Chemical-Scale Studies of the Nicotinic Acetylcholine Receptor: Insights from Amide-to-Ester Backbone Mutagenesis

Thesis by

Kristin Rule Gleitsman

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California
2010

(Defended April 12, 2010)

Acknowledgements

First and foremost I would like to thank my advisor, Professor Dennis Dougherty, who has supported me throughout my graduate career both personally and professionally. Although it took me longer than most to find my way in the lab, I have come to appreciate the level of intellectual freedom he affords his graduate students. While this meant plenty of room to make (and learn from) mistakes in the first few years of my graduate career, it has ultimately fostered a degree of independence that I believe will serve me well throughout my scientific career. There is nothing like graduate school to instill a strong sense of humility, occasionally trending toward insecurity, so these acknowledgements would not be complete without thanking Dennis for being there with words of encouragement at critical moments during the last five and a half years.

The expertise of Professor Henry Lester and his laboratory were critical to the completion of the research contained in this thesis. Henry's depth of knowledge and experience in the field of electrophysiology provided essential perspective for interpreting unusual results. In addition, Henry served as a wellspring of experimental suggestions, whose value lay not only in the ideas themselves, but also in honing my critical thinking skills in deciding which experiments to pursue. Of the Lester lab, I am particularly indebted to Dr. Rigo Pantoja for helping me design and execute my single-molecule TIRF experiments.

I would also like to thank my committee, Professors Peter Dervan, Harry Gray, and Shu-ou Shan. Their guidance and advice have been invaluable, especially when planning the next steps in my career. In addition, it has truly been a pleasure to interact with people who are not only brilliant, but also kind, encouraging, and interesting. I only wish there had been more opportunities for interaction.

Past and present members of the Dougherty lab have certainly shaped my graduate school experience. In particular, the candid humor of Dr. Michael Torrice was incredibly important in getting me through some rough patches. Likewise, Jai Shanata has been an incredible colleague and friend. Several chapters of this thesis (4 and 5, in particular) would not have been possible without his intellectual contribution, as well as moral support. Sean Kedrowski has also been a pleasure to work with since he joined the lab. More recently, he and Ethan Van Arnam have been extremely tolerant of the not infrequent baby-induced disruptions to our otherwise quiet bay. I owe my deepest gratitude to the entire lab for their support of me and their enthusiastic acceptance of my daughter, Malina, over the past year, as I attempted to juggle new motherhood with research and thesis writing. In particular, Ariele Hanek not only helped in proofreading parts of this document, but also brought us numerous delicious meals in the early weeks of Malina's life. Nyssa Puskar has been an exceptional colleague over the last year, acting as a sounding board for proposal ideas and other professional quandaries, as well as being an excellent copy editor. Although I wouldn't accuse Nyssa of being quiet, her unassuming manner has come to define the meaning of "still waters run deep" for me. Finally, I must thank Angela Blum for indulging my desire to do one last project as I was finishing up in the lab by agreeing to tackle the experimental component of the research described in Chapter 7 (and for proofreading that chapter).

Since moving to Pasadena more than five years ago, I have been blessed in the friendships that I have made here. I specifically want to mention Heidi Privett, Michael Adams, and Valerie Scott. Their friendship over the years has had a tremendous impact on my life, more than I can even begin to describe here.

Several people from my pre-Caltech existence also deserve acknowledgement. My mother has provided support and encouragement my entire life. Her sacrifices through difficult

times in our family's history ensured that I had the opportunity to pursue my passions. My awe of what she was able to accomplish as a single parent has only increased over the years, especially since becoming a parent myself. My biology teacher from high school, Spike Black, initially sparked my interest in science. My freshman chemistry professor, Dr. Bob Olsen, is the reason that I decided to major in chemistry in the first place. Karen Wovkulich, herself currently a graduate student in the physical sciences, has been with me every step of the way- choosing the seat next to her in first semester freshman year is one of those happy twists of fate that likely changed the course of my life. Not only did she help inspire my early passion for chemistry, by providing help with problem sets and friendly competition, she also introduced me to my husband.

I also owe an enormous debt of gratitude to my husband, Wolfe Gleitsman. Beginning with his willingness to follow me to California in the fall of 2004, Wolfe has supported me in innumerable ways throughout my graduate career. Most recently, he has been sharing in the adventure of new parenthood, whether that meant staying up with Malina so that I could get some much needed sleep or filling in for gaps in childcare so that I could complete some final experiments. And to Malina, who will not remember this somewhat hectic chapter in our life, thank you for the excited smiles when I come home in the evening, for the occasional night of good sleep, and for just being the charming little person you are.

Abstract

This thesis describes the use of peptide backbone amide-to-ester mutations to study the structure and function of ligand-gated ion channels. The research described herein has been done on the muscle nicotinic acetylcholine receptor, a prototypical ligand-gated ion channel in the cys-loop superfamily. Backbone mutagenesis in these proteins provides insight into specific intermolecular interactions that are critical to function, as well as answering more fundamental questions about the role of the peptide backbone in long-range conformational changes in these allosteric receptors.

Chapter 2 describes the identification of a key hydrogen bond near the binding site that is involved in the gating pathway. We found that the backbone N-H of a loop C residue makes a hydrogen bond to an anionic side chain of the complementary subunit upon agonist binding. The hydrogen bonding partner is not the residue predicted by structural data, but instead an aspartate that was originally believed to participate directly in agonist binding.

In chapter 3 we consider the involvement of the peptide backbone in the binding-induced conformational changes that lead to channel gating. Single backbone mutations in the β-sheet-rich extracellular domain were well tolerated, whereas two proximal backbone mutations led to nonfunctional receptors. These results support a model in which backbone movements in the outer β-sheet are important for receptor function.

Chapter 4 describes a new method - **e**lucidating **l**ong-range **f**unctional **c**oupling in **a**llosteric **r**eceptors (ELFCAR) - that should be broadly applicable to determining functional roles of residues in allosteric receptors.

Chapters 5 and 6 describe electrophysiological and computational investigations into the role of amide-to-ester mutations in the aromatic binding box of the nicotinic receptor.

Echoing the results of chapter 3, these mutations largely reveal an overall tolerance of backbone mutations in the binding site.

Finally, in chapter 7, we explore the use of ester and N-methyl backbone modifications to uncover the role of conformational changes at an unusual vicinal disulfide bond near the tip of the C-loop. Using ab initio calculations, we demonstrate that N-methylation and esterification of this ring structure in model peptides dramatically impacts its *cis-trans* conformational preferences.

Table of Contents

List of Figures

List of Tables