Influence on Proline Upon the Folding and Geometry of a Model Transmembrane Peptide

Rachel Thomas
University of Arkansas, Fayetteville

Follow this and additional works at: http://scholarworks.uark.edu/inquiry

Part of the Biochemistry Commons

Recommended Citation
Available at: http://scholarworks.uark.edu/inquiry/vol10/iss1/5
INFLUENCE OF PROLINE UPON THE FOLDING AND GEOMETRY OF A MODEL TRANSMEMBRANE PEPTIDE

By Rachel Thomas
Department of Chemistry and Biochemistry

Faculty Mentor: Roger Koeppe II
Department of Chemistry and Biochemistry

Abstract

The orientations, geometries and lipid interactions of designed transmembrane (TM) peptides have attracted significant experimental and theoretical interest. Because the amino acid proline will introduce a known discontinuity into an alpha-helix, it is important to measure the extent of helix kinking caused by a single proline within an isolated TM helical domain. For this purpose, acetyl-GWWLALALAP<sup>10</sup>ALALALWWA-ethanolamide was synthesized, and pairs of deuterated alanines were included by using 60-100% deuterated fluorenylmethoxycarbonyl-L-alanine (fMoc-d<sub>4</sub>-L-Ala) at selected sequence positions. Solid-state deuterium (H) magnetic resonance spectra from oriented, hydrated samples (1/40, peptide/lipid; using several lipids) reveal signals from many of the alanine backbone deuterons as well as the alanine side-chain Ca methyl groups, whereas signals from Ca deuterons have not been observed for similar peptides without the proline. It is conceivable that altered peptide dynamics may be responsible for the apparent “unmasking” of the backbone resonances in the presence of the proline. Data analysis based upon the method known as Geometric Analysis of Labeled Alanines (“GALA”) revealed that the peptide helix is significantly distorted due to the presence of the proline. In order to make available valuable additional data points for evaluating the segmental tilt angles of the two halves of the peptide, it may be advisable to substitute selected leucines with d<sub>4</sub>-alanine. Together the results suggest that the central proline influences not only the geometry but also the dynamics of the membrane-spanning peptide. The results are important for understanding the functional role of proline in several biological families of membrane proteins, including ion channels and others.

Introduction

Transmembrane proteins are integral components of lipid bilayer membranes and have a variety of functions in biological systems, including acting as channels for ion transport and as receptors for cell signaling. Indeed, about 20-30% of the human genome encodes membrane proteins. In spite of their prevalence, the fundamental molecular interactions between membrane lipids and membrane proteins are poorly understood. For this reason, model systems consisting of designed membrane-spanning peptides are needed to understand the complex properties of biological membranes. The WALP family of peptides is one such model system that is useful for exploring the nature of protein-lipid interactions (Killian et al., 1996). WALP peptides are transmembrane peptides consisting of the sequence acetyl-GWW(LA),LWWA-ethanolamide, in which a variable length core sequence of n residues is built using alternating aliphatic leucine (L) and alanine (A) residues. This core generally assumes an alpha-helical configuration (Van der Wel et al., 2002).

The tryptophan (W) residues at either end of the peptide interact with the lipid bilayer’s membrane/water interface, producing an anchoring and stabilizing effect on the peptide helix within the membrane. Peptide anchoring probably is accomplished through hydrogen bonding and orientation preferences of tryptophan’s aromatic indole rings with the lipid phosphocholine head groups and/or water. Peptide motion is still allowed somewhat, and more motional freedom is provided for amino acid side chains at the C-terminus due to the necessity for reorientation of the C-terminal indole rings to interact properly with the lipid bilayer (van der Wel et al., 2007). Hydrophobic mismatch, namely the difference between the lengths of the hydrophobic regions of membrane lipids and membrane peptides, has been observed in many studies to have consequences for the orientations and motions of both the lipid and peptide components. Nevertheless, research has shown that WALP peptides in general produce a lower than expected response to hydrophobic mismatch. A possible explanation for this result lies in the presence of the two pairs of anchoring tryptophans.

WALP19 is a 19-residue-long member of the WALP peptide family (with “n” = 6; above sequence). Having no helix-breaking residues, WALP19 remains a regular helix (unkinked) within lipid bilayers formed by several membrane lipids, including phosphatidylcholines with 14-, 16- or 18-carbon fatty acyl chains. WALP19 peptides also exhibit very little tilt (~4°) from the bilayer normal (Figure 1A).

