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Positions of Polar Amino Acids Alter Interactions between Transmembrane Segments and Detergents

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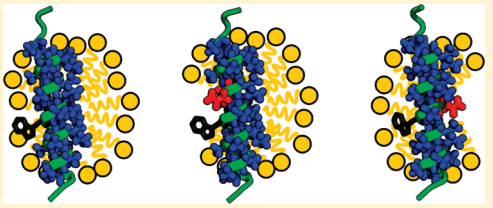
Supporting Information

ABSTRACT: α-Helical transmembrane (TM) segments in membrane proteins are comprised primarily of hydrophobic amino acids that accommodate insertion from water into the nonpolar membrane bilayer. In many such segments, however, polar residues are also present for structural or functional reasons. These latter residues impair the local favorable acyl interactions required for solvation by hydrophobic media such as phospholipids in native bilayers or detergents used for in vitro characterization. Using a series of Lys-tagged designed TM-like peptides (typified by KK-YAAAIAAIAWAIAAIAAAIAA-KKK) in which single-Asn residue substitutions (from Ile or Ala) were made successively from the center of the hydrophobic region toward the C-terminus, we demonstrate that polar residues strongly alter the nature of the interaction between TM segments and the solvating detergent. Through the application of sodium dodecyl sulfate−polyacrylamide gel electrophoresis, circular dichroism spectroscopy, and tryptophan fluorescence, we observed drastic differences in the structures of the detergent−peptide complexes that contain relatively minor sequence differences. For example, the blue shift of the Trp fluorescence (indicating local detergent solvation at this location) differs by as much as ∼10 nm depending upon the position of a single Asn substitution in an otherwise identical segment. The overall results suggest that polar point mutations occurring in a biological membrane will elicit comparable effects, placing a significant refolding burden on the local protein structure and potentially leading to disease states through altered protein−lipid interactions in membrane proteins.



Although α-helical transmembrane (TM) segments in proteins are ubiquitous in nature and essential for a multitude of cellular functions, the structural characterization of their sequence-dependent protein−protein and protein−lipid interactions remains a challenging pursuit. To render biophysical analysis more accessible, structural characterization of TM segments is typically performed in a suitable mimetic of the native membrane bilayer, such as lipid vesicles or detergent micelles. Detergent-solubilized states have been particularly useful for structural characterization by X-ray crystallography and solution NMR spectroscopy.[1−3] In addition, a reductionist approach is often used to study the details of TM protein assembly wherein individual TM segments are treated as independently folded units, conforming to the Popot and Engelman model of membrane protein folding.[4] Accordingly, systems composed of single α-helical TM segments have been extensively used to elucidate aspects of membrane protein folding (reviewed in refs 5 and 6).

In natural membranes, individual TM segments are most often inserted via the translocon. This process is largely irreversible, particularly if a TM segment is between hydrophilic domains that would be incapable of being retranslocated across the membrane.[7] By contrast, the environment of a detergent micelle is considerably more malleable and potentially has smaller kinetic and or energetic barriers to interconversion between fully inserted, partially inserted, or noninserted (aqueous-interactive) states. Recent work has shown the overall detergent−transmembrane segment complex is dependent on the hydropathy of the segment.[8,9] Such variations in how detergents interact with TM segments may alter the fine-tuning of helix−helix interactions, in turn altering overall membrane protein folding and/or susceptibility to denaturation in detergents, yet this versatility, in turn, allows in-depth assessment of the structural consequences, both local and longer range, of introducing a point mutation into a given TM segment.

As many as 25% of polytopic membrane proteins have at least one TM segment not expected to be capable of efficient membrane insertion, because of relatively low segment hydropathy, in the absence of the remainder of the protein.[10] Furthermore, disease-phenotypic nonpolar to polar mutations are common in TM domains[11] and in some cases have been shown to prevent translocon-mediated insertion of TM segments.[12] Although strongly polar residues can promote oligomerization of otherwise highly hydrophobic TM segments,[13,14] little is known about the structural consequences of such residues when they occur in low-hydropathy TM segments, where they would be predicted to become destabilizing toward insertion. In this work, we have systematically investigated how detergent solvation is affected by the sequence of the spanning protein segment,

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focusing primarily upon the positional dependence of the introduction of a strongly polar residue (Asn) into a moderately hydrophobic TM segment. We have chosen to study the anionic detergent sodium dodecyl sulfate (SDS) because of its widespread use in identifying helix−helix association interfaces,[15−18] as well as its known sensitivity to protein sequence.[19] Our findings suggest that single polar residues in TM segments may have a fundamental impact on membrane protein folding.

