Membrane-Anchoring Interactions of M13 Major Coat Protein†

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ABSTRACT: The response to hydrophobic mismatch of membrane-bound M13 major coat protein is measured using site-directed fluorescence and ESR spectroscopy. For this purpose, we investigate the membrane-anchoring interactions of M13 coat protein in model systems consisting of phosphatidylcholine bilayers that vary in hydrophobic thickness. Mutant coat proteins are prepared with an AEDANS-labeled single cysteine residue in the hinge region of the protein or at the C-terminal side of the transmembrane helix. In addition, the fluorescence of the tryptophan residue is studied as a monitor for the N-terminal side of the transmembrane helix. The fluorescence results show that the hinge region and C-terminal side of the transmembrane helix hardly respond to hydrophobic mismatch. In contrast, the N-terminal side of the helical transmembrane domain shifts to a more apolar environment, when the hydrophobic thickness is increased. The apparent strong membrane-anchoring interactions of the C-terminus are confirmed using a mutant that contains a longer transmembrane domain. As a result of this mutation, the tryptophan residue at the N-terminal side of the helical domain clearly shifts to a more polar environment, whereas the labeled position 46 at the C-terminal side is not affected. The phenylalanines in the C-terminal part of the protein play an important role in these apparent strong anchoring interactions. This is demonstrated with a mutant in which both phenylalanines are replaced by alanine residues. The phenylalanine residues in the C-terminus affect the location in the membrane of the entire transmembrane domain of the protein.

The membrane-bound major coat protein of M13 bacteriophage is an excellent model system to study fundamental aspects of protein−lipid interactions. These interactions are important for the formation of the correct membrane assembly of not only the M13 major coat protein but also membrane proteins in general. A main determinant in the formation of the correct membrane assembly will be the anchoring interactions of the protein with the membrane. Anchoring can be accomplished by several factors. Important are the hydrophobic interactions, which will be mainly determined by the length and hydrophobicity of the transmembrane helix. Then there are the amino acid residues tryptophan and tyrosine, which are often found at the membrane−water interface, and are thought to play a role in the interactions of a protein with the polar headgroup region of the membrane. A comparable function can be ascribed to the presence of charged amino acid residues in the interface [reviewed in Killian and von Heijne (*1*)].

In recent years, several chemosynthetic peptide analogues were produced to gain insight in the function of specific amino acids for membrane-anchoring interactions with model membranes. In these experiments, a mismatch is induced between the length of the hydrophobic domain of the protein and the hydrophobic thickness of the membrane. Studies on lysine-flanked peptides revealed that a too short α-helix under strong mismatch conditions moves to a nontransmembrane orientation (*2*−*4*). On the contrary, a too long α-helix has the tendency to form oligomers (*2*, *3*). Mismatch did not result in significant adaptations of the peptide backbone conformation in the fluid phase (*5*). However, changes in the phase transition temperature in response to mismatch have been reported indicating that the acyl chain order is affected to some extent (*6*).

De Planque et al. showed that synthetic peptides, flanked with tryptophan or lysine residues, induce nonbilayer phases under mismatch conditions at high peptide to phospholipid ratio. At low peptide to phospholipid ratio, the main response on increasing mismatch is occlusion of the peptide material from the membrane. Especially, in the case of negative mismatch, in which the peptide is too short to accommodate the membrane, occlusion is a dominant response (*7*, *8*). The phenomenon of hydrophobic mismatch has been studied in biological membranes as well. With a "glycosylation mapping" technique (*9*), it is shown that tryptophan pulls a polyleucine segment toward the membrane−water interface. An affinity for the hydrophobic core was demonstrated for the phenylalanine residue (*10*). This difference in behavior of both tryptophan and phenylalanine contradicts the observation that these residues display an almost identical apparent hydrophobicity on transfer from water to membrane (*11*).

