THE DEVELOPMENT OF A MODEL OF ALPHA HELIX FORMATION FOR TRANSMEMBRANE PEPTIDES

A Senior Honors Thesis

By

GEOFFREY ALEXANDER FUNK

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements of

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

April 2000

Group: Molecular Genetics

THE DEVELOPMENT OF A MODEL OF ALPHA HELIX FORMATION FOR TRANSMEMBRANE PEPTIDES

A Senior Honors Thesis

By

GEOFFREY ALEXANDER FUNK

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements
For the Designation of

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOW

Approved as to style and content by:

J. Martin Scholtz
(Fellows Advisor)

Edward A. Funkhouser
(Executive Director)

April 2000

Group: Molecular Genetics

THE DEVELOPMENT OF A MODEL OF ALPHA HELIX FORMATION FOR TRANSMEMBRANE PEPTIDES

A Senior Honors Thesis

By

GEOFFREY ALEXANDER FUNK

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements
For the Designation of

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOW

Approved as to style and content by:

J Martin Scholtz

J. Martin Scholtz
(Fellows Advisor)

Edward A. Funkhouser
(Executive Director)

April 2000

Group: Molecular Genetics

ABSTRACT

The Development of a Model of Alpha Helix Formation

for Transmembrane Peptides. (April 2000)

Geoffrey Alexander Funk
Department of Biochemistry & Biophysics
Texas A&M University

Fellows Advisor: Dr. J. Martin Scholtz
Department of Biochemistry & Biophysics /
Department of Medical Biochemistry & Genetics

Researchers have studied the folding and binding properties of peptides in water for many years, but only recently has anyone attempted to explore those same tendencies in an environment similar to that of a transmembrane protein incorporated into the phospholipid bilayer of the cell. To this end, we have been working on the synthesis of a group of peptides with the general sequence $^{+}H_3N$-Ala_2-Leu_3-Ala_7-Trp-Ala-X-Ala_{10}-Lys_6-COOH, where we will substitute all twenty naturally occurring amino acids into position X. We have successfully synthesized and purified the peptide in which the guest position X is filled by an isoleucine—due to the difficulty of the sequence involved, we have been unable to synthesize and purify eighteen of the remaining nineteen. Peptides, once synthesized, are characterized by MALDI mass spectrometry and HPLC and purified peptides are studied using circular dichroism (CD) spectroscopy to determine the α-helicity. Initial results suggest that the transmembrane environment will indeed alter the propensities of the various amino acid residues to form α-helices,

though to what degree still remains to be seen. Once complete, this study should make it possible to determine a system for predicting α-helix formation in membrane proteins and determine the basic rules that guide such helix formation.

ACKNOWLEDGEMENTS

I would like to thank both Dr. J. Martin Scholtz, my advisor, and Dr. Michael Merchant, our collaborator from Texas Woman's University, for the opportunity to do this research during my time at Texas A&M.

I would also like to thank all the members of the Scholtz and Pace labs that have guided me in one way or another since I first entered the lab in the summer of 1997. I have learned a great deal during my time here because of their efforts. Specifically, I would like to mention the following members:

Scott Celinski

Mary Elizabeth Huffines

Eric Nicholson

Ron Peterson

Jennifer Ross

Jason Schmittschmitt

TABLE OF CONTENTS

TABLE OF FIGURES

Page

INTRODUCTION

Studies in recent years have shown that the propensity of certain residues to form α-helices or β-sheets is altered from their tendencies in water when studied in membrane proteins. Residues that lend themselves to one type of secondary structure can reverse these inclinations from water to a lipid bilayer according to Li and Deber (1994) and thus lend no data when studying an environment similar to that which would normally be found. As such, while large amounts of data have been compiled, these data do not necessarily lend themselves to practical applications. A different approach to the problem is necessary to answer some of the questions in this much-researched area of biochemistry.

To this end, I have helped to begin the work to develop a model of α-helix stability in a membrane environment similar to that of the cell. By studying a series of synthetic peptides using techniques such as circular dichroism spectropolarimetry (CD) and fluorescence and modifying a single guest residue position between the various peptides, it should be possible to determine a model of the propensity of various residues to form α-helices while situated in the bilayer.