MATERIALS AND METHODS

Assessment of the Hydrophobicity and Hydrophobic Moment. The relative hydrophobicities of the model TM segments were assessed on the basis of predicted free energies of translocon-mediated membrane insertion (ΔG_{ins}) (http://dgpred.cbr.su.se),[10,20] water to lipid bilayer interfacial region partitioning (ΔG_{wif}),[21] water to octanol partitioning (ΔG_{woct}),[22] and a hydrophobicity scale derived by reverse phase high-performance liquid chromatography.[23] Hydrophobic moments ($\mu_{H\Phi}$) of the peptide sequences (not including the flanking Lys residues) were calculated from individual amino acid hydrophobicities (H_i) with eq 1:[24]

$$\mu_{H\Phi} = \{[H_i \sin(100i)]^2 + \sum[H_i \cos(100i)]^2\}^{1/2} \quad (1)$$

For the translocon-mediated scale, H_i values were obtained from the aforementioned prediction server, specific to the predicted transverse positioning of each residue in the central core, where residue A11 is in the center of a 21-residue TM segment (Y^1−A^{21}). For other scales, H_i values were not sensitive to position. The i = 0 position was chosen at the central Trp residue in the primary sequence, so that this residue projected directly onto the positive x-axis. The magnitude of the hydrophobic moment in the direction of the Trp residue was calculated with eq 2:

$$\mu_{H\Phi(Trp)} = \sum[H_i \cos(100i)] \quad (2)$$

Peptide Synthesis and Purification. Peptides were synthesized using standard Fmoc [*N*-(9-fluorenyl)methoxycarbonyl] chemistry[25] on a PAL-PEG-PS [4′-aminomethyl-3′,5′-dimethoxyphenoxyvaleric acid-poly(ethylene glycol)] polystyrene resin (Applied Biosystems). Peptides were cleaved from the resin during a 2 h incubation period with an 88% trifluoroacetic acid, 5% phenol, 5% ddH_2O, 2% triisopropyl cleavage cocktail, producing an amidated C-terminus. Cleaved peptides were purified by reverse phase high-performance liquid chromatography on a C4 preparative column (Phenomenex), using an acetonitrile/water gradient (with 0.1% trifluoroacetic acid). Mass spectrometry was used to identify the molecular weight of the purified peptides. All peptides were lyophilized following purification, resuspended in ddH_2O, and stored in aliquots at −20 °C. Peptide concentrations were determined using quantitative amino acid analysis of SDS-solubilized samples performed by the Advanced Protein Technology Centre of the Hospital for Sick Children. On the basis of replicate analysis of the wild-type protein and select variants, as well as comparison to other concentration determination methods (UV absorbance), the estimated error in concentration determination is 10%.

Circular Dichroism Spectroscopy. Freshly thawed aliquots of peptides were diluted to a final concentration of 15−40 μM in 10 mM Tris-HCl buffer (pH 8.0), with or without 34.7 mM SDS. Spectra were recorded in a 1 mm path length cuvette on a Jasco J-810 circular dichroism (CD) spectropolarimeter. All spectra were background subtracted and converted to mean residue ellipticity [MRE (degrees square centimeters per decimole)]. For each peptide, CD spectra were recorded at a minimum of two peptide concentrations. For most peptides, the standard deviation between replicate circular dichroism measurements was on the same order of magnitude as or smaller than the estimated error in the concentration determination.

Tryptophan Fluorescence Measurements. Freshly thawed aliquots of peptides were diluted to a final concentration of 10 μM in 10 mM Tris-HCl buffer (pH 8.0), with or without 34.7 mM SDS. Additional samples were prepared by dilution of those used for circular dichroism in a 1:1 ratio with buffer and used at a final concentration between 10 and 20 μM. Fluorescence emission spectra of peptides were recorded on a Hitachi F-400 Photon Technology International C-60 fluorescence spectrometer at an excitation wavelength of 295 nm with a 2 nm slit width, and emission was measured between 310 and 400 nm with a 4 nm slit width. All spectra were corrected for light scattering effects by subtraction of background, and by the correction function of FELIX provided by the manufacturer. The wavelength of maximal emission intensity was obtained from these spectra, and the blue shift was calculated as the difference between the average wavelength of maximal emission in aqueous buffer and SDS-containing buffer.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis Analysis. SDS−PAGE was performed using precast 12% acrylamide NuPAGE gels in MES buffer (Invitrogen) according to the manufacturer's protocols. Silver stain (Invitrogen) was used to visualize proteins on all gels. Twenty nanograms of each peptide was loaded in a total volume of 10 μL in each lane. At this concentration, the wild-type peptide was previously shown to be a monomer.[8] Apparent molecular weights were estimated based upon R_f analysis using Mark12 molecular standards (Invitrogen). Migration distances were determined using NIH Image. Gel shift values (ΔMW_{app}) were calculated as (estimated MW from gel)/(formula MW from sequence) and normalized to the wild-type peptide on each gel.

