to the polar region under conditions of positive mismatch, suggesting that the energy cost of hydrophobic mismatch, at least for $(Leu\text{-}Ala)_n$, is small as compared to that of the various mismatch responses [11].

2.5. Effects on lipids

Lipids could adapt to a positive mismatch by changing their effective length, as suggested by the so-called mattress model [19]. Indeed ^{2}H NMR experiments indicated a systematic but very small increase in chain order when WALP peptides with increasing hydrophobic length were incorporated in diacylphosphatidylcholine (PC) bilayers [18]. For KALP peptides, no significant stretching of the acyl chains was observed [11,22]. These results are in line with strong interfacial anchoring interactions of Trp, but not of Lys. Importantly, the increase in chain ordering observed with the WALP peptides was insufficient to compensate for the mismatch, implying that there is also a significant energy cost to acyl chain stretching.

3. Consequences of negative mismatch for protein and lipid organization

3.1. Aggregation

Like with positive mismatch, also under negative mismatch conditions, when the hydrophobic part of the peptide is small as compared to the bilayer thickness, lysine-flanked peptides have a tendency to oligomerize, as shown by fluorescence measurements [6,10], and the amount of peptide that can be stably incorporated in a lipid bilayer decreases with the extent of negative mismatch [9]. For WALP peptides fluorescence measurements [20] and visualization of striated domains by AFM [21] suggested the formation of linear peptide aggregates under conditions of negative mismatch.

Like with positive mismatch, at higher *P*/*L* and/or at increasing mismatch, the tendency to aggregate leads to the formation of macroscopic aggregates, and peptide-rich aggregates can be isolated. Under negative mismatch conditions these aggregates were found to consist of a well-defined non-lamellar phase: the inverted hexagonal (H_{II}) phase [8]. Formation of such non-lamellar phases can be a way to adapt to mismatch. This is explained in a model of the H_{II} phase [8] in which the WALP peptides span the distance between adjacent tubes, and are surrounded by lipids that, due to the geometry of the H_{II} phase, are much more disordered than in a bilayer and therefore have a reduced hydrophobic length. Also in this model the peptides form large linear aggregates.

Non-lamellar phases can be induced both by WALP and KALP peptides under conditions of negative mismatch whereby the type of non-lamellar phase is determined by the precise extent of negative mismatch [22]. An important implication of this observation is that effects of peptides on phase behavior at high *P*/*L* can be used as a tool to determine the effective length of peptides under negative mismatch conditions (see Section 3.3).

3.2. Backbone conformation

In principle, the peptide backbone could adapt to a negative mismatch by stretching to form a 3_{10} helix. However, as with positive mismatch, also under a range of negative mismatch conditions, a very stable α-helical conformation of backbone was observed for WALP peptides [9], further supporting the notion that backbone adaptation is an unfavorable mismatch response.

3.3. Side chain reorientation and interaction of flanking residues with interfacial region

Analogous to the situation of positive mismatch, reorientation of side chains near the lipid/water interface under negative mismatch conditions could result in an increase of the effective hydrophobic length of the peptide. The contribution of Trp and Lys to the effective peptide length could be investigated in detail by comparison of the effects of WALP and KALP peptides on lipid phase behavior in different lipid systems [9,22–24]. For WALP peptides, the effective length was found to be independent of the type of lipid used [24], indicating that the Trp residues do not change their orientation. However, for KALP peptides large lipid-dependent differences in the effective length were observed: in lipids, which by themselves have a tendency to form non-lamellar phases, the effective length was much smaller than in PC, which is a typical bilayer forming lipid [24]. This suggested that in PC, but not in the other lipids, upon inducing a negative mismatch, Lys increases the effective length of the transmembrane segment by snorkeling. This means that the backbone Cα is buried in the hydrophobic region of the bilayer and the side chain stretches out with its hydrophobic part still interacting with the hydrophobic region of the bilayer, but with its positively charged amino group extended outwards into the more polar interfacial region. In systems that by themselves have a tendency to form non-lamellar phases, it was found that the system reacts to a mismatch by promoting the formation of these non-lamellar phases, rather than by snorkeling. This behavior is illustrated in Fig. 3. This interpretation was supported by experiments with modified KALP peptides, containing Lys derivatives with a shortened side chain [23,24]. From these and related experiments, the energetic cost of snorkeling of Lys side chains was estimated and was found to be rather low, less than 0.7 kcal/mol [25].

Comparison of the effective lengths of WALP and KALP peptides in PC lipids furthermore suggested that upon decreasing the peptide length, Trp resists partioning with its indole NH group below the carbonyl region, while the positively charged amino group of Lys resists partioning below the level of the phosphate [22].