This thesis follows the style and format of *Analytical Biochemistry*.

The peptide we have been developing is a 31-residue peptide with the sequence $^{+}H_3N$-Ala_2-Leu_3-Ala_7-Trp-Ala-X-Ala_{10}-Lys_6-COOH, a peptide similar to that used by Liu and Deber (1997) in some of their work. This sequence is particularly well suited to the task for a number of reasons:

1) The highly polar poly-Lys at the N-terminus of the peptide provides a terminal portion of the peptide that will orient itself into the extra-lipid and polar environment, thus favoring a transmembrane orientation.
2) Conversely, the highly hydrophobic poly-Ala and poly-Leu stretches should cause the majority of the peptide to bury itself within the hydrophobic lipid layer, similarly favoring a transmembrane orientation.
3) The guest residue position, the X-site, is in a central location within the peptide. This will decrease the possibility that the polar environment around the lipid layer will affect the secondary structural tendencies of the guest residue.
4) The centrally located Trp absorbs ultraviolet light at a wavelength of 275nm, allowing for easier purification using the techniques of HPLC and FPLC, as well as opening up the possibility for fluorescence studies when that phase of this project is reached.
5) Alanine and leucine both have high α-helix forming propensities in water-based studies. With such a high concentration of these residues in the peptide, we can be quite certain that the peptide will show high helical content in our initial non-membrane-associated CD work.

6) The peptide is also long enough that it should be able to fold effectively and extend across the membrane so that a transmembrane configuration may be more favorable than a surface configuration.

Thus, this peptide should be able to meet all of the necessary requirements for the studies we are attempting to perform.

The lipid membrane may consists of any standard phospholipid so long as the phase transition temperature of the lipid is low enough that we do not have to worry about whether or not the lipid is undergoing phase transition and possibly leaving the liquid phase. If this were to happen, we would be unable to determine whether or not any data that we might acquire was the result of the crystalline phase or the actual transmembrane peptide in the appropriate configuration in the liquid phase.

The important feature of the lipid membrane is that of thickness—as mentioned earlier, if the peptide extends too far beyond the membrane after folding (too long), there is a good chance that a transmembrane configuration would force the hydrophobic core of the peptide into the polar extra-membrane solvent. Conversely, if the peptide folds such that it cannot extend across the entire membrane (too short), the hydrophilic termini of the transmembrane peptide will be exposed to the hydrophobic interior of the lipid bilayer. Figure 1 demonstrates these concepts:

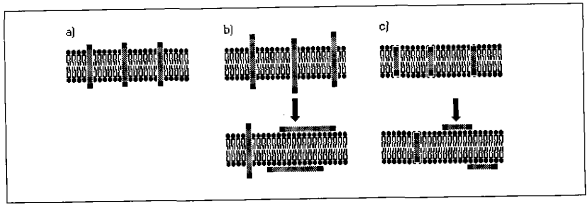

Figure 1: Tendencies for peptide orientation as a result of membrane thickness. In a) the folded peptide extends such that only the hydrophilic regions (black) of the peptide are exposed to the polar exterior environment, while the hydrophobic regions (gray) reside solely in the non-polar interior of the bilayer. In b) the bilayer is too thin, causing exposure of the hydrophobic peptide core to the polar environment outside the bilayer; in this situation, a surface configuration may be favorable. Likewise, in c) the membrane is too thick, causing the hydrophilic termini of the peptide to be exposed to the hydrophobic bilayer core, resulting in a surface configuration.

In either of these situations, such exposure is highly unfavorable—as a result, the more favorable surface configuration for the peptide would most likely be favored. This is not to say that there will be no instances of the transmembrane configuration, but instead that it is disfavored in comparison to the surface orientation.

BACKGROUND

Many years of study have gone into the propensity of various amino acid residues to form α-helices in water. In such an environment it is now possible both to predict the α-helical propensity of a given sequence and to design a specific sequence so as to induce helix formation. Little attention, however, had been paid to the realm of membrane proteins and helix formation in such a hydrophobic environment until recent years.