RESULTS

Peptide Design and TM Insertion Prediction. To address the membrane insertion requirements of prototypic TM strands in a systematic manner, we designed peptides based, in part, upon a moderately hydrophobic TM segment.[8] The hydrophobic core of these peptides consists primarily of Ile and Ala residues, as well as a central Trp residue (sequence of the "wild-type" (WT) peptide, termed AI5, shown in Figure 1A). The wild-type peptide is predicted to have a ΔG of translocon-mediated insertion of −0.55 kcal/mol,[10] a ΔG of octanol partitioning of −1.4 kcal/mol,[22] a ΔG of POPC interfacial partitioning of −2.0 kcal/mol,[21] and a Liu−Deber average hydrophobicity of 1.2 (greater than the predicted threshold value for membrane insertion of 0.4).[23] The WT peptide has been shown to be capable of insertion and dimerization in a native bilayer; it is able to adopt a helical structure in SDS micelles but does not dimerize at micromolar concentrations.[8] In addition, the presence of Lys tags confers aqueous solubility to the peptides, allowing us to characterize their secondary structure in the absence of detergent.[26]

In this work, a number of variants of this peptide were produced by introducing single Asn substitutions, beginning at

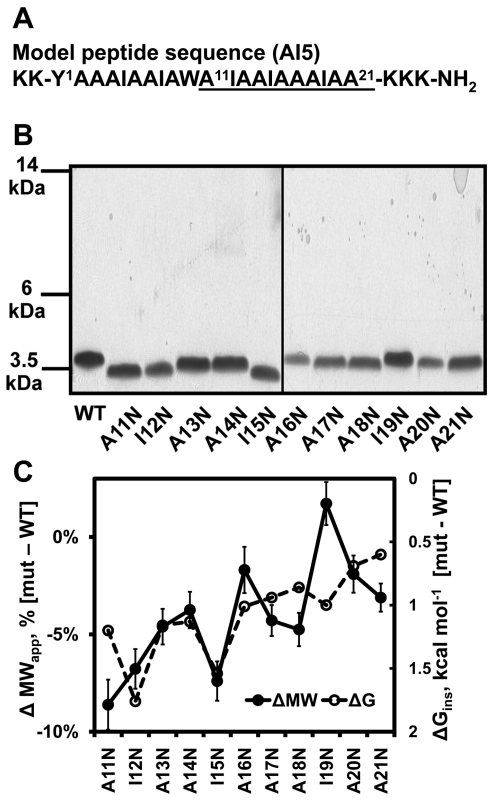

Figure 1. Peptide sequence and SDS−PAGE analysis of Asn peptide variants. (A) Amino acid sequence of the base peptide (WT) under investigation. The region in which Asn substitutions were made is underlined. (B) Representative SDS−PAGE of WT and single-Asn peptides. (C) Plotted on the primary axis are the mean relative gel shifts (see Materials and Methods) compared to that of the wild type (ΔMW_{app}[mut−WT]) shown as filled circles connected with a solid line. Error bars represent the standard deviation in the calculated gel shift from a minimum of four gels for this set of peptides. Plotted on the secondary axis is the difference in the contribution to the free energy of translocon-mediated insertion between an Asn residue and the native amino acid (ΔG_{ins}[mut−WT]) at that location (empty circles and dashed lines).

the central position (residue 11 of the hydrophobic core) and proceeding toward the C-terminus. The inclusion of this polar residue reduces the hydrophobicity by incremental amounts, depending upon (i) whether the Asn is substituted for an Ala or Ile residue and (ii) the position of the substitution in the context of predicted translocon-mediated insertion (see Table S1 of the Supporting Information). In many cases, this change lowers the net hydrophobicity below the predicted insertion threshold. The position of the Asn residue may further produce large differences in local hydrophobicity on one side of the helix versus the other. To mathematically quantify the asymmetry of the degree of hydrophobicity between opposite helix surfaces, we calculated the "hydrophobic moment" of each peptide using various hydrophobicity scales, as described in Materials and Methods. In all cases, the WT peptide had a relatively large hydrophobic moment (indicating one surface has more hydrophobicity than the other), with decreased hydrophobicity in the direction of the Trp residue. Substitutions made close to the position of the Trp residue about the helical axis increased the hydrophobic moment up to ∼50% without significantly changing its direction, while substitutions on the opposite side of the helix decreased the magnitude by up to ∼50%, indicating that local hydrophobicity of individual surfaces was highly dependent on the position of this single substitution (see also Table S1 of the Supporting Information).