With respect to membrane-anchoring interactions, the M13 major coat protein possesses interesting characteristics. The M13 coat protein can be roughly divided into three structural domains (Figure 1). First there is the helical transmembrane domain. The second is the amphipathic N-terminal arm, which is thought to be mainly parallel to the surface of the membrane (*12*, *13*). Finally, there is a hinge region connecting both the helical transmembrane domain and the amphi-

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pathic N-terminal arm. The helical transmembrane domain contains four lysine amino acid residues at the C-terminus at positions 40, 43, 44, and 48. The lysine residue at position 40 is buried within the hydrophobic core of the membrane. The side chain of this residue is probably located in the polar headgroup region (*14*). The C-terminal side of the protein also contains two phenylalanine residues at positions 42 and 45, which prefer to be in the hydrophobic core of the membrane (*1*). A tryptophan residue at position 26 is present at the N-terminal side of the helical transmembrane domain. The hinge region contains two tyrosine residues at positions 21 and 24 (Figure 1). These aromatic and positively charged residues could provide the anchoring interactions, which influence the position of the protein within the membrane (*1*, *15*). M13 coat protein possesses an additional anchoring interaction via the N-terminal segment of the protein. The arm is in equilibrium between a membrane-bound and a water-phase configuration. The ratio between both configurations can be altered by a mutation of Leu14 and/or Phe11 (*16*, *17*).

In this paper, additional information is acquired about the role of specific amino acids at the membrane−water interface by studying the M13 major coat protein under mismatch conditions. The relative depth of Trp26 is followed by fluorescence spectroscopy. Furthermore, site-specific information is acquired about the relative depth of positions in the membrane by attaching an ESR spin-label or fluorescence probe to a cysteine residue of single cysteine mutants.

MATERIALS AND METHODS

Materials. Dimyristoleoylphosphatidylcholine (DMoPC),[1] dioleoylphosphatidylcholine (DOPC), dieicosenoylphosphatidylcholine (DEiPC), and dierucoylphosphatidylcholine (DEuPC) were purchased from Avanti Polar Lipids. Fine chemicals were purchased from Merck.

Preparation and Purification of Labeled Single Cysteine Mutants. Site-specific cysteine mutants were prepared with the QuikChange Site-Directed Mutagenisis Kit from Stratagene. PT7-7, with geneVIII as insert, was used as the template for this procedure (*16*). The oligonucleotides were purchased from Amersham Pharmacia Biotech. The sequence of the mutant DNA was verified using automated DNA sequencing. Correctly mutated plasmid DNA was transformed into competent *E. coli* BL21(DE3) cells (*18*).

Mutant M13 coat protein was purified, and labeled with IAEDANS as is described in detail by Spruijt et al. (*16*). First, mutant protein was overexpressed in *E. coli*, and the membrane fraction was collected. Second, the membrane proteins were extracted from the membrane, and separated by hydrophobicity using reversed phase chromatography. After this step, the about 85% pure mutant coat protein was added to a mixture of 2-propanol/water and 0.1% triethylamine (v/v). The mutant coat protein was subsequently labeled with IAEDANS. Slight modifications were introduced in the procedure for the attachment of maleimidoproxyl spin-label to the single cysteine mutants. An excess of spin-label was added to the protein fraction after reversed phase chromatography. The label was allowed to react with the SH- groups of the mutants for 20 min at room temperature at pH 7. In the final step, free label and remaining impurities were removed from the labeled mutant coat proteins using size exclusion chromatography. The about 95% pure protein, labeled with AEDANS or proxyl, was now in a buffer containing 50 mM cholate, 10 mM Tris-HCl (pH 8), 1 mM EDTA, and 150 mM NaCl.

Reconstitution of Labeled M13 Coat Protein. Labeled M13 major coat protein was reconstituted into PC phospholipids using the cholate-dialysis method (*19*). The amount of protein was chosen such that the lipid to protein molar ratio (L/P) exceeded 200. Typically, samples contained about 4 mg of phospholipids for ESR spectroscopy and about 1 mg for fluorescence spectroscopy. Chloroform was evaporated from the desired amount of phospholipid solution, and the residual traces of chloroform were removed by drying under vacuum for at least 2 h. The lipids were subsequently solubilized in 400 mM cholate, 1 mM EDTA, 150 mM NaCl, 10 mM Tris-HCl, pH 8, and mixed with labeled protein. In the following step, the lipid−protein mixture was dialyzed for 60 h against a 100-fold excess of buffer containing 1 mM EDTA, 150 mM NaCl, and 10 mM Tris-HCl, pH 8. The buffer was replaced every 12 h.

Steady-State Fluorescence Spectroscopy. Fluorescence spectra of the labeled mutants were collected at 20 ± 0.5 °C using an excitation wavelength of 340 nm for AEDANS and 290 nm for tryptophan on a SPEX Fluorolog 3-22 fluorometer equipped with a 450 W xenon lamp as an excitation source. The background samples contained PC bilayers with and without wild-type protein. Excitation and emission bandwidths were set to 1 and 2 nm. The spectra were recorded between 400 and 600 nm for AEDANS, and between 300 and 400 nm for tryptophan. The integration time was set to 0.5 s. Samples of 1 mL were in 1 cm light-path fused silica cuvettes (Hellma model 114F-QS), and the optical density at 340 or 280 nm never exceeded 0.1. The spectra were corrected for background signal and sensitivity of the photomultiplier.