No definite mechanism has been provided for the integration of transmembrane proteins into membranes, but it is known that α-helices are extremely good structures in such proteins. This is due to the intrinsic ability of the α-helix to bury the polar backbone groups from the hydrophobic environment of the lipid bilayer. The data regarding the α-helix propensity of the various residues in water, however, do not lend themselves to solid conclusions about the same propensity in a lipid bilayer (Li and Deber, 1994). Thus, a definitive model needs to be developed for membrane proteins.

As we desire to isolate and study membrane proteins, it will be important to maintain the transmembrane configuration of the proteins. Liu and Deber (1997) demonstrated that the length of the folded peptide and the fluidity of the bilayer have effects on the conformation of the protein in relation to the bilayer. With thin layers, addition of decane (incorporated into the center of the membrane) or cholesterol leads to an increase in the number of peptides in the transmembrane configuration, while in thick layers such addition drives the proteins to the surface configuration. This can lead to

effective isolation of either configuration, but will more profoundly affect my studies in the section of transmembrane peptides.

SPECIFIC AIMS

As was mentioned prior to the start of this project, we were aware that the scope of the project was quite large and it was unlikely that the work would be completed within the time allotted. Still, any progress that could be made would be worth the effort and would hasten the completion of the work. From the outset, the following goals were set forth:

1) Synthesize the transmembrane peptides with each of the twenty amino acids substituted in at the guest position.

2) Conduct preliminary studies using CD and fluorescence spectroscopies in water to confirm the helical propensity of the peptides. In addition, studies in non-polar solvents, such as methanol, would be completed at this time.

3) Determine a refined technique for inserting the peptide into vesicles and for isolating the transmembrane configuration by altering bilayer width and fluidity.

4) Perform similar CD and fluorescence studies with the various forms of the transmembrane peptide.

5) Begin to formulate hypotheses on helical propensity in membranes.

MATERIALS AND METHODS

Peptide Synthesis:

Peptide synthesis was attempted using three different methods: 1) automated Fmoc solid phase chemistry; 2) automated FastMoc solid phase chemistry; and 3) FastMoc solid phase chemistry by hand.

Automated syntheses were carried out on an Applied Biosystems 431A Peptide Synthesizer either by continuous-flow Fmoc or FastMoc solid phase chemistry. In either method, peptides were synthesized upon Rink resin, activation of the amino acid residues occurs using N-methylpyrrolidone (NMP) and 1-hydroxybenzotriazole (HOBt), and coupling uses Fmoc-protected amino acids. The notable difference between Fmoc and FastMoc chemistry involves the use of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in the HOBt/NMP activation, as well as *in situ* activation by diisopropylethylamine (DIEA), in FastMoc chemistry, resulting in higher efficiency and shorter coupling times.

Hand syntheses of peptides were carried out using the same FastMoc solid phase chemistry as discussed above, though coupling times and reagent concentrations (specifically molar ratios of amino acid : desired synthesis scale) were increased in the hopes of increasing coupling efficiency and synthesis accuracy further. A 0.03 mmol reaction scale was used (in terms of resin volume). The following method table highlights the three reactions of the hand synthesis:

TABLE 1 Non-automated chemical synthesis of peptides

Reaction	Reagents	Reaction Time
Deprotection	- 20% Piperidine in NMP with 1% Triton X-100	5 minutes, flip stack, 5 minutes
Coupling	- 40 molar excess of desired amino acid residue - 552 mg HOBt - 1365 mg HBTU - 1.260 mL DIEA in NMP with 1% Triton X-100	2-6 hours
Capping	- 5% Pyridine - 5% Acetic anhydride in NMP with 1% Triton X-100	15 minutes, flip stack, 15 minutes

This series of three reactions is repeated for every residue of the peptide. Cleavage from the resin occurs after the final capping reaction.

After each reaction, all samples undergo five consecutive washes in NMP with 1% Triton X-100 in order to remove any remaining reagents before beginning the next reaction.

Cleavage of the peptide from the resin involves two hours in a gyrorotary bath with an adequate volume of trifluoroacetic acid (TFA) / scavenger solution (5% anisole, 95% TFA), followed by extraction with approximately 40 mL cold tert-butyl methyl ether and lyophilization.