![Figure 1: Schematic structures for (A) WALP19; (B) WALP19-Pro (Proline shown in green)](image-url)
In countless families of biological systems, proline residues buried within the membrane-spanning portion of integral membrane proteins have been found to be highly conserved. For example, membrane ion channels such as the acetylcholine receptor M1, yeast cytochrome b, and the lac carrier of E. coli contain proline residues in the central region of the transmembrane portions of the proteins (Cordes et al., 2002). The transport of a substrate through the channel requires a transient, reversible conformational change in portions of the protein. It has been hypothesized that proline’s function is to catalyze the cis-trans isomerization of one of proline’s peptide bonds within the helix, thus providing the necessary reversible conformational change in the channel protein. In addition, integral non-transport proteins also have been found to contain proline, such as the IKe phage protein and the avian sarcoma virus glycoprotein, and it has been suggested that the proline may serve as a ligand site for protein-cation interactions (Brandl and Deber, 1986). It has also been found that the introduction of a possible kink-inducing proline residue results in a global “loosening” of the entire WALP helix, not just in the vicinity of the proline itself (Demmers et al., 2001). Structures of various proline-containing transmembrane peptides determined by X-ray crystallography have been found to exhibit various kink angles (Cordes et al., 2002) as shown below in Figure 2.

![Figure 2. Various kink angles found through analysis of protein crystal structures (Cordes et al., 2002). The labels 1C3W, etc. are code names for structures in the Protein Data Bank (see http://www.rcsb.org/pdb).](image)

Despite the highly conserved nature of proline and its potential for a variety of biological roles, the true function of the residue remains to be determined. The presence of proline, with its rigid ring structure, within the transmembrane complex could enhance the response to hydrophobic mismatch. Due in part to its rigid ring structure, and to the removal of a hydrogen bond, proline will tend to interrupt the backbone structure within a standard α-helix, creating “kinks” (Figure 1B). The 5-membered ring is formed from the aliphatic side chain being joined to both the α-carbon and the amino group, restricting the torsion angle, Φ (Figure 3). In addition, proline is an imino acid and therefore will not hydrogen bond with the (i - 4) residue in the peptide sequence (Cordes et al., 2002). The “kinks” that are created by the proline residues are not merely geometric anomalies but are believed to be essential to the voltage-dependent ion transport abilities of several transmembrane protein channels (Sansom and Weinstein, 2000).

Within the above framework, an improved method for analyzing the function of proline within membranes is needed. A simplified, model system such as WALP19, within an oriented lipid bilayer, provides a direct method of examining proline’s contribution to membrane-spanning segments. Further, solid-state NMR spectroscopy provides a sensitivity that allows for insight into the dynamics as well as the structure of the peptide. To employ this method, the model peptides will need to incorporate deuterium (2H) labels in specific positions. Combining the WALP-19 model system with solid-state deuterium NMR spectroscopy will enable the determination of whether or not proline has indeed created a kink or influenced the motions within the WALP α-helix. The results may provide insight concerning the theory that kinking in transmembrane complexes produces greater flexibility for biological function. By altering the amino acids within model membrane-spanning peptide α-helices, much can be learned about the peptide/lipid interactions within biological membranes.

For the above reasons, I have synthesized WALP19 containing proline as the tenth residue, acetyl-GWWLALAPALALALWWA-ethanolamide. Different versions of WALP19-P10 (Figure 1B and Figure 3), were synthesized, each containing two deuterium labeled alanine residues (selected from among the underlined positions). These designed peptides were then analyzed through solid-state deuterium NMR spectroscopy to examine the influence of the proline within the helix.

**Methods**

**Preparation of Fmoc-L-Ala-d4**

Deuterated L alanine was first derivatized with an N-terminal fluoroaryl methoxy-carbonyl (Fmoc) protecting group. Five mmol (445 mg) of deuterated alanine (L-Ala-d4, Cambridge Isotope Labs, Inc.) were dissolved in 5 mL water containing 5 mmol (700 μl) triethylamine (TEA, Mallinckrodt) in a 250-mL round bottom flask. A stir bar was added and the solution was mixed briefly using a magnetic stir plate.

Next, 4.8 mmol (1.62 g) N-(9-fluorenyl methoxy carbonyl oxycarbonyloxy) succinimide (Fmoc-ONSu) (Novabiochem) was dissolved in 5 mL acetonitrile in a 20-mL....
beaker with gentle heating. This solution was added to the predissolved alanine in water/TEA and stirred for 4 hours. TEA was added as necessary during the reaction to maintain the pH between 8.5 and 9.