ESR Spectroscopy. ESR spectra of the labeled mutants were collected at 20 ± 0.5 °C. A comparable experimental setup was used as is described in Stopar et al. (*20*). Samples of 1 mL of reconstituted mutant coat protein were freeze-dried overnight, and dissolved in 10 mM Tris-HCl, 1 mM EDTA, and 150 mM NaCl, pH 8, yielding multilamellar vesicles. The vesicles were concentrated by centrifugation at 30 000 rpm in a Beckman Ti75 rotor at 20 °C. Fifty microliter glass capillaries were filled up to 5 mm with vesicles containing labeled mutant coat protein. These capillaries were inserted into standard 4 mm diameter quartz tubes. ESR spectra were recorded on a Bruker ESP 300E ESR spectrometer equipped with a 108TMH/9103-microwave cavity. The temperature was regulated with a nitrogen gas-flow temperature system. The ESR settings for all recorded spectra were the following: 6.38 mW microwave power, 0.1 mT modulation amplitude, 40 ms time constant, 160 s scan time, 10 mT scan width, and a 342.0 mT center field. Up to 20 recorded spectra were accumulated.

[1] Abbreviations: IAEDANS, *N*-(iodoacetyl)aminoethyl-1-sulfonaphthylamine; L/P, lipid to protein molar ratio; SDS, sodium dodecyl sulfate; λ_{max}, wavelength of maximum emission; PC, phosphatidylcholine; DMoPC, di14C:1Δ9c PC (dimyristoleoyl-PC); DOPC, di18C:1Δ9c PC (dioleoyl-PC); DEiPC, di20C:1Δ11c PC (dieicosenoyl-PC); DEuPC, di22C:1Δ13c PC (dierucoyl-PC).

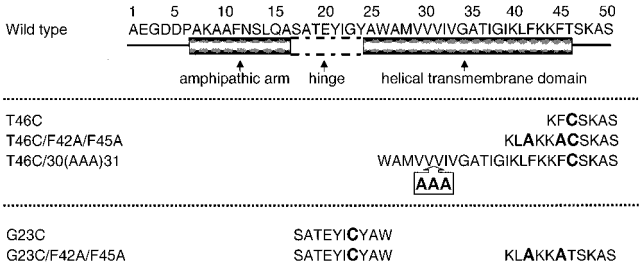

FIGURE 1: Primary structure of wild-type (*37*) and mutant M13 major coat proteins. The amino acid residues that differ from the wild-type protein are printed in boldface type. The structural domains are indicated below the primary structure of the wild-type protein.

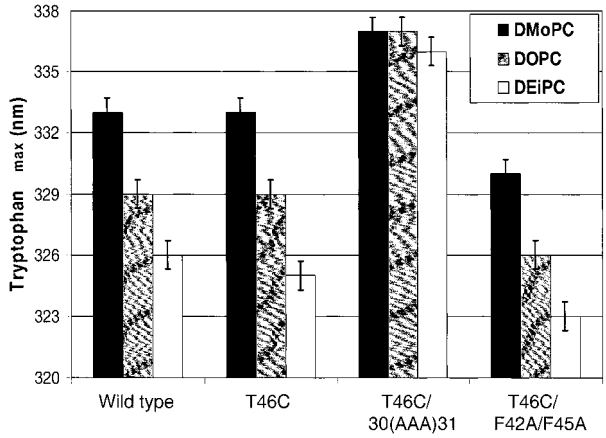

FIGURE 2: Tryptophan emission maximum of wild-type and mutant proteins, reconstituted in PC bilayers with varying thickness. The three bars for each mutant represent in black DMoPC, in gray DOPC, and in white DEiPC. The vesicles were in 150 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8, at 20 °C.