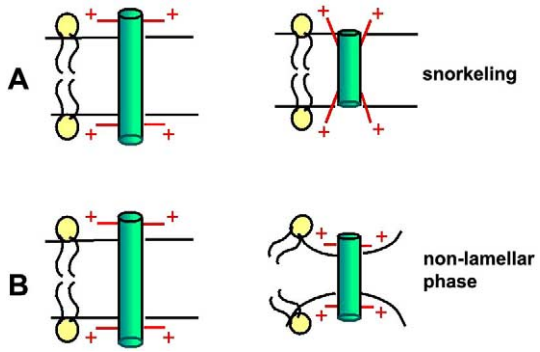

Fig. 3. Schematic illustration of the differences in the effective length of KALP peptides in bilayer lipids (A) and lipids that have a tendency to form non-lamellar phases (B). Under matching conditions, Lys is not snorkeling and the lipids are in a bilayer organization. Upon introducing a small negative mismatch, in system A the Lys side chain adapts by snorkeling, thereby increasing the effective length of the peptide, while the lipids remain in a bilayer organization. In system B the lipids react by forming a non-lamellar phase, thereby effectively decreasing their acyl chain length, and the Lys side chain does not snorkel.

3.4. Effects on lipids

^{2}H NMR experiments showed that WALP peptides cause a systematic, but only slight decrease in chain order under conditions of negative mismatch [18]. Since this response is small as compared to what would be required to fully compensate for a mismatch, this suggests a significant energy cost to disordering of the lipids, at least when present in a bilayer. Formation of non-lamellar phases, which can occur in PC lipids at high *P*/*L* [8,22], is an effective response to a negative hydrophobic mismatch and may be regarded as an ultimate lipid chain disordering effect.

4. Conclusions and perspectives

From the results discussed here, it is clear that even in single-lipid systems and using simple synthetic peptides, many different mismatch responses can be observed, each with its own energy cost. The final mismatch response, or combination of responses, will be determined by the balance of the energy costs of the individual responses and the energy cost of hydrophobic mismatch itself. This will obviously depend on many factors. Not only the extent of mismatch will contribute, but also the precise composition of the proteins, including their total hydrophobicity, the distribution of side chains along the helix axis, and the nature of their flanking residues, as well as the precise composition of the lipids, including the size and charge of their head groups and the length and degree of unsaturation of their acyl chains. In the studies discussed here, only the role of the flanking residues was investigated systematically, by comparison of the effects of WALP and KALP peptides. The results illustrate that the flanking residues play an important role in determining mismatch responses and that they do so in three different ways. First, the orientation of the side chains of the flanking residues can influence the effective hydrophobic length of the peptides. Second, also the preferred site of interaction of these side chains at the interface will influence the effective length of the peptides. Third, the strength of the interfacial anchoring interactions, or the energy cost of moving the side chain away from its preferrred position will influence the energy cost of mismatch and hence the strength of the mismatch response. Studies using peptides with other flanking residues suggested that there are subtle differences between the effects of the positively charged residues Lys, Arg and His (at low pH) on one hand, and between the effects of the aromatic Trp, Tyr and Phe on the other hand [23,24]. A number of studies have been carried out to investigate the importance of other parameters, such as hydrophobicity [26], but still more systematic studies are required to shed light on how all these different factors may influence mismatch responses.

The results discussed here have illustrated how studies on model peptides can provide detailed insight into the various mismatch responses. One may wonder how well synthetic peptides represent 'real' membrane proteins. The contribution of the transmembrane domains to the various mismatch responses as observed for the model peptides is likely to be similar to responses of single-span membrane proteins. However, synthetic single-span peptides may be less suitable as models for multi-span proteins. For example, in multi-span proteins the effects on lipids may be larger and molecular sorting may occur more easily [27], or tilting may be a more favorable response, because it may less disturb lipid packing. On the other hand, the lipid exposed parts will sense mismatch effects similar to single-span proteins and may cause responses like aggregation and reorientation of side chains near the interface, similar to those observed for the model peptides. One indication that anchoring residues behave very similar in synthetic peptides as in 'real' membrane proteins came from a comparison between model membrane studies as discussed in this review and studies on interfacial anchoring of proteins upon insertion into microsomal membranes [28].

Finally, it should be noted that systematic studies on model peptides also can be useful to investigate general principles of peptide/peptide and peptide/lipid interactions. An example is a recent study on the effects of transmembrane segments of proteins on lipid flop, in which WALP and KALP peptides were used as models [29]. Such studies will lead to a better understanding of the way in which proteins integrate in membranes, of how their behavior can be influenced by the surrounding lipids, and of how proteins can affect lipid organization and dynamics.

Acknowledgements: I thank the many PhD students, post-docs, and international collaborators who have contributed to the work described here, and I would like to thank Prof. Ben de Kruijff for helpful comments on the manuscript.