The mixture was then filtered through a Buchner funnel, with filter paper and the precipitate being discarded. The round bottom flask was rinsed with methanol and the filtrate was returned. The sample was then concentrated on a rotary evaporator until the Fmoc-L-Ala-d4 dried to a thick, yellowish gel.

Sixty mL of HCl (1.5 N) was added to the concentrated filtrate in 10-mL aliquots, while swirling the flask. The flask was then sonicated for 5-7 minutes to further dissolve the pellet. This suspension was then filtered with a Buchner funnel, the HCl poured off, and cold methyl-t-buty! ether (MtBE) was used for rinsing. The cleaned precipitate was then dried on the vacuum line overnight.

A recrystallization step was necessary to obtain a sample of acceptable purity. The Fmoc-Ala-d4 was dissolved in 10-15 mL of ethyl acetate while heating in a water bath. The undissolved material was removed by filtration through a glass frit. The filtrate was added to a crystallization vial and approximately 4 mL hexane (in 1 mL aliquots) was added to the solution. The sample was then put into the freezer for 48 hours and checked periodically for crystals. If no crystals had formed, the volume was reduced by blowing dry nitrogen gas, and the sample was returned to the freezer. Once crystals had formed, they were filtered on a Buchner funnel and dried under vacuum overnight.

**Solid-Phase Peptide Synthesis**

The WALP19-P10 peptides were synthesized by “FastMoc” solid-phase synthesis methods using an Applied Biosystems 433A synthesizer (Table 1). Commercially available fluorenlymethoxy-carbonyl (Fmoc) protected L amino acids (Novabiochem) were weighed out and placed into synthesis cartridges. (However, because of its position at the N-terminal, the acetyl-glycine residue does not possess the Fmoc protective group). For the deuterium labels, one cartridge contained 60% Fmoc-Ala-d4 (40% non-deuterated Fmoc-Ala) and the other 100% Fmoc-Ala-d4 (synthesized above). The amino acid residues were assembled into peptides using the automated synthesizer and an Fmoc-Ala-Wang resin (Novabiochem).

The final peptide was then removed from the resin through an ethanolamine cleavage, which is the final deprotection step for the peptide. The peptide-containing resin was mixed in a 20% ethanolamine mixture (8 mL dichloromethane and 2 mL ethanolamine). The mixture was flushed with nitrogen gas, covered with parafilm, and wrapped in foil. The sample was then placed on a mixer for 48 hours.

The resulting slurry was then filtered through a 10-mL glass filter and rinsed 4 times each with 5 mL each time with dichloromethane (DCM) and trifluoroethanol (TFE). In a 250-mL round bottom flask, the sample was placed on a rotovapor and allowed to concentrate to a minimal volume. It was then precipitated overnight in deionized H2O in the refrigerator. The sample was then centrifuged at 14,000 RPM for 90-120 min. After a pellet had formed, the supernatant was poured off and the pellet was dried under vacuum.

Table 1. Synthetic Peptide Sequences with Ala-d4 residues underlined.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-GWWL&lt;sup&gt;AA&lt;/sup&gt;ALA&lt;sup&gt;AA&lt;/sup&gt;ALA&lt;sup&gt;AA&lt;/sup&gt;WWW&lt;sup&gt;AAA&lt;/sup&gt;-ethanolamide</td>
<td>1 mg/mL</td>
</tr>
<tr>
<td>Acetyl-GWWL&lt;sup&gt;AA&lt;/sup&gt;ALA&lt;sup&gt;AA&lt;/sup&gt;ALA&lt;sup&gt;AA&lt;/sup&gt;WWW&lt;sup&gt;AAA&lt;/sup&gt;-ethanolamide</td>
<td>1 mg/mL</td>
</tr>
<tr>
<td>Acetyl-GWWL&lt;sup&gt;AA&lt;/sup&gt;ALA&lt;sup&gt;AA&lt;/sup&gt;ALA&lt;sup&gt;AA&lt;/sup&gt;WWW&lt;sup&gt;AAA&lt;/sup&gt;-ethanolamide</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>

Quantitation steps were performed after dissolving the peptide pellet in 4 mL TFE. Then, 10 μL of WALP19-P10 in TFE was mixed with 4 mL methanol. An Agilent 8453 model UV-Visible Spectrophotometer set at 280 nm (absorption wavelength for tryptophan) was used to determine the average molar and mass concentrations. A 20 μL aliquot of the peptide was diluted with TFE to the concentration of 1 mg/mL prior to analysis by reversed-phase high-performance liquid chromatography (HPLC). The HPLC sample was analyzed to determine the purity of the peptide. Each peptide produced a single peak, illustrating good sample purity, at an elution time of approximately 4.9-5.0 min (Figure 4A).