SDS−PAGE Migration Rates of Asn-Containing Peptides. SDS−PAGE migration is commonly used to identify the monomeric molecular weight (MW) of a protein, although membrane protein oligomeric states may be retained.[27−29] SDS−PAGE is also sensitive to protein conformation,[30,31] and we have previously demonstrated that gel migration rates may, in part, be traced to conformational changes in membrane protein structure.[32,33] To a large degree, migration rates of soluble proteins in SDS−PAGE are determined by particle size because of the sieving effects of a polyacrylamide gel.[34,35] As an increasing amount of SDS binds to the segment, the mass/negative charge ratio will decrease, an effect that should, in principle, increase the gel migration rate. However, on the basis of previous studies by our lab using a membrane protein model composed of two TM segments, an increasing level of detergent binding is in fact correlated with a decreasing rate of gel migration, indicating that particle size is the dominant factor.[19]

When we performed SDS−PAGE migration analysis on the present series of peptides containing Asn mutations (Figure 1B), all peptides migrated as a single species at a rate consistent with a monomeric state. Although these Asn-containing peptides differ at only one position, and all accordingly have MWs very similar to that of the WT version, nearly all migrated significantly faster than the WT peptide. The migration rates of the peptides followed two general trends. (i) The addition of Asn increased the rate of migration, with Ile-to-Asn substitutions increasing the rate more than Ala-to-Asn substitutions. (ii) The observed increase in the migration rate was more pronounced in substitutions located at or near the center of the TM helix sequence. Additionally, it has been shown that central polar residues are more deleterious to translocon-mediated insertion of transmembrane segments and therefore behave as less hydrophilic the closer they are to the helix ends.[10] Thus, both of these effects observed upon gel migration are consistent with the changes in hydrophobicity induced by these substitutions, where less hydrophobic sequences have faster gel migration as we have observed in previous studies.[19] Accordingly, the introduction of a polar residue at different positions produces similar trends in both gel migration rate and hydrophobicity changes as shown in Figure 1C.

Peptide Helicity in Aqueous versus Detergent Environments. Circular dichroism was used to assess the adoption of α-helical secondary structure upon solubilization of members of this peptide library in either aqueous buffer or SDS. The WT peptide contained significant helical structure in aqueous solution (Figure 2A, left panel). The introduction of an Asn residue at any location leads to a significant decrease in helicity [evidenced by a weaker signal at 222 nm and a shift of the minimum near 208 nm to lower wavelengths, indicative of a population of "random coil" structure,[36] as predicted from Chou−Fasman parameters[37] (shown for representative spectra in Figure 2A, left panel)]. In SDS, the WT peptide as well as all variants had increased levels of helicity compared to that of the aqueous buffer (shown for representative spectra in Figure 2A, right panel). The majority of mutations contained amounts of helical secondary structure that were within 10% of WT values (θ_{222} = −33000 deg cm^2 dmol^{-1}). Of all variants, only three had a significant decrease in helicity in SDS buffer compared to that in the wild type (Figure 2B).

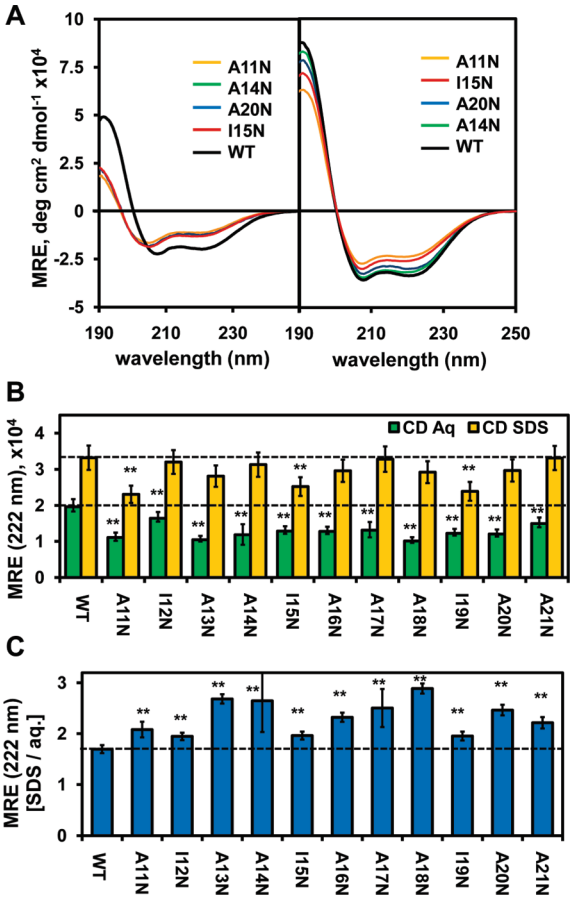

Figure 2. Circular dichroism of peptides. (A) CD spectra of the wild type and selected variants in aqueous (left) or SDS-containing buffer (right). In each panel, mutants are listed in order of increasing helicity at 222 nm. (B) Mean residue ellipticity [θ] (degrees square centimeters per decimole) at 222 nm of peptides in aqueous or SDS buffer. Error bars represent the combined standard deviation from both the CD measurements and concentration determination. CD measurements were taken for three to six samples for each peptide under each buffer condition. (C) Fold increase in helicity from aqueous to SDS buffer (measured by MRE at 222 nm). Error bars represent the combined standard deviation from the CD measurements. In panels B and C, WT levels are indicated by a dashed line. Two-sided *t* tests were performed between WT and each peptide variant. Asterisks indicate $p < 0.01$.