RESULTS

The primary structures of the mutants, which were constructed for this study, are shown in Figure 1. These mutants were prepared to reach the following goals. (a) A cysteine residue, which is labeled with AEDANS, is introduced at position 46 to monitor the changes in relative position at the C-terminal side of the transmembrane helical domain. (b) The changes in position of the hinge region are followed via a labeled cysteine residue at position 23. (c) The effect of a longer transmembrane domain is studied using a T46C/30(AAA)31 mutant, in which three alanines are introduced. (d) The mutants G23C/F42A/F45A and T46C/F42A/F45A are prepared to determine the importance of the Phe42 and Phe45 for the position of the protein in the membrane. The changes in the wavelength of maximum emission (λ_{max}) of Trp26 are monitored to measure the response on the N-terminal side of the helical transmembrane domain of the wild-type and mutant proteins.

Figure 2 shows the λ_{max} of the tryptophan residue from the wild-type and mutant proteins that are reconstituted into DMoPC, DOPC, and DEiPC. The hydrophobic thickness of these bilayers is defined as the distance between the acyl chain C-2 atoms of two opposing lipids. This results in a hydrophobic thickness of 20 Å for DMoPC, 27 Å for DOPC and 30.5 Å for DEiPC (*7*, *21*). The λ_{max} of the wild-type's tryptophan residue decreases from 329 to 326 nm when the bilayer thickness is increased. This suggests a shift of the tryptophan residue to a more apolar environment. Comparable results are found for the tryptophan residue of the AEDANS-labeled T46C mutant. Apparently, the presence of the AEDANS probe at position 46 has no influence on the position of the tryptophan residue in the membrane. Increasing the thickness of the bilayer has hardly any effect on the λ_{max} of the tryptophan of the T46C/30(AAA)31 mutant. The λ_{max} exhibits a value of about 337 nm in all PC bilayers. The T46C/F42A/F45A mutant again shows a shift of the tryptophan residue to a more apolar environment, when the bilayer thickness is increased (330−323 nm). The tryptophan fluorescence intensity of the G23C and G23C/F42A/F45A mutants was low. The close proximity of the AEDANS-labeled cysteine at position 23 probably causes a quenching of the tryptophan fluorescence via energy transfer and/or quenching by the sulfur group. Because of this effect, the tryptophan wavelength of maximum emission could not be reliably determined.

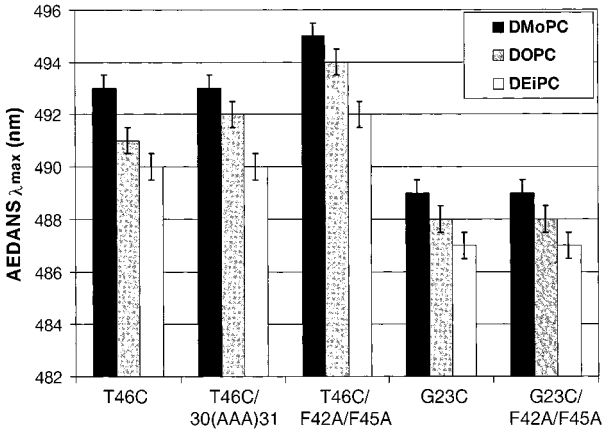

FIGURE 3: AEDANS emission maximum of the labeled mutant protein, reconstituted in PC bilayers with varying thickness. The three bars for each mutant represent in black DMoPC, in gray DOPC, and in white DEiPC. The vesicles are in 150 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8, at 20 °C.

Figure 3 shows the λ_{max} of AEDANS from the mutant proteins, which are reconstituted into DMoPC, DOPC, and DEiPC. The λ_{max} decreases for all labeled mutants when the hydrophobic thickness is increased. The T46C and T46C/30(AAA)31 mutants show similar values of λ_{max} in the PC bilayers. Apparently, the longer transmembrane helix has no effect on the location of position 46 in the membrane. The T46C/F42A/F45A mutant exhibits a systematically higher value of λ_{max} (with about 2 nm) as compared to the T46C mutant. This suggests a shift of position 46 to a more polar environment as a result of the phenylalanine mutations. The AEDANS-labeled position 23 of both G23C and G23C/F42A/F45A is equal in all PC bilayers. Therefore, position 23 is insensitive to the phenylalanines at the C-terminal side of the protein.