From each HPLC sample, 50 μL was transferred to a second vial and diluted once more with 450 μL of TFE (~0.1 mg/mL, resulting solution). Electrospray ionization mass spectrometry was used to verify the peptide molecular mass (Figure 4B).

**Oriented Sample Preparation**

Portions of each synthetic, deuterated peptide were incorporated into hydrated lipid bilayers of dimyristoylphosphatidylcholine (DMPC; 14 C), dilauroylphosphatidylcholine (D LPC; 12 C) or dioleoyl-phosphatidylcholine (DOPC; 18 C). A peptide-lipid mixture at a 1/40 peptide/lipid ratio (95% methanol, 5% water) was evenly applied on 40 glass slides and dried under vacuum (<1.5 Pa) for 48 hours. The slides were
then hydrated 45% (w/w) with $^2$H-depleted water, stacked and sealed in a cuvette. The samples were allowed to orient into a liquid crystalline phase, incubated at 40 °C for at least 48 hours (until the peptide-lipid mixture was no longer cloudy) prior to measurements (Figure 5).

Solid-State NMR Spectroscopy

Solid-state NMR technology involves the placement of a sample in a strong magnetic field and sending rapid pulses of radio waves to interact with the molecules. All solid-state nuclear magnetic resonance experiments were conducted using Bruker Avance spectrometers with a proton frequency of 300 MHz. Phosphorus NMR spectroscopy ($^{31}$P nucleus) with broadband $^1$H decoupling was used to confirm that the bilayer lipids were quite well oriented at 323 °K. Spectra were recorded using two sample orientations, with the bilayer normal either parallel ($\beta$ =0°) or perpendicular ($\beta$ =90°) to the applied magnetic field. Spectra of lipid bilayers containing WALP19-P10 were compared to those of pure lipid to determine if the introduction of the peptide was disrupting the bilayer formation.

Deuterium NMR (2H) experiments were performed at both $\beta$=0° and $\beta$=90° orientations using quadrupolar echo pulse sequence with full phase cycling. Eight hundred thousand free induction decays were obtained for these spectra, compared to only 128 for the $^{31}$P spectra.

Circular Dichroism Spectra

A circular dichroism (CD) spectrum, obtained using polarized light, indicated that WALP19-P10 maintained an alpha-helical conformation (Figure 6). Of particular importance are the characteristic minima at 208 nm and 222 nm, as well as the peak of maximum intensity near 195 nm.

GALA Analysis

The $^2$H NMR data of the peptides were analyzed using a program designed in the Koeppe laboratory, GALA (Geometric Analysis of Labeled Alanines) implemented in Microsoft Excel. The quadrupolar splittings ($\Delta\nu_q$) from the spectra were determined, quadrupolar splittings of CD$_2$ groups in Alanine residues are defined as distances between corresponding peaks.

The splittings are dependent upon the carbon-deuteron bond orientation, thus providing insights into the structure of the peptide itself. The following expression in Equation 1 shows this relationship:

$$\Delta\nu_q = \frac{3}{2}S_\alpha \epsilon^2 qQ \left( \frac{1}{2} \cos^2 \theta - 1 \right) \left( \frac{1}{2} \cos^2 \beta - 1 \right)$$

Where:

$$\frac{\epsilon^2 qQ}{h}$$ - static coupling constant (~168 kHz for aliphatic C-D bond)

$S_\alpha$ - order parameter (~0.87 for WALP peptides)

$\theta$ - angle between the applied magnetic field and alanine Ca-C$\beta$ bond

$\beta$ - angle between membrane normal and magnetic field direction (0° or 90°)

$\theta$ depends on the orientation of the peptide itself, and is expressed by the following relationship:

$$\theta = f(\tau, \rho, \epsilon)$$

Where:

$\tau$ - tilt of the peptide

$\rho$ - rotation of the peptide with respect to the Ca of Gly

$\epsilon$ - angle between alanine Ca-C$\beta$ bond and the peptide axis

The $\Delta\nu_q$ term is observable and all other variables are constant except $\theta$. This is a variable term dependent upon $\tau$, $\rho$, and $\epsilon$. Using these relationships, the GALA program varied the $\tau$, $\rho$, and $\epsilon$ values to give the best agreement between the calculated and observed quadrupolar splitting values, $\Delta\nu_q$ (Van der Wel et al., 2002). A 3-D matrix was created in Excel and using the Grid-Search method, the terms with the lowest root-mean-squared deviation (RMSD) values were determined, indicating the best-fit between the calculated and observed splittings.