To make further quantitative comparisons among mutants, we measured the ratio of helicity in SDS buffer to that in aqueous buffer. This method eliminates any variation introduced by inaccuracy in concentration determination, which in many CD studies can be the largest source of error (see Materials and Methods). The WT peptide has an SDS/aqueous ratio of 1.7, indicating that SDS increased the helicity of this peptide by 70%. All Asn-containing variants had significantly ($p < 0.05$) larger ratios, ranging from 2.0 to 2.9 (Figure 2C). This increase in ratio indicates that Asn was more detrimental to the formation of helical structure in aqueous buffer than in SDS, and therefore, in a sense the "folding competency" of these segments is retained in SDS. An additional trend in these data is that all Ile-to-Asn mutants displayed a ratio of ~2, while all Ala-to-Asn mutants had larger ratios. This result indicates that removal of Ile is more detrimental to the formation of helical structure than removal of Ala in a membrane environment compared to an aqueous environment. Although Ala is predicted to be a superior helix former than Ile even in membrane mimetics,[38] in this situation hydrophobicity appears to be the dominant factor in helix formation. The exception to this trend was the most central Ala-to-Asn mutation at position 11, which had a ratio of 2.1 and was more similar to the Ile-to-Asn mutations in this regard.

Detergent Binding as a Dominant Factor in SDS−PAGE Migration. While there are some apparent differences in secondary structure among the peptide Asn variants in SDS, there was only a moderate inverse correlation between gel migration rate and helical structure ($R^2 = 0.34$; $p = 0.05$) (Figure S1A of the Supporting Information), indicating that changes in peptide structure alone were not a major factor in the phenomena observed. The Asn scanning results (Figure 1) are thus consistent with a reduction in the level of detergent binding because of the decreased hydrophobicity presented by polar residues. To confirm that differences in hydrophobicity are a determining factor here, we synthesized a peptide with a central Ile-to-Asp mutation (I12D), and a further I12N analogue also containing two Ala-to-Ile substitutions designed to "restore" hydrophobicity (Figure 3A). In addition, as a direct test of the dependence on sequence versus composition, we synthesized a peptide variant [termed "scrambled" (scr.)] that has a composition identical to that of the wild type but differs in sequence in a manner such that Ile residues are distributed evenly around the helix axis, as well as positioned toward the helix ends (helical wheel diagram in Figure 3B). All additional peptides were found to have a similar level of helicity as the WT peptide (data not shown). As shown in Figure 3C, the Asp mutant migrated like the Asn mutation at this position, while the more hydrophobic variant (I12N+2I) now migrated at a rate much closer to the WT rate in accordance with its increased hydrophobicity with respect to the other polar variants. Interestingly, the scrambled version migrated at a relative rate that was ~7% slower ($p < 0.01$) than the WT rate, indicating the SDS−PAGE approach can detect sequence-dependent differences in detail in the overall detergent−peptide complex.

Taking into account all the peptide variants that were studied, we observe a strong correlation of gel migration rate with net hydrophobicity as assessed by translocon-mediated membrane insertion[10] ($R^2 = 0.71$; $p < 0.001$) (Figure 3D). In contrast, the correlation between gel migration and helicity was no longer statistically significant upon inclusion of these additional variants ($R^2 = 0.01$; $p = 0.71$) (Figure S1B of the Supporting Information). We thus hypothesize that the majority of the observed variance in gel migration between detergent−peptide complexes is therefore due to differences in detergent binding as the source of variation in overall particle size.

