Chemical scale investigations of ligand-gated ion channels using unnatural amino acids

Thesis by

Darren Lee Beene

In Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2005

(Defended December 9, 2004)

for V.A.B. and E.S.B.

. . . everyday.

Acknowledgments

so much depends
upon

a red wheel
barrow

glazed with rain
water

beside the white
chickens.
W.C. Williams

Well somehow, despite my reluctance to venture beyond the academic confines, I will soon no longer be a student. The salad days are over. Having spent nearly thirty years of my life in school, there are more than a few to thank. First I must give credit to public education. From elementary school to university, I have received quality instruction at these state institutions. I also must give thanks to the Caltech community for creating a research institute truly different from the rest. Much thanks and appreciation goes to my advisor, Dennis Dougherty. In addition to creating a research environment where it was always a pleasure to work, his scientific reasoning and interpretive insight never failed to amaze me. An added bonus of joining the Dougherty group has been the opportunity to work with Henry Lester, who is a dedicated mentor and a firm believer in scientific discussion. I have also had the pleasure of a very fruitful collaboration with the Sarah Lummis group at the University of Cambridge. I am very thankful for the time and effort they have contributed to our joint projects. I would also like to thank my committee, Peter Dervan, Richard Roberts, and Robert Grubbs for their time and commitment to my education.

One of the great pleasures of my time at Caltech has been working with the members of the Dougherty group, who are, to the last, some of the most positive and creative

people I know (and anyone who says different is itchin' for a fight). Of the historical members who were there when I arrived, Joshua Maurer and Gabriel Brandt deserve special mention. It is not hyperbole to say these two have had a lasting influence on group culture. During my time in the group, Josh and Gabe were the stewards of technical and scientific knowledge. Josh is perhaps the most generous and helpful curmudgeon I have had the pleasure of knowing, and Gabe is truly a renaissance man. I am also indebted to other past members of the group. Lintong Li (at least in my time) broke the baby barrier for the group and was an excellent example of how to balance research and family. Niki Zacharias is an incredibly humane person and I have always been impressed with her social commitment. Don Elmore, the other literature major, was consistently a source of interesting conversation and positive cheer. David Dahan and I became first-time parents around the same time and got to share the joys and trials of parenthood.

In addition to past members, I also owe gratitude to the current line-up of Dougherty all-stars. I entered the group with E. James Petersson, Sarah May (nee´ Monahan), and Steve Spronk. Whether it involves suppression in mammalian cells, swimming or photography, Sarah is one of the most indefatigable people I know, and I have often wished I had one-tenth of her energy. Steve and I were the resident sport fanatics and were it not for a bum knee on my part, would have likely rivaled Stockton-to-Malone in the Caltech Intramural Basketball League. While at Caltech, I have had the pleasure of becoming good friends with James. I am greatly appreciative for the support and assistance he has provided, and must say that due in large part to his expertise and generous nature, group research did not miss a beat with Josh and Gabe's departure.

Tingwei Mu with his good nature never seemed to mind when my teetering mess encroached on his desk. Amanda Cashin always made work enjoyable, in particular when trying to diagnose the sometimes fickle Opus. Lori Lee has kindly volunteered to ensure the thioamide work will not die an ignoble death and will in my absence, I am sure, keep Michael on the straight and narrow. Michael Torrice never seemed to mind my (I hope not too) relentless ribbing and was a welcome addition to the lunch crew. Amy