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Polar Networks Control Oligomeric Assembly in Membranes

Chad D. Tatko, Vikas Nanda, James D. Lear, and William F. DeGrado*

Department of Chemistry and Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received September 20, 2005; E-mail: wdegrado@mail.med.upenn.edu

Despite the central importance of membrane proteins to cell biology and pharmaceutical science, our understanding of the features determining their structures is at a primitive state when compared to that of water-soluble proteins. However, polar interactions are beginning to emerge as important features for folding of both natural proteins as well as designed peptides.[1] Polar interactions involving Asn, Gln, and protonated Asp and Glu residues have been shown to contribute significantly to the thermodynamics of assembly of model transmembrane peptides.[2] Their introduction into natural membrane proteins can lead to aberrant associations with pathological consequences.[3] Previously, we showed that the introduction of an Asn into an otherwise hydrophobic transmembrane peptide provided a strong driving force for association, but that the assembly process lacked specificity and led to the formation of dimers and trimers.[4] Here we show that both the overall affinity and specificity of the interaction can be increased through the introduction of a Ser or Thr side chain at a single position of the peptide to mimic a "polar clamp" spatial motif, such as those observed in natural proteins.[5] Although Ser and Thr are individually unable to induce association, they can cooperate with the Asn side chain to clamp the peptide into a trimeric conformation.[6] Thus, hydrogen-bonded interactions can be efficiently networked to yield highly stable, specific structures that fold cooperatively.

The 29 amino acid MS1 peptide has provided a useful system for studying the structural basis for membrane protein assembly.[7] Residues 3−20 define an aliphatic core of a well-folded helical peptide.[8] An Asn at position 14 is critical for oligomerization, and when this residue is converted to Val, Thr, or Ser, the peptide fails to assemble appreciably.[2b] Examination of a computational model of MS1 indicated that the full hydrogen-bonding potential of the Asn side chain was not satisfied in the trimeric conformation.[9] On the basis of predictions from a computational search of side chains in low-energy rotamers, polar residues were introduced that could form additional hydrogen bonds to the Asn carboxamides.[10] A Thr or Ser positioned one residue prior to the critical Asn appeared capable of receiving a hydrogen bond from an Asn side chain on a neighboring helix, while simultaneously donating a hydrogen bond to a carbonyl at position $i-3$ in its own helix, thereby creating an intricate network of hydrogen bonds that could only be formed in a trimeric conformation. Although related polar clamps have been observed in membrane proteins,[5] the designed i, $i-1$ motif described herein was not observed in a search of an updated database. Thus, the i, $i-1$ motif is a novel design.

Analytical ultracentrifugation of these variants indicated that the Ser and Thr mutations indeed increased the affinity as well as the specificity of the association. The association is measured in C_{14}-betaine micelles, and the molecular mass contribution of the detergent is eliminated by matching the density of the aqueous buffer to that of the betaine micelle with 12.5% D_2O.[11] The

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                d    a  d    a  d    a  d    a
MS1         BQLLIA VLLLIAV NLILLIA VARLRYL VG
MS1-V13S    BQLLIA VLLLIAS NLILLIA VARLRYL VG
MS1-V13T    BQLLIA VLLLIAT NLILLIA VARLRYL VG
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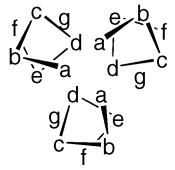

Figure 1. The sequences of the membrane peptides investigated; the mutated position 13 is shown in bold. The "B" residue is a β-alanine.

association constant and aggregation number were determined from global fits to multiple peptide-to-detergent ratios and centrifuge rotor speeds.

Previous models of MS1 assembly based on ultracentrifugation in micelles required a dimeric intermediate between the monomer and the trimer.[12] In contrast, mutation of MS1 to either MS1 V13T or V13S resulted in a completely cooperative monomer/trimer equilibrium that did not require the incorporation of a dimeric species to describe the data. In addition to improved cooperativity, the stability of the ternary complex increased by over 2 orders of magnitude. The pK_{ass} of trimerization for MS1 is 5.0, while MS1−V13T and MS1−V13S are 7.3 ± 0.1 and 7.2 ± 0.2, respectively. The resulting trimeric complex is stabilized by 10.0 kcal mol^{-1}, which is 3.0 and 3.2 kcal mol^{-1}, respectively, more than MS1. This amounts to more than 1 kcal mol^{-1} per Ser/Thr side chain. van der Waals contacts have been implicated in membrane association; however, the isosteric substitution from valine to threonine provides a substantial change in stability and specificity, suggesting additional membrane-embedded hydrogen bonds.[13]