Tryptophan Fluorescence Reveals Variations in the SDS Distribution. In previous studies of the WT peptide, Trp fluorescence was used to demonstrate that the central Trp residue was in an aqueous environment, while the positioning of an Asn residue across from this Trp caused it to become more micelle-buried (greater blue shift).[8] In the study presented here, we extended this analysis to all Asn-containing variants between positions 11 and 21. Although all mutants effectively lowered the overall hydrophobicity, at some positions the addition of Asn resulted in a greater blue shift of the Trp residue. There is a periodicity in the pattern of the Trp blue shift as the Asn is placed at various locations across the helical axis, up until position 17, after which the blue shifts become similar to that of the wild type (Figure 4A). We also calculated the magnitude of the hydrophobic moment vector in the direction of the Trp residue as an indicator

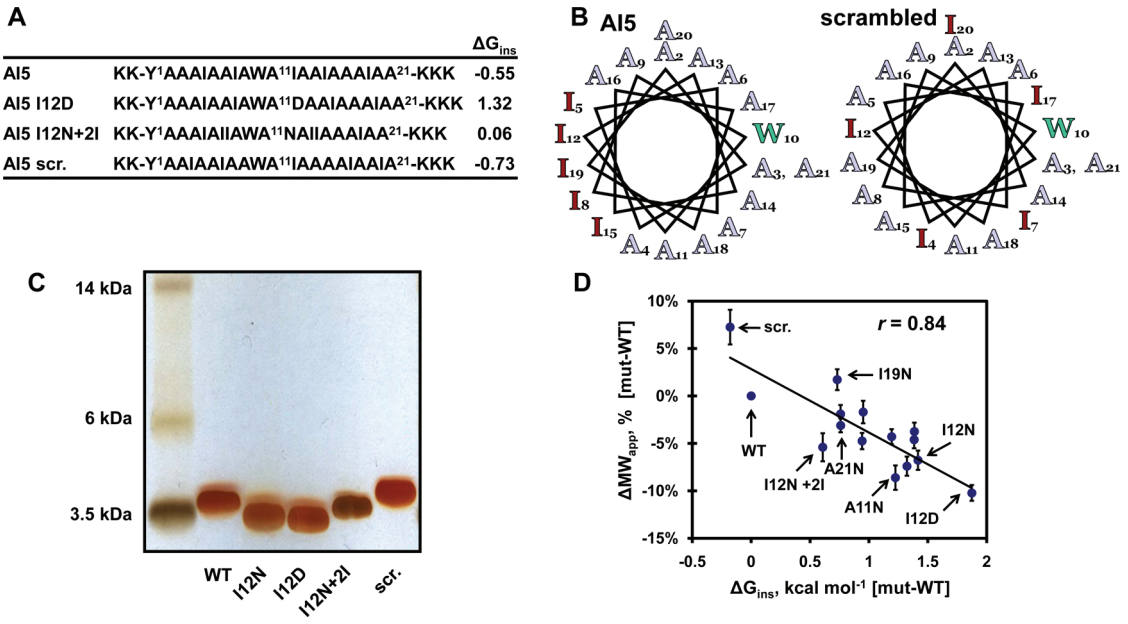

Figure 3. Sequence and SDS−PAGE analysis of additional peptide variants. (A) Amino acid sequences of the additional peptide variants studied, along with predicted free energies of translocon-mediated membrane insertion.[10] (B) Helical wheel diagram of both the WT peptide (termed AI5[8]) and the scrambled variant. (C) Representative SDS−PAGE of these variants. (D) Correlation between the observed SDS−PAGE gel shift of all peptide variants (panel C, Figure 1B) and the predicted free energy of translocon-mediated insertion is shown with a correlation coefficient ($r = 0.84$). The trend line p value is 0.0001. Selected Asn-containing variants, as well as all segments shown in panel C, are labeled. Error bars indicate the standard deviation of gel shifts for each variant obtained for three to eight independent gels.

of how "hydrophobic" this face is compared to the remainder of the segment. We found that peptides that were relatively more hydrophobic on the side opposite the Trp residue (a larger value on this scale) had smaller Trp blue shifts, while those that had a more even distribution of hydrophobicity (a smaller value) had larger blue shifts (Figure 4A). This provides evidence that there is an unequal distribution of SDS about the helix and that this distribution may be sensitive to alteration by polar substitutions. Indeed, while the scrambled peptide variant (with a minimal hydrophobic moment) had a level of helicity similar to that of the WT peptide (data not shown), it had a greater blue shift (11 nm vs 6 nm for WT), a value more typical of insertion into the center of a micelle (Figure 4B).

DISCUSSION

Alteration of Detergent Solvation of Hydrophobic Peptides Induced by Polar Residues. The side chain of an Asn residue typifies a strongly polar functional group that would be intrinsically unfavorable for hydrophobic interactions. When we placed the Asn residue in an otherwise hydrophobic sequence (characteristic of a protein TM segment), it is evident from the biophysical analysis performed that this residue may have a drastic effect on how the resulting segment interacts locally with the lipid environment of a detergent. Such large variation is observed even in model TM segments of identical composition, highlighting the nuances of such differences.