For additional information about the location of position 46, ESR spectra were acquired of spin-labeled T46C and T46C/F42A/F45A reconstituted in DOPC (Figure 4). The ESR spectrum of the T46C mutant displays a superposition of two components, which was also found by Stopar et al. (*14*). The spectrum consists of a sharp three-line spectrum representing mobile probes on the ESR time-scale (10^{-11} s $\leq \tau_c \leq 3 \times 10^{-9}$ s), and a broad spectral component indicative for motion in the slow regime ($\tau_c \geq 2 \times 10^{-9}$ s)

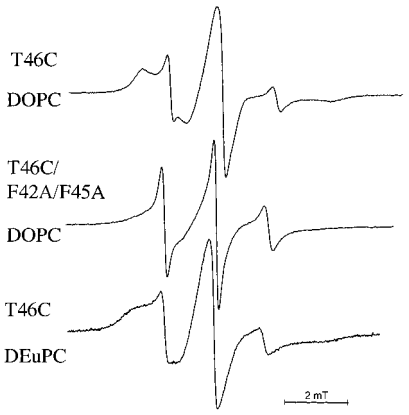

FIGURE 4: ESR spectra of proxyl-labeled position 46 of the T46C and T46C/F42A/F45A mutants, reconstituted into DOPC, and the T46C mutant reconstituted into DEuPC. The vesicles are in 150 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8, at 20 °C.

(*22*). The replacement of both phenylalanines by alanines in the T46C/F42A/F45A mutant produces a spectrum in which the broad component has almost completely disappeared.

Figure 4 also shows the spin-labeled T46C mutant reconstituted into DEuPC membranes, which exhibits a hydrophobic thickness of 34 Å. ESR experiments on mutants, reconstituted in DEuPC, display these abnormal broad spectra, indicating a large variety of spectral components, and perhaps spin−spin interactions as a result of protein aggregation. Probably, the protein exists in several orientations and/or configurations in this model membrane as was also found for Lys-flanked polypeptides under strong mismatch conditions (*2*−*4*). This result shows that the natural M13 coat protein displays similar behavior as the peptides when the hydrophobic thickness is too large.

Under unfavorable conditions, M13 major coat protein has the ability to form irreversible aggregates, which show up as a β-sheet conformation in CD spectra. This phenomenon is not found in vivo, and is therefore regarded as an artifact (*23*). Since the hydrophobic mismatch experiments and the use of mutants could induce this β-sheet formation, the conformation of the proteins was checked with CD spectroscopy. For all mutants in the PC bilayer systems, the results showed similar spectra typical for α-helices (data not shown), indicating the absence of irreversible protein aggregation.

DISCUSSION

To record possible adaptations of the protein to mismatch conditions and/or specific mutations, changes at both sides of the transmembrane helical domain (residues 25−46) were monitored in our work (Figure 1). As a monitor for the N-terminal side of the helical transmembrane domain, we selected the intrinsic fluorescence probe tryptophan at position 26, whose wavelength of maximum emission is sensitive to the polarity of the environment. This is reflected in a red-shifted emission maximum in the aqueous phase as compared to the value of λ_{max} in the hydrophobic phase. This property provides a tool to measure the relative depth of Trp26 in the membrane. A similar approach was used for chemosynthetic peptides described by Ren et al. (*2*, *3*).

To monitor the hinge region and the C-terminal side of the transmembrane helical domain, key positions were labeled with the fluorescence probe AEDANS. This probe is designed to be sensitive to the polarity of the environment (*24*). Moreover, it was shown that the λ_{max} of AEDANS is highly sensitive to small changes in penetration depth in the membrane, when attached to single cysteine mutants of the M13 major coat protein (*16*, *25*). There is no quantitative relation between the emission maxima of tryptophan and AEDANS with respect to the actual depth of the fluorophores in the membrane. Therefore, an equal change in the λ_{max} of both fluorophores does not imply an equal change in membrane depth.

As a monitor for the C-terminal side of the helical domain, a cysteine residue is introduced at position 46 by site-specific mutagenesis. The cysteine residue is subsequently labeled with AEDANS. Earlier studies with the cysteine-scanning approach indicated that position 46 is located near the edge of the transmembrane domain (Figure 1) (*25*, *26*). To monitor the hinge region of the protein, a mutant is selected with a cysteine at position 23. NMR experiments on the protein dissolved in SDS showed that the hinge is composed of amino acid residues 17−24, which show a relatively high flexibility (*27*, *28*). Not much is known about the structure of the hinge region in phospholipid bilayers. Spruijt et al. showed that the hinge is not located in the water phase, but is buried within the membrane, close to the polar headgroup region of the bilayer (*16*).