Helical wheel plots were then produced that illustrated the RMSD values (or the average difference between the observed and calculated values) as well as the Ca positions for each alanine label (in degrees).

Results

Phosphorus ($^{31}$P) NMR
The phosphorus NMR spectra indicated that the lipids were orienting well into parallel bilayers. At $\beta=0^\circ$, this was shown by a tall peak (oriented peak) at approximately 20-23 ppm depending upon the lipid. A much smaller peak at approximately -20 ppm indicated a small fraction of the lipids were not orienting (non-oriented peak), probably representing randomly oriented bilayers. Nevertheless, the smaller peak was seen in both pure and peptide-containing lipid samples, indicating that the peptides were not disrupting the bilayer (Figure 7). All samples produced comparable spectra.

Deuterium ($^2$H) NMR Spectra

Deuterium NMR results produced two sets of peaks for the CD$_3$ groups of the two different labeled alanines in each designed peptide, the taller being representative of the 100% label, and the shorter peak of the 60% label. Figure 8 shows the $^2$H NMR results for samples oriented at $\beta=90^\circ$ in each of the lipids.

The quadrupolar splitting values were determined as the distances between each symmetrical pair of peaks in each spectrum. These values for the deuterated alanine side chains in WALP19-P10 are tabulated in Table 2. The large changes in the quadrupolar splittings that are induced by proline-10 indicate that the proline has significant influence upon the helix geometry and orientation.

Table 2. Quadrupolar splittings values ($\Delta\nu_q$) for alanine methyl side chains from $^2$H NMR spectra. The $L10$ columns refer to WALP19 itself (van der Wel et al., 2002). The $|\Delta\nu_q|$ columns show the absolute difference in signal between WALP19 and WALP19-P10.

<table>
<thead>
<tr>
<th></th>
<th>DLPC</th>
<th>DMPC</th>
<th>DOPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>L10</td>
<td>P10</td>
<td>L10</td>
</tr>
<tr>
<td>5</td>
<td>7.2</td>
<td>17.3</td>
<td>10.1</td>
</tr>
<tr>
<td>7</td>
<td>3.3</td>
<td>2.6</td>
<td>0.7</td>
</tr>
<tr>
<td>9</td>
<td>11.3</td>
<td>16.6</td>
<td>7.3</td>
</tr>
<tr>
<td>11</td>
<td>2.6</td>
<td>10.7</td>
<td>8.1</td>
</tr>
<tr>
<td>13</td>
<td>14.0</td>
<td>24.3</td>
<td>10.3</td>
</tr>
<tr>
<td>15</td>
<td>0.0</td>
<td>15.7</td>
<td>15.7</td>
</tr>
</tbody>
</table>

$|\Delta\nu_q|$ values for the $\alpha$ peaks were not included in the GALA analysis at this stage of the project.

GALA Analysis

The GALA analysis was attempted by treating WALP19-P10 as a single, non-distorted $\alpha$-helix. It was not surprising to find that doing so produced high root mean squared deviation (RMSD) values. The resulting very poor agreement between observed and calculated quadrupolar splitting values (Figure 10) confirmed a disruption within the $\alpha$-helix, illustrating proline's role as a "helix-breaking" residue. It should be noted that the curves in Figure 10 represent the "best" fits to the data. Even so, many of the
data points fall far from the curves in Figure 10, making it impossible to fit a regular alpha helix to the deuterium NMR data. This feature represents the key evidence that proline is distorting the helix. Residue 9, in particular—immediately preceding proline and adjacent to the missing hydrogen bond—fit very poorly and therefore was removed from the data set. Nevertheless, the exclusion of the 9th residue continued to result in unsatisfactorily high RMSD values: 2.5 kHz (DLPC), 3.0 kHz (DMPC), and 3.2 kHz (DOPC). This analysis suggests that proline significantly distorts the position of not only the 9th but also other residues as well.

**Discussion**

From a survey using X-ray crystal structures of transmembrane α-helices in transmembrane proteins, Cordes et al. (2002) found that the presence of proline, which was most often found near the center of the α-helices, was correlated with kinks (referred to as “hinges”) in the systems. One way in which the authors compared the systems was to align them at the proline residue. When this was done, the C-termini showed good alignment beyond the aligned five-membered proline rings; however, the N-termini exhibited a wide variety of directions and kink angles, ranging from 5° to 70° and demonstrating the increased complexity for this portion of the proteins (Cordes et al., 2002).