References

[1] Lee, A.G. (2003) Biochim. Biophys. Acta 1612, 1–40.
[2] Dumas, F., Lebrun, M.C. and Tocanne, J.F. (1999) FEBS Lett. 458, 271–277.
[3] De Planque, M.R.R. and Killian, J.A. (2003) Mol. Membr. Biol. 20, 271–284.
[4] Davis, J.H., Clare, D.M., Hodges, R.S. and Bloom, M. (1983) Biochemistry 22, 5298–5305.
[5] Webb, R.J., East, J.M., Sharma, R.P. and Lee, A.G. (1998) Biochemistry 37, 673–679.
[6] Ren, J., Lew, S., Wang, J. and London, E. (1999) Biochemistry 38, 3905–3912.
[7] Zhang, Y.P., Lewis, R.N., Hodges, R.S. and McElhaney, R.N. (1995) Biochemistry 34, 2362–2371.
[8] Killian, J.A., Salemink, I., de Planque, M.R.R., Lindblom, G., Koeppe II, R.E. and Greathouse, D.V. (1996) Biochemistry 35, 1037–1045.

[9] De Planque, M.R.R., Goormaghtigh, E., Greathouse, D.V., Koeppe II, R.E., Kruijtzer, J.A.W., Liskamp, R.M.J., De Kruijff, B. and Killian, J.A. (2001) Biochemistry 40, 5000–5010.
[10] Mall, S., Broadbridge, R., Sharma, R.P., East, J.M. and Lee, A.G. (2001) Biochemistry 40, 12379–12386.
[11] De Planque, M.M.R., Bonev, B.B., Demmers, J.A.A., Greathouse, D.V., Koeppe II, R.E., Separovic, F., Watts, A. and Killian, J.A. (2003) Biochemistry 42, 5341–5348.
[12] Van der Wel, P.C.A., Strandberg, E., Killian, J.A. and Koeppe II, R.E. (2002) Biophys. J. 83, 1479–1488.
[13] Harzer, U. and Bechinger, B. (2000) Biochemistry 39, 13106–13114.
[14] Yau, W.M., Wimley, W.C., Gawrisch, K. and White, S.H. (1998) Biochemistry 38, 14713–14718.
[15] Persson, S., Killian, J.A. and Lindblom, G. (1998) Biophys. J. 75, 1365–1371.
[16] White, S.H. and Wimley, W.C. (1999) Annu. Rev. Biophys. Biomol. Struct. 28, 319–365.
[17] Demmers, J.A.A., Van Duijn, E., Haverkamp, J., Greathouse, D.V., Koeppe II, R.E., Heck, A.J.R. and Killian, J.A. (2001) J. Biol. Chem. 276, 34501–34508.
[18] De Planque, M.R.R., Greathouse, D.V., Koeppe II, R.E., Schäfer, H., Marsh, D. and Killian, J.A. (1998) Biochemistry 37, 9333–9345.
[19] Mouritsen, O.G. and Bloom, M. (1984) Biophys. J. 46, 141–153.
[20] Bogen, S.T., De Korte-Kool, G., Lindblom, G. and Johansson, L.B.A. (1999) J. Phys. Chem. B 103, 8344–8352.
[21] Rinia, H.A., Kik, R.A., Demel, R.A., Snel, M.M.E., Killian, J.A., Van der Eerden, J.P.J.M. and De Kruijff, B. (2000) Biochemistry 39, 5852–5858.
[22] De Planque, M.R.R., Kruijtzer, J.A.W., Liskamp, R.M.J., Marsh, D., Greathouse, D.V., Koeppe II, R.E., De Kruijff, B. and Killian, J.A. (1999) J. Biol. Chem. 274, 20839–20846.
[23] De Planque, M.R.R., Boots, J-W.P., Rijkers, D.T.S., Liskamp, R.M.J., Greathouse, D.V. and Killian, J.A. (2002) Biochemistry 41, 8396–8404.
[24] Strandberg, E., Morein, S., Rijkers, D.T.S., Liskamp, R.M.J., Van der Wel, P.C.A. and Killian, J.A. (2002) Biochemistry 41, 7190–7198.
[25] Strandberg, E. and Killian, J.A. (2003) FEBS Lett. 544, 69–73.
[26] Lewis, R.N., Zhang, Y.P., Liu, F. and McElhaney, R.N. (2002) Bioelectrochemistry 56, 135–140.
[27] Sperotto, M.M. and Mouritsen, O.G. (1991) Biophys. J. 59, 261–270.
[28] Killian, J.A. and Von Heijne, G. (2000) TIBS 25, 429–434.
[29] Kol, M.A., De Kroon, A.I.P.M., Rijkers, D.T.S., Killian, J.A. and De Kruijff, B. (2001) Biochemistry 40, 10500–10506.