In DOPC, the tryptophan residue of the wild-type protein exhibits a λ_{max} of 329 nm (Figure 2). A larger value of λ_{max} is expected for a tryptophan residue at the interface of a DOPC bilayer. De Planque et al. reported values of 337 nm for WALP peptides, which suggested that the tryptophan residues are in the interface (*29*). In the other PC bilayers, a value of 333 nm is obtained in DMoPC, and 326 nm in DEiPC. Apparently, the tryptophan residue is located deeper in the membrane as compared to the WALP peptides. Trp26 moves to a more apolar environment, when the hydrophobic thickness is increased from 20 to 30.5 Å. The protein is unable to adapt to a hydrophobic thickness of 34 Å as is demonstrated with ESR experiments on the T46C mutant. Reconstitution into DEuPC results in aggregation and/or exclusion of the protein from the bilayer (Figure 4).

A comparable result is obtained for the AEDANS-labeled T46C and G23C mutants (Figure 3). The λ_{max} of AEDANS, attached to position 46, changes from 493 to 490 nm going from DMoPC to DEiPC. The λ_{max} of AEDANS at position 23 shows a blue shift of 2 nm in these bilayers. These results show that the general response to an increased hydrophobic thickness appears to be a deeper penetration of the monitored positions of the protein in the membrane. However, a much stronger response of positions 23 and 46 would be expected when the hydrophobic thickness is increased. Both positions 23 and 46 are located at the membrane−water interface (*16*, *25*, *26*). This region of the membrane exhibits a steep polarity gradient on going from the outside to the inside of the membrane (*30*). A change in the penetration depth of AEDANS in this region of the membrane will result in a large change in λ_{max}. Nevertheless, the labeled positions 46 and 23 show only a change in λ_{max} of 3 and 2 nm, respectively. These experiments suggest that position 46 and

23 show a small change in relative depth under mismatch conditions.

The finding that the C-terminal side (position 46) of the protein is less liable to a shift to a deeper position in the membrane under mismatch conditions is further supported by results obtained from the T46C/30(AAA)31 mutant. The AEDANS-labeled T46C and T46C/30(AAA)31 mutants produce comparable values of λ_{max} in all PC bilayers (Figure 3). Therefore, the relative depth in the membrane of position 46 is not significantly affected by the insertion of the three alanines. However, a strong response is observed at the N-terminal side of the helical transmembrane domain as monitored by the tryptophan residue. The comparison between the λ_{max} of the tryptophan residue from the wild type and the insertion mutant shows systematically higher values in all PC bilayers for the T46C/30(AAA)31 mutant (Figure 2). This result implies that the tryptophan residue shifts to a position closer to the water phase when three alanines are inserted into the protein.

No change is measured in the λ_{max} of the tryptophan of the T46C/30(AAA)31 mutant between DMoPC and DOPC, and a small shift is visible in DEiPC. De Plangue et al. also found such an unchanged λ_{max} for WALP peptides, which varied in hydrophobic length (*29*). Apparently, the tryptophan residue is now located in the interface of the membrane, where it has strong anchoring interactions with the polar headgroup region of the bilayer. From the above-mentioned experiment, it can be concluded that the C-terminal side of the helix (position 46) exhibits stronger anchoring interactions than the N-terminal side (position 26).

The C-terminal side contains two phenylalanine and four lysine amino acid residues. In an earlier study, it is shown that especially Lys40 is buried within the membrane hydrocarbon core. It is suggested that the charged side chain can just reach the polar headgroup region of the membrane, which is referred to as the so-called "snorkeling" effect (*31*, *32*). On the contrary, phenylalanine residue 42 and especially residue 45 are suggested to be located in the more polar headgroup region. These residues probably bend back toward the hydrophobic core of the membrane (*14*, *25*, *26*). This arrangement of the amino acids would explain the strong membrane-anchoring interactions of the C-terminal part of the protein.

To study the role of the phenylalanine residues in these interactions, a mutant is prepared in which both phenylalanines are replaced by alanine residues. Interestingly, in this mutant position 46 clearly moves to the water phase, as can be concluded from the red shift of the AEDANS emission maximum (Figure 3). The relocation of position 46 as a result of the mutation is confirmed by ESR spectroscopy (Figure 4). The spectrum of the proxyl-labeled position 46 exhibits a broad component in agreement with Stopar et al. (*14*). The consequence of the mutation of both phenylalanines is an almost complete disappearance of the immobile component, which in turn suggests a movement of this position toward the water phase. The result of the mutation of the phenylalanines is a blue shift of the tryptophan residue as compared to the wild-type protein in all PC bilayers (Figure 2). This suggests a deeper burial of Trp26 in the membrane. The combination of these movements of positions 46 and 26 suggests a shift of the entire helical transmembrane domain to the C-terminal side when both phenylalanines are replaced by alanines. Apparently, the phenylalanine residues provide an inside directed force on the helical domain.