While perhaps not considered classically amphipathic because of a lack of a truly hydrophilic surface, there is still a modest hydrophobic moment present in the TM helices studied here that dictates the relative conformation of these peptides within the detergent micelles. Specifically, if the net hydrophobicity is directed away from the Trp residue, as in the WT or variants in which the Asn is on the same surface of the helix, the Trp has a relatively small blue shift, indicating some exposure of this surface to water and/or the headgroup regions of the micelle. Conversely, a substitution opposite the Trp (such as I12N and A16N) causes further burial of this same helix surface in the center of the micelle. These findings support the notion of conformational sensitivity of a helix such as the present WT sequence that contains both a strongly hydrophobic surface and a moderately hydrophobic surface. Thus, in contrast to the situation in which a helix contains a hydrophobic face and a demonstrable hydrophilic face, one could term the WT sequence "lipopathic" because of the presence of two inherently hydrophobic faces with differing preferences to interact with lipid-like environments such as the core versus the surface of a detergent micelle.

Although the incorporation of polar residues at most positions does not have a major effect on membrane-based helicity, they do alter the solvation of SDS in two ways.

(i) Detergent binding is lost, likely in the area where the polar residue is located, as manifested by a faster peptide gel migration rate, indicative of a smaller particle size. This phenomenon depends primarily on hydrophobicity as most Ile-to-Asn substitutions display greater migration differences versus the WT peptide than Ala-to-Asn substitutions. It is also noted that the introduction of an Asn residue at locations closest to the Trp residue decreases the blue shift, indicating less detergent present locally in these areas.

(ii) The distribution of SDS changes, as evidenced by the observed systematic variations in Trp blue shifts. This phenomenon is related to the lipopathy of the helix in that more SDS is distributed to the more hydrophobic surface, creating a situation

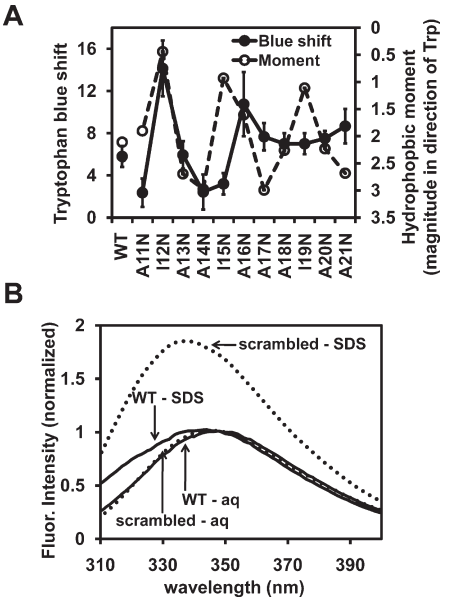

Figure 4. Tryptophan blue shifts induced by SDS solvation and hydrophobic moments of peptides. (A) Pattern of the blue shift (filled circles and solid line), compared to the hydrophobic moment along the axis in the direction of the Trp residue (empty circles and dashed line). The blue shift values for the WT peptide and for variants between and including A11N and I15N have been previously reported.[8] (B) Trp emission spectra of WT (—) and a scrambled peptide variant (· · ·) in aqueous solution (gray lines) or SDS-containing buffer (black lines). For each spectrum, the fluorescence intensity has been normalized to the maximal intensity in aqueous buffer.

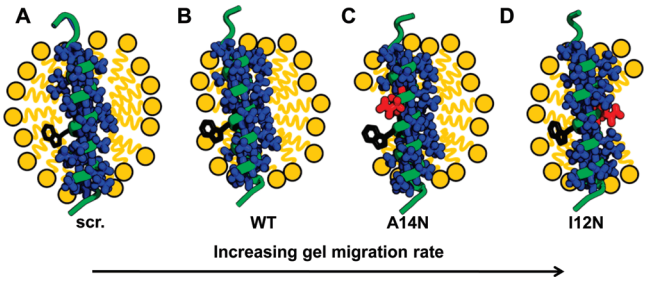

Figure 5. Schematic representation of detergent−peptide complexes. Detergent−peptide complexes are arranged from left to right according to decreasing relative particle size based upon SDS−PAGE migration rates. Ala and Ile residues are colored blue; Trp is colored black and Asn red. In this schematic, peptides that have faster gel migration rates are depicted as having less detergent bound. For WT (B), Trp fluorescence results show that SDS is not distributed evenly along the peptide sequence and that the side containing the Trp along with Ala residues is less solvated than the Ile side. The role of the lipopathic nature of these peptides (see Discussion) is highlighted by analysis of the scrambled variant (A), for which the even distribution of side chain hydrophobicity allows for an increased overall level of detergent binding, in turn giving rise to a slower migration rate for the scrambled variant vs WT peptide despite their identical composition. Also depicted are the situations expected for a typical Ala-to-Asn mutation (A14N) (C) vs a typical Ile-to-Asn mutation (I12N) (D). In both latter cases, less detergent is bound at the location of the Asn residue. For situations in which Asn is not located on the same surface of the Trp residue (as is the case for I12N), we infer that the loss of detergent occurs locally where hydrophobicity is decreased. Additionally, the large change in the hydrophobic moment produced in the I12N variant produces an altered distribution of detergent in comparison to WT, where the Trp residue is now more "buried" in the micelle (D). Images of the TM segments were produced using PyMol (Schrödinger LLC).

in which the helix adopts a type of "interfacial" conformation. This situation allows for the polar residue to be accommodated in a less hydrophobic environment, even if it is surrounded by hydrophobic residues.