Peptide Purification:

Initially, peptide purifications were carried out on a reverse-phase LCC-500 PLUS from Pharmacia with a SpectraSERIES UV100 from Thermo Separation Products

and FPLCdirector software using an acetonitrile gradient in 0.1% TFA. Later purifications were carried out on the Pharmacia AKTA HPLC unit with a Waters 25mm column and the Unicorn software program, again using an acetonitrile gradient in 0.1% TFA. Fractions were collected according to absorbance at 275nm (absorbance maximum of Trp) for FPLC and at 275nm, 254nm, and 225nm for HPLC. To determine purity of peptides, reverse-phase FPLC, HPLC, and MALDI-TOF mass spectrometry using a Perspectives Voyager mass spectrometer were used.

Phospholipid Vesicles:

Peptide incorporation used a solution of the peptide in chloroform/methanol (2:1) and mixed with the lipid dioleoylphosphatidylcholine (DOPC) in the same solvent at a ratio of 1 mole peptide : 100 moles lipid. The solution is then evaporated under nitrogen using a RotoVap system, thereby creating a lipid film covering the surface of its container. This lipid film must then be rehydrated for 30 minutes in 10 mM Tris-HCl buffer, 10 mM NaCl, pH 7.0, with vortexing. After rehydration the lipids are sonicated on ice using a bath sonicator made by Laboratory Supplies Co., Inc., until the solution moves from an opaque white to a slightly more translucent bluish-white. The final concentration of the mixture should be 30 μM for CD studies and 4 μM for fluorescence spectroscopy.

Circular Dichroism Measurements:

CD spectra were recorded using an Aviv 62DS CD spectrapolarimeter using 1 cm quartz cuvettes at room temperature. Helicity was measured by observing the characteristic minima at 222nm and 208 nm in wavelength scans. The final spectra are analyzed with a number of available programs to determine α-helical content. Measurements were carried out in both polar and non-polar solvents in order to determine differences in peptide behavior between the two environments.

Cloning:

Cloning attempts were made using the pZH3 plasmid vector containing ampicillin resistance. Sequential restriction digests at the Sal*I* and BamH*I* sites were carried out to remove a 2 kb stuffer fragment, after which a 123-base fragment containing appropriate codons for our peptide and the restriction sites for BamH*I* and Sal*I* was restricted and then ligated into the cleaved plasmid. The sequence of that 123-base fragment is contained in Figure 2:

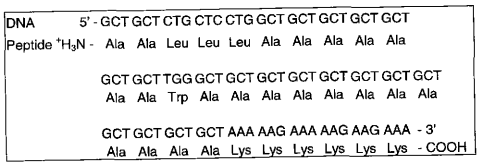

Figure 2: Coding sequence for transmembrane peptide insert and corresponding amino acid residues. The above DNA sequence corresponds to the desired DNA fragment to be inserted into the pZH3 plasmid. The template strand corresponding to the above coding sequence was ordered from Integrated DNA Technologies, Inc.

Transformations took place using electroporation and heat shock methods into a competent *E. coli* cell line. Transformed cells were plated on Luria broth (LB) agar plates containing ampicillin and allowed to grow for 24 hours. Any colonies found after 24 hours were transferred into test tubes containing a small volume of LB and growth-prohibitive concentrations of ampicillin and allowed to grow for a further 24 hours. Aliquots of these preparations were then purified using the Qiagen DNA mini-prep kits and subjected to Sal*I* and BamH*I* restriction digests. Agarose gel electrophoresis (1.2% agarose gels) was used after each digestion in this process to determine the presence or absence of appropriate sized fragments. Appropriate bands were purified from the agarose gel and used for further restrictions or ligations.

DISCUSSION AND RESULTS

Peptide Synthesis by Chemical Means:

To date, we have been able to synthesize and purify the isoleucine variant of our target sequence successfully. Some initial CD work has been done and will be discussed later. We may also have been able to synthesize a second variant, though that has yet to be confirmed. As such, we are currently attempting to chemically synthesize the peptide by hand (not using the automated synthesizer) and also to use a modified cloning procedure, the latter being undertaken by Jason Schmittschmitt, one of the graduate students in the lab.