Although the tryptophan residue moves to a deeper location in the membrane as a result of the phenylalanine mutations, the AEDANS probe at position 23 in the hinge region is not affected. Apparently, a change in the local structure in this region of the protein takes place. The fixed location of position 23 confirms the strong anchoring interactions of the hinge region with the membrane. There are several possibilities that can explain these interactions. The amino acid composition in the hinge can be important with, for instance, the tyrosine residues at positions 21 and 24. It is suggested that tyrosine residues, similar to tryptophan, prefer to be localized in the interface region of the membrane (*33*, *34*). Another possibility can be that the amphipathic N-terminal arm on the one side and the helical transmembrane domain on the other side are responsible for the apparent fixed location of the hinge region.

It is not straightforward to construct a model of the membrane-bound M13 major coat protein that agrees with the behavior of the wild-type protein under mismatch conditions. A number of possible adaptations can be suggested when the hydrophobic thickness of the bilayer is increased. For instance: the elastic lipid bilayers can adapt, the local conformation of the protein can change (undetectable with CD spectroscopy), and the tilt angle of the helical transmembrane domain can vary with the hydrophobic thickness (*15*). Probably, all these effects will play a role when the M13 protein is studied under mismatch conditions. However, there are two arguments that suggest the presence of a tilt angle of the helical transmembrane domain of M13 protein. First, a tilt angle of $20° \pm 10°$ has been deduced from ^{13}C-MAS NMR spectroscopy on the protein, reconstituted into dimyristoylphosphatidylcholine (*35*). The apparent strong anchoring interactions of the C-terminal side of the protein and the hinge region can very well induce an increased tilt angle when the thickness of the bilayer decreases. Such an increased tilt angle has also been suggested for the lysine-anchored peptides by Harzer et al. (*36*). Second, a tilt angle is more energetically favorable for the lysine- and phenylalanine-rich C-terminus. In a helical conformation, the lysines are opposite to both Phe42 and Phe45. The presence of a tilt of the helix brings the amide group of Lys40 closer to the polar headgroup region. On the other hand, both phenylalanines (especially Phe45) on the opposite face of the helix can more easily reach the hydrophobic core of the membrane. Furthermore, Trp26 is at the same face of the helix as the lysine residues. As a result of a tilted arrangement, the tryptophan residue is directed toward the hydrophobic core of the membrane, which could explain the relatively deep burial of this residue.

CONCLUSION

The C-terminal side of the transmembrane helix and the hinge region of M13 protein show hardly any response to hydrophobic mismatch. Apparently, these protein regions have strong anchoring interactions with the membrane−water interface. The existence of strong anchoring interactions of the C-terminus is confirmed using the T46C/30(AAA)31 mutant. The phenylalanines play an important role in these

interactions. They affect the location of the entire transmembrane domain as is shown with the T46C/F42A/F45A mutant. The hinge region is not affected by the phenylalanine mutations, which demonstrates the strong anchoring interactions of this region of the protein to the interface. In conclusion, the protein is kept in position in the membrane not only by the hydrophobic domain itself, but also by a combination of forces brought about by specific amino acid residues, which reside in the membrane−water interface.