![Helical wheel plots for each of three lipids based upon GALA analysis using a canonical undistorted helix. The lipids are dilaurylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), and dioleoylphosphatidylcholine (DOPC).](http://scholarworks.uark.edu/inquiry/vol10/iss1/5)

Figure 10. Helical wheel plots for each of three lipids based upon GALA analysis using a canonical undistorted helix. The lipids are dilaurylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), and dioleoylphosphatidylcholine (DOPC).

The results from 3H NMR analysis for WALP19-P10 agree with the general picture found by the survey of Cordes et al. (2002) and confirms the function of proline as a helix-breaking residue. Indeed it was impossible to perform a complete GALA analysis for the six labeled core alanines when treating the peptide as one single α-helix. The presence of the proline at the central position 10 disrupts the helix and disrupts the analysis. To proceed further, it will be interesting to determine individually the tilt of each half of the WALP19-P10 peptide, before and after the proline. The individual segmental tilt values will enable us to evaluate the magnitude of the proline-induced “kink” in WALP19-P10. To complete such an analysis, definitively, will require more than three labeled alanines in each segment. For this purpose, I would suggest introducing some single mild mutations, thus changing Leu to d4-Ala at several selected sequence positions, but changing only one leucine at a time. As a control, one already known alanine (data in Figure 8 and Table 2) could be deuterated along with the newly introduced alanine (also deuterated, but with a different extent of isotope enrichment).

A strategy of introducing additional deuterium-labeled alanines (by leucine replacement) could potentially allow the segmental tilt to be determined for portions of WALP19-P10 before and after the proline. This approach also could allow the kink angle induced by proline to be evaluated in several different lipid bilayer membranes.

In fundamental agreement with the finding of Cordes et al. based upon a survey of 199 proline-containing transmembrane helices from protein crystal structures, proline’s effect on the N-terminus of WALP19-P10 is complex. Likely, the disruption in the hydrogen bonding of the N-terminus is a cause for complications, namely the particular lack of fit for Ala-9 within any helix defined by any of the other alanines to date. The presence of what are believed to be the Cα-deuteron peaks further suggest that there is a change in the dynamics of the protein segment, which could be caused by an increase or decrease in the flexibility of the peptide. Hydrogen bonds are responsible for producing the α-helical structure of the peptide and removal of particular bonds would be expected to have an impact on the protein’s motion. A complete analysis of dynamics as well as peptide geometry will require further research.

An additional approach to solving this problem (if necessary) would be to alter the model system substantially by mutating all of the leucines of the N-terminal portion of the peptide to alanines. The new system would be created with and without the presence of proline at residue 10 in order to compare the two peptides. Then, each alanine could be labeled — single residues or in pairs — and analyzed in the same manner as before. This approach would eliminate any difficulties that could arise due to leucine-to-alanine mutations and might allow a full characterization of the N-terminus.

In conclusion, my results have shown that the presence of proline in the center of an α-helix causes a disruption in the geometry of the peptide, a “kink,” while still allowing at least a majority of the helical conformation to be maintained. Peptide dynamics also are influenced by proline. Because the effects upon the two halves of the peptide may be different, the introduction of additional deuterated alanine labels should be considered. Complete characterization of the influence of proline within the WALP19-P10/lipid bilayer model system will require more experiments, yet is likely within reach using available methods.

**References**


Mentor Comments:

Professor Roger Koeppe uses the term "groundbreaking" in writing about the importance of Rachel Thomas' undergraduate research and the foundation it provides for additional investigations:

In her undergraduate research, Rachel Thomas did groundbreaking work to characterize the amino acid proline in a model peptide helix that spans a lipid bilayer membrane. The work is of fundamental importance for understanding proteins that function in biological membranes. In addition to characterizing the influence of proline upon the peptide geometry, Rachel found surprising and novel results: Deuterium labels on the alanine backbone alpha carbons (C-alpha deuterons), that had previously been "silent" in the magnetic resonance spectra recorded for related model peptides, seemed to become "unveiled" by the presence of the proline residue. These unexpected findings suggest that proline may alter not only the geometry but also the time scale and extent of motions within the transmembrane peptide helix. Her results therefore pave the way for a series of follow-up experiments. It is notable also that Rachel proposes future strategies for extending her landmark work. Indeed she continued the project several more weeks in order to complete several of the additional experiments that she proposes in the text.