Fluorescence resonance energy transfer is used to confirm the tight association of MS1−V13T.[14] The peptide is labeled at the N-terminus with 4-nitrobenzo-2-oxa-1,3-diazole (NBD) or tetramethylrhodamine, which can quench NBD fluorescence. The binding of membrane peptides is influenced by the mole fraction of detergent or lipid.[15] Therefore, examination of the fluorescence intensity at multiple ratios of peptide to detergent concentration can be used to determine the oligomerization affinity. Three different peptide concentrations were examined over a range of detergent ratios from 1:25 to 1:1500 (peptide:C14 betaine). The fluorescence of MS1−V13T is globally fit to determine the trimeric binding constant of 6.7 ± 0.7, which is within experimental error of ultracentrifugation data.[16] While polar interactions have been implicated as potential sources of transmembrane stabilization, few studies have directly probed their role in stabilizing membrane proteins. Multiple, networked polar interactions seem to reflect natural methods of transmembrane helix stabilization. The aspartate receptor has a QxxS motif that is believed to be important for dimerization.[17] Transmembrane peptides with multiple Ser residues

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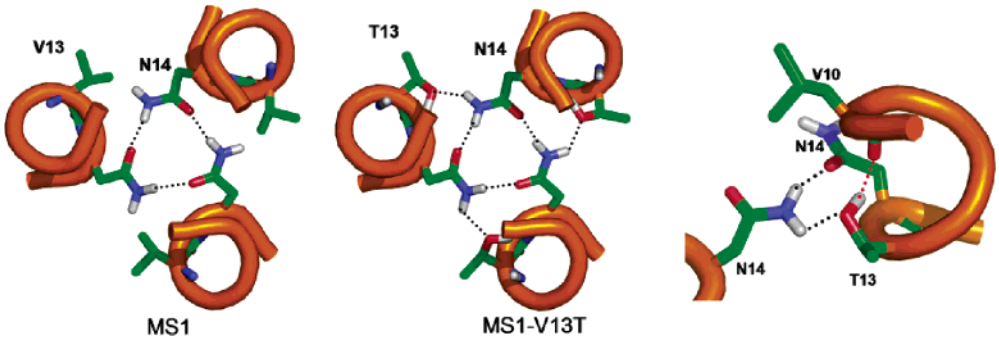

Figure 2. Model of the trimeric structures of MS1 and MS1−V13T. Side chains from residues 13 and 14 are shown. All others are omitted for clarity. Hydrogen bonds are represented as dashed lines. The polar network is shown in detail on the right. Interhelical hydrogen bonds are shown as black dashes, and the intrahelical hydrogen bond is shown in red.

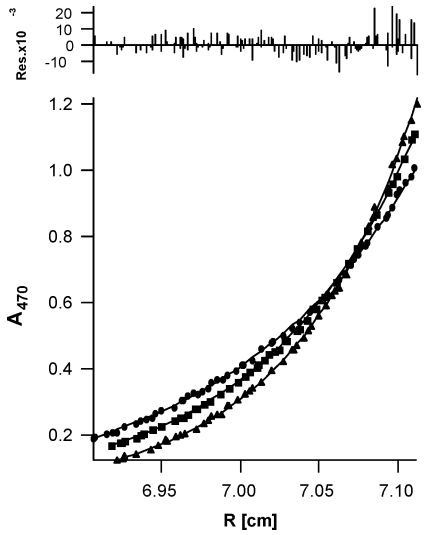

Figure 3. Analytical ultracentrifugation of NBD-labeled MS1−V13T. The peptides are solubilized in C14 betaine micelles. The samples are spun at 46, 48, and 50K rpm. The micelles are density matched with 12.5% D_2O. MS1−V13T NBD is 26.6 μM and the betaine is 4 mM, giving a 1:250 peptide-to-detergent ratio.

can also form dimers in bacterial membranes.[18] Substitution into the GxxxG motif of GpA has shown that small, polar residues, such as Ser, can also stabilize a dimeric conformation.[19] The reported findings here show that maximizing the hydrogen-bonding potential of residues into polar networks can strongly influence membrane protein folding. Often a hydroxyl alone is insufficient to nucleate assembly; however, as a hydrogen bond acceptor, Ser or Thr can be designed to synergistically stabilize and specify membrane protein assembly. In conclusion, de novo designed peptides with a novel motif of networked polar interactions successfully show effective control of membrane protein organization. Further studies are currently being carried out to fully explore the structural and energetic roles of polar interactions and hopefully improve our understanding of intermolecular forces in the membrane.

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Supporting Information Available: Experimental details, CD analysis, and fluorescence resonance energy transfer data. This material is available free of charge via the Internet at http://pubs.acs.org.

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