These two effects are also potentiated by the position of the mutated residue with respect to the core of the hydrophobic sequence. The overall interpretation of this work with respect to polar residue substitutions within a detergent-solubilized system is shown schematically in Figure 5.

We further observed that introduction of polar residues at most positions has only minor effects on membrane-based helicity, and the relatively low resolution of circular dichroism spectra limits our ability to detect minor kinks, or some local helix fraying. Interestingly, the A11N variant is unique in that it has a migration rate faster than that of any Ile-to-Asn variant, appearing as an outlier based solely upon its hydrophobicity. It is also the least helical among the Ala-to-Asn mutants and thus displays the smallest relative increase in helicity upon detergent solvation. Likely at this most central position, loss of hydrophobicity leads to local unfolding of the helix, which has previously been observed in other TM segments.[39,40] The I19N variant is another example in which loss of helical structure is detected by CD. This variant has a gel migration rate somewhat greater than that of the wild type, an observation that cannot be directly explained by a loss of hydrophobicity. We note, however, that substitution drastically decreases the local hydrophobicity at the C-terminus, removing any strongly hydrophobic residue for an extended stretch (e.g., sequence AAANAAKKK for residues 16−24), and leads to some loss of helical structure. Given that these peptides have been Lys-tagged for synthesis, this result may arise from extant electrostatic interactions that the Lys residues have with the anionic SDS detergent. Ala-to-Asn substitutions in this region may not produce the same effect because of preservation of the most terminal Ile, therefore yielding a less drastic hydrophobicity change and the accompanying preservation of the helical structure. These outliers indicate that detergent binding as a function of hydrophobicity alone is not the sole determinant of the gel migration rate, and a complex interplay of interactions is necessary to fully describe the detergent−peptide structures.

Features of SDS Solvation That Mimic Membrane Protein Folding. Strongly polar residues have been shown in many cases to have drastic effects on membrane protein folding, such as TM segment insertion,[10] as well as transverse positioning as observed in model bilayers.[41,42] These effects are often dependent upon the position of the residue in relation to the midpoint of the transmembrane segment sequence. The underlying sources of these effects have been observed in computational studies that show that polar residues near the ends of helices are less perturbing to membrane insertion, as the hydrophilic moieties may snorkel into the interfacial region; in contrast, polar residues positioned in more central TM regions may form H-bonds with the helix backbone or in extreme cases (i.e., for charged residues) lead to local bilayer defects.[43,44] In this work, SDS−PAGE migration rates are shown to be similarly sensitive to this positioning, and indeed, there is a corresponding correlation between the predicted position-dependent in vivo insertion propensities (Figure 3D). Our overall results therefore suggest that the variation in sequence-dependent peptide−SDS interactions observed in biophysical

processes may be similar to the variation in biological TM segment insertion and/or folding.

Our work also indicates that detergent systems are capable of stabilizing individual TM segments that contain both micelle- and aqueous-interactive surfaces. Such conformations have been similarly observed in studies of antimicrobial peptides,[45,46] as well as for the structures of various TM segments derived from membrane proteins.[47,48] In unfolding studies of full-length proteins, SDS-solvated helices are often considered as the "denatured" state.[49,50] It is clear from our studies that polar residues can greatly alter this state, a factor that must be taken into consideration for obtaining free energies of unfolding. Of equal importance is the implication from our work that in locations where "lipopathicity" occurs in natural TM segments, a single polar mutation in an otherwise hydrophobic face can propagate potentially drastic changes in local folding patterns, perhaps most significantly in proteins that have a membrane-spanning aqueous pore or channel that may attract the polar side chain. These revelations reinforce the notion that many of the same forces driving membrane protein folding in vivo are mirrored by detergent solvation.

■ ASSOCIATED CONTENT

Supporting Information. A figure showing the lack of correlation between SDS−PAGE gel shifts and peptide secondary structure and a table containing hydrophobicities, predicted free energies of partitioning of the TM segments studied here into various hydrophobic media, and calculated values of the hydrophobic moment of these segments. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

TM, transmembrane; SDS, sodium dodecyl sulfate; CD, circular dichroism spectroscopy; MRE, mean residue ellipticity; PAGE, polyacrylamide gel electrophoresis.

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