The majority of our attempts to synthesize the peptides were made using the automated peptide synthesizer and Fmoc chemistry—unfortunately this method ran into a recurring problem in that the product of these syntheses was only ~80% pure, the rest of the product being of slightly lower mass when mass spectrometry was used to analyze the results of the synthesis. Calculations led to the determination that this impurity peak was a form of the peptide missing one alanine residue (ΔAla) but otherwise intact. Repeated attempts to purify using both FPLC and HPLC were unsuccessful in eliminating the impurity—both the transmembrane peptide and the ΔAla version came off the chromatography column at the same time and were inseparable by our techniques. With such a large impurity, any analytical data obtained is dubious. If there were a way to determine which alanine had been lost, it may have been possible to use this new sequence to create the rest of the peptides. With twenty alanine positions

involved, however, it is not possible to determine the exact sequence of the ΔAla peptide.

It is highly likely that the large hydrophobic content of this peptide is the very problem causing the synthesis difficulties. With up to a possible twenty-four hydrophobic residues (20 Ala, 3 Leu, and 1 at the X-position), this peptide is probably attempting to bury as many of the hydrophobic residues as possible before synthesis is even complete. The peptide is also quite long for synthesis: 20-mers and below are not uncommon for lab synthesis, but as the desired peptide gets longer, the efficiency of the syntheses generally go down.

This is a result of a number of factors—first, of course, the length of the peptide; second, the proximity of the active sites upon the resin bead on which the peptide is built; and third, the secondary structural characteristics of the sequence itself, to name a few. In combination, these can create an environment in which long syntheses become extremely difficult. If there is not an adequate distance between the active sites on the resin beads, it becomes possible for the highly hydrophobic regions of the transmembrane peptide to favor burying those hydrophobic regions by either aggregation of a number of strands of the growing peptide or simply by folding back upon itself. Either of these, one can reason easily, will result in a decrease in the coupling efficiencies—as a result, it is not unthinkable that a single coupling will not succeed in a number of strands.

This suggests several solutions that are now being incorporated into the current synthesis efforts or may be used in future efforts: 1) increase length of time given for

coupling reactions, thereby increasing the likelihood of even unfavorable couplings; 2) increase the concentrations of the activated amino acid residues, again increasing the chances of coupling through brute force; 3) use a detergent to decrease favorable secondary structure folding during synthesis and thus leave the ends of the peptides in construction more accessible for successful coupling reactions rather than being buried in some form of self- or inter-chain aggregates; and 4) use a resin with an increased distance between active sites to decrease the chance of interchain dimer- or oligomerization (though this would not have an effect if the problem is one of secondary structure during synthesis).

Due to problems with the apparatus itself, we have not been able to complete a FastMoc automated synthesis successfully, though the higher coupling efficiency associated with this chemistry led us to attempt FastMoc hand synthesis. This pursuit, due to a shortage of NMP, will not be completed prior to the submission of this thesis, though we should have indications as to whether or not chemical synthesis could be successful shortly. Of the possible solutions discussed above, we are incorporating the use of a detergent (1% Triton X-100) and increasing coupling times and amino acid : resin ratios.

That it is possible to generate this peptide via automated chemical synthesis is not in doubt—mass spectrometry and liquid chromatography have confirmed that the isoleucine variant of the transmembrane peptide is of the correct mass and is extremely pure. This may not be the most efficient way to produce the peptides, however, as the final yield full-length peptide is rarely so high or so pure as in the case of the isoleucine

variant. As such, it is still very important to pursue other approaches in order to optimize the production of the peptides.

Peptide Synthesis Using Molecular Genetics:

While initial results using a cloning approach for the synthesis of the peptide were not entirely promising, a current attempt headed by graduate student Jason Schmittschmitt seems to suggest that it may indeed be possible to create this peptide by creating a plasmid containing the coding sequence for the transmembrane peptide and using *E. coli* cells to mass-produce it. This work is also currently in progress and should be advanced enough to determine whether not this is a viable solution to the problem in the near future.

Initial attempts to clone the peptide into a vector used the pZH3 plasmid from Dr. Hu's lab in the Department of Biochemistry and Biophysics—this plasmid had an easily removed "stuffer" sequence of about 2 kb that could easily be cleaved with restriction enzymes. After purification to remove the 2kb stuffer fragment, it should have been possible to ligate our template fragment into the plasmid. For some reason, however, this never seems to have occurred successfully. We were able to obtain growth on ampicillin-resistant media of *E. coli* carrying the vector, but we were unable to successfully isolate our insert when analyzing the DNA-purification product from those cells. Sequencing also became a problem, though we attempted it on several different occasions in several different sequencing labs—the fragment we submitted seemed to aggregate, thereby making sequencing impossible.