REFERENCES

1. Killian, J. A., and von Heijne, G. (2000) *Trends Biochem. Sci. 25*, 429−434.
2. Ren, J., Lew, S., Wang, Z., and London, E. (1997) *Biochemistry 36*, 10213−10220.
3. Ren, J. H., Lew, S., Wang, J. Y., and London, E. (1999) *Biochemistry 38*, 5905−5912.
4. Webb, R. J., East, J. M., Sharma, R. P., and Lee, A. G. (1998) *Biochemistry 37*, 673−679.
5. Zhang, Y. P., Lewis, R. N., Hodges, R. S., and McElhaney, R. N. (1995) *Biochemistry 34*, 2362−2371.
6. Zhang, Y. P., Lewis, R. N., Hodges, R. S., and McElhaney, R. N. (1992) *Biochemistry 31*, 11579−11588.
7. De Planque, M. R. R., Greathouse, D. V., Koeppe, R. E., Schafer, H., Marsh, D., and Killian, J. A. (1998) *Biochemistry 37*, 9333−9345.
8. de Planque, M. R. R., Kruijtzer, J. A. W., Liskamp, R. M. J., Marsh, D., Greathouse, D. V., Koeppe, R. E., de Kruijff, B., and Killian, J. A. (1999) *J. Biol. Chem. 274*, 20839−20846.
9. Nilsson, I., Saaf, A., Whitley, P., Gafvelin, G., Waller, C., and von Heijne, G. (1998) *J. Mol. Biol. 284*, 1165−1175.
10. Braun, P., and von Heijne, G. (1999) *Biochemistry 38*, 9778−9782.
11. White, S. H., and Wimley, W. C. (1998) *Biochim. Biophys. Acta*, 339−352.
12. Marassi, F. M., Ramamoorthy, A., and Opella, S. J. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8551−8556.
13. McDonnell, P. A., Shon, K., Kim, Y., and Opella, S. J. (1993) *J. Mol. Biol. 233*, 447−463.
14. Stopar, D., Spruijt, R. B., Wolfs, C. J. A. M., and Hemminga, M. A. (1996) *Biochemistry 35*, 15467−15473.
15. Killian, J. A. (1998) *Biochim. Biophys. Acta*, 401−416.
16. Spruijt, R. B., Meijer, A. B., Wolfs, C. J. A. M., and Hemminga, M. A. (2000) *Biochim. Biophys. Acta 1509*, 311−323.
17. Meijer, A. B., Spruijt, R. B., Wolfs, C. J. A. M., and Hemminga, M. A. (2001) *Biochemistry 40*, 5081−5086.
18. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorf, J. W. (1990) *Methods Enzymol. 185*, 60−89.
19. Spruijt, R. B., Wolfs, C. J. A. M., and Hemminga, M. A. (1989) *Biochemistry 28*, 9158−9165.
20. Stopar, D., Spruijt, R. B., Wolfs, C. J. A. M., and Hemminga, M. A. (1998) *Biochemistry 37*, 10181−10187.
21. Lewis, B. A., and Engelman, D. M. (1983) *J. Mol. Biol. 166*, 211−217.
22. Marsh, D. (1981) in *Membrane Spectroscopy* (Grell, E., Ed.) pp 51−142, Springer-Verlag, Berlin, Heidelberg, and New York.
23. Hemminga, M. A., Sanders, J. C., Wolfs, C. J. A. M., and Spruijt, R. B. (1993) *New Compr. Biochem. 25*, 191−212.
24. Hudson, E. N., and Weber, G. (1973) *Biochemistry 12*, 4154−4161.
25. Spruijt, R. B., Wolfs, C. J. A. M., Verver, J. W. G., and Hemminga, M. A. (1996) *Biochemistry 35*, 10383−10391.
26. Stopar, D., Jansen, K. A. J., Pali, T., Marsh, D., and Hemminga, M. A. (1997) *Biochemistry 36*, 8261−8268.
27. Papavoine, C. H. M., Remerowski, M. L., Horstink, L. M., Konings, R. N. H., Hilbers, C. W., and van de Ven, F. J. M. (1997) *Biochemistry 36*, 4015−4026.
28. Papavoine, C. H. M., Konings, R. N. H., Hilbers, C. W., and Vandeven, F. J. M. (1994) *Biochemistry 33*, 12990−12997.
29. De Planque, M. R. R. (2000) Thesis, Utrecht University.
30. Wiener, M. C., and White, S. H. (1992) *Biophys. J. 61*, 437−447.
31. Tanford, C., and Reynolds, J. A. (1976) *Biochim. Biophys. Acta 457*, 133−170.
32. Mishra, V. K., Palgunachari, M. N., Segrest, J. P., and Anantharamaiah, G. M. (1994) *J. Biol. Chem. 269*, 7185−7191.
33. White, S. H., and Wimley, W. C. (1999) *Annu. Rev. Biophys. Biomol. Struct. 28*, 319−365.
34. White, S. H., Wimley, W. C., Ladokhin, A. S., and Hristova, K. (1998) *Methods Enzymol. 295*, 62−87.
35. Glaubitz, C., Grobner, G., and Watts, A. (2000) *Biochim. Biophys. Acta*, 151−161.
36. Harzer, U., and Bechinger, B. (2000) *Biochemistry 39*, 13106−13114.
37. Van Wezenbeek, P. M. G. F., Hulsebos, T. J. M., and Schoenmakers, J. G. G. (1980) *Gene 11*, 129−148.

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