Current results suggest that the problem we initially encountered was indeed during insertion of the transmembrane peptide coding sequence into the vector. Using a commercial kit expressly for the purpose of enhancing the ligation efficiency of low-yield insertions such as this, we have now been able to clone the *transmembrane* peptide sequence into a high-efficiency ligation vector successfully. After successful sequencing of the necessary region of the plasmid, we will then continue to isolate this plasmid after amplification and isolate our insert in high yield. This will allow us to then use this high-yield product to increase the likelihood of a successful insertion into the pZH3 plasmid.

Assuming successful insertion into pZH3 and assuming successful amplification, it should be possible to isolate the transmembrane peptide product by using an ion-exchange column to separate the peptide out based on the histidine-tag that is added to the peptide as a result of the plasmid. While this will add another residue to the length of the peptide, its effect on folding should not be too worrisome. If it is possible to progress to this stage with the cloning approach, it is highly likely that synthesis of the rest of the transmembrane peptides will follow shortly thereafter by using site-directed mutagenesis on the original vector.

This method of peptide synthesis may prove successful by the end of this semester, though more work has to be done.

Creation of Small Unilamellar Vesicles:

Various techniques were studied to determine the best method of creating unilamellar vesicles for our experiments. Key to our studies are homogeneity of the vesicles produced and small enough vesicles that interference with CD spectra is minimized to regions below approximately 200 nm. To this end, we attempted to use two different extrusion techniques, as well as both probe and bath sonication.

Extrusion methods, involving the repeated passage of the lipid sample through a membrane or small opening, yielded mixed results. A hand-held device developed by Avestin was tested but did not seem to produce the required vesicle size—the pores in the membrane through which the lipid was passed were far too large (50 nm in diameter), resulting in significant light scattering during CD experiments. A second procedure using a French pressure cell was also tested, but indications from a BioRad Bio-Gel A size exclusion column indicated that these were also far too large and not as homogeneous as desired.

Probe sonication was discarded relatively quickly due to the introduction of fine metallic particles from the sonicating probe. While centrifugation can remove these particles relatively easily, we moved onto bath sonication as it seemed to achieve similar results without the added step. At the time of the probe sonication experiments, we were also using dimyristoylphosphatidylcholine (DMPC) as our lipid of choice and it was difficult, if not impossible, to keep the lipid above its phase transition temperature while at the same time keeping the probe on ice so as not to overheat the instrument.

Bath sonication has proven the most effective method in terms of both homogeneity of vesicle size qualitatively and in terms of decreasing CD light-scattering problems. Size exclusion experiments of this product indicated one peak (studying absorbance at 254 nm) of relatively small vesicles, and CD scattering appeared at approximately 200 nm and shorter wavelengths but it did not interfere at wavelengths greater than 205 nm needed to characterize α-helical structure.

As noted earlier, original experiments were conducted using DMPC, with a phase transition temperature at approximately 23°C. We later chose to switch to dioleoylphosphatidylcholine (DOPC), as its phase transition occurred at –22°C, allowing us to discount such transitions as a source of difficulty.

Studies involving peptide orientation and cholesterol/decane addition were not performed at the time of writing, though this will certainly be one of the next steps in the vesicular studies. Also, electron microscopic techniques have generally been employed by other researchers in this area to determine accurately both size and homogeneity of vesicles produced for transmembrane studies—this too will need to be done in the near future. Currently, however, optimization of peptide synthesis remains the highest priority.

Isoleucine Variant of the Transmembrane Model Peptide:

The isoleucine variant of the transmembrane model peptide, with the sequence $^{+}H_3N$-Ala$_2$-Leu$_3$-Ala$_7$-Trp-Ala-Ile-Ala$_{10}$-Lys$_6$-COOH, was successfully synthesized using the Applied Biosystems 431A Peptide Synthesizer and purified using the

Pharmacia AKTA HPLC unit with a Waters 25mm column. As with all other automated syntheses, initial mass spectrometry experiments indicated a ΔAla impurity qualitatively estimated at approximately 15% of the final product. In this case, however—using the standard acetonitrile gradient in water with 0.1% TFA—the peaks representing the ΔAla form and the full-length transmembrane model peptide were disparate enough that separation was possible. Further mass spectrometry indicated a single peak at a mass of approximately 2817, corresponding to the isoleucine variant of the transmembrane model peptide. Figure 3 contains the mass spectrum corresponding to that purification, obtained from the Perspectives Voyager mass spectrometer:

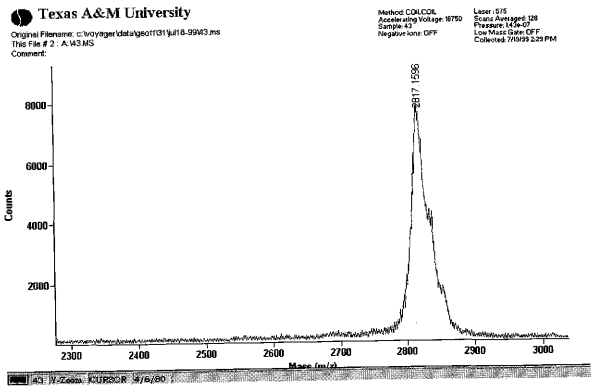

Figure 3: Mass spectrum from the isoleucine of the transmembrane model peptide. Purification was performed using the Pharmacia AKTA HPLC and an acetonitrile gradient with 1% TFA. Note the absence of any peak corresponding to a ΔAla form, which would have fallen at approximately 2747 mass units.

CD studies were performed of that purified peptide at concentrations of 1mM, 5mM, and 10mM in polar and non-polar environments—water and methanol, respectively. This allowed us to determine that the peptide was indeed helical in both environments. CD spectra corresponding to such data follow in Figures 4 and 5. Definitive evidence that the peptide is indeed helical allows us to continue on with experimentation—we know that our current sequence will indeed form a helix in order to bury the hydrophilic backbone of the transmembrane sequence and will thus allow us to use the transmembrane model peptides, once synthesized, to mimic natural

transmembrane helices in order to determine the contributions of the various amino acids to secondary structure.

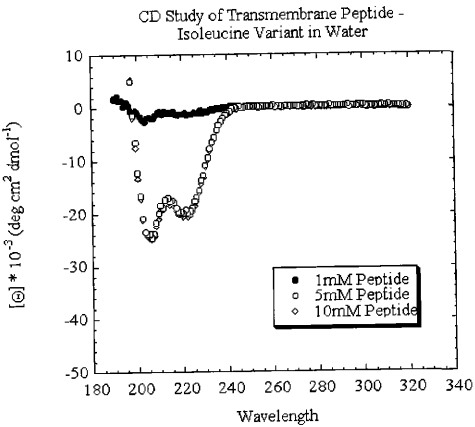

Figure 4: CD spectra of the transmembrane model peptide at three different concentrations in water. Characteristic minima at 208 and 222 nm in circular dichroism experiments are associated with α-helical conformation of the analyte.

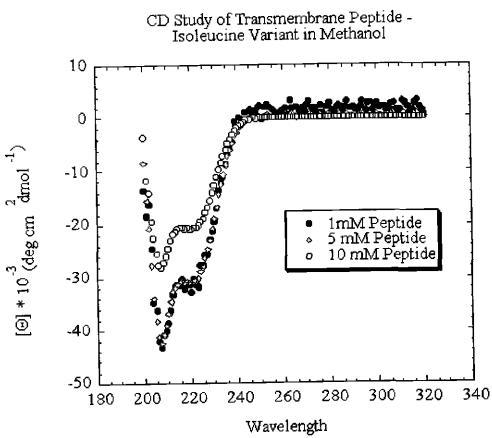

Figure 5: CD spectra of the transmembrane model peptide at three different concentrations in methanol. Characteristic minima at 208 and 222 nm in circular dichroism experiments are associated with α-helical conformation of the analyte.

Also of interest is the differing response to the change of peptide concentration as seen in Figures 4 and 5. Note that in water the peptide's ellipticity increases as concentration increases, but the exact opposite is true for the methanol study. Little can be said regarding this data, but it is possible that the differing effects of concentration may be the result of the solvent in which the peptide is dissolved. This hints at some of the interesting discoveries that may be made regarding transmembrane proteins and helical propensities as our studies continue.

CONCLUSIONS

We have been able to demonstrate that the synthesis of the transmembrane model peptide is possible chemically, though the low efficiency suggests that there is probably a better method for its production. As such, work will continue on both chemical and molecular genetic approaches to enhance the yield and purity of the peptide so that the synthesis of the rest of the transmembrane model peptides will be far easier than this first one. Almost from the outset, we were aware that this would be a difficult sequence with which to work, so the relative lack of success in actual synthesis is mitigated by the fact that we were able to successfully complete at least one peptide, demonstrating that the transmembrane sequence as delineated earlier can be synthesized and is a viable sequence to study for our helical propensity study.

Initial CD data are also encouraging—the isoleucine variant of the transmembrane model peptide is indeed helical in both polar and non-polar environments. CD studies have yet to be performed with the transmembrane peptide inserted in the lipid vesicles, but the fact that the peptide is highly helical in these initial studies is very encouraging for later work. Now it only remains to be seen the differences between the transmembrane peptide variants to start determining differences in helical propensity among the twenty naturally occurring amino acids. These studies will be conducted in polar and non-polar solvents initially, as with the transmembrane model peptide isoleucine variant, and then proceed on to the vesicle-integrated level.

Vesicular work to this point has also been quite encouraging. As soon as the rest of the peptides are synthesized, work can be completed to determine the optimum vesicular preparation for our peptide and also whether or not the addition of a long-chain hydrocarbon will be necessary for the transmembrane model peptide to insert appropriately in the transmembrane configuration. Fluorescence work will be used to confirm these orientations using methods similar to those performed by Li and Deber (1997).

There is still a great deal of work to be done—this project was never intended to be completed in the short time available through the University Undergraduate Research Fellows program. But the groundwork has been laid so that soon it will be possible to finish the synthesis and purification of the transmembrane model peptide variants and begin the full-fledged effort to determine a helical propensity scale in transmembrane environments based on the data from the transmembrane model peptide experiments.

REFERENCES

1. Hamilton, Jr., R. L. Goerke, J., Guo, L. S. S., Williams, M. C., and Havel, R. J. (1980) *J. Lipid Res.* **21**, 981-992.

2. Hunt, J. F., Rath, P., Rothschild, K. J., and Engelman, D. M. (1997) *Biochemistry* **36**, 15177-15192.

3. Lew, S. and London, S. (1997) *Anal. Biochem.* **251**, 113-116.

4. Li, S.-C. and Deber, C. M. (1994) *Struct. Biol.* **1**, 368-373.

5. Liu, L.-P. and Deber, C. M. (1997) *Biochemistry* **36**, 5476-5482.

6. Liu, L.-P., Li, S., Goto, N. K., and Deber, C. M. (1996) *Biopolymers* **39**, 465-470.

7. Ren, J., Lew, S., Wang, Z., and London, E. (1997) *Biochemistry* **36**, 10213-10220.

8. Zakim, D. and Scotto, A. W. (1989) *Methods Enzymol.* **171**, 253-265.

VITA

GEOFFREY ALEXANDER FUNK

1507 Austin Avenue

College Station, TX 77845

My educational background consists of four years of education at Texas A&M University pursuing two degrees—a B.S. in biochemistry and a B.S. in genetics—and two minors—English and chemistry. I have also attended North Harris Community College and received my paramedic licensure in the state of Texas as a result. This fall I will matriculate to the Texas A&M University System Health Science Center College of Medicine to pursue an M.D.

I have been inducted into the honors societies of Phi Eta Sigma, Golden Key, Phi Kappa Phi, Gamma Sigma Delta, and Sigma Tau Delta. I am a repeated member of the Dean's List and the Dean's Honor Roll. I was selected as a participant in the Texas A&M Health Science Center 1998 Summer Research Program, culminating with a poster presentation covering similar material to that covered in this thesis. I am also a Biochemistry Research Scholar, again pertaining to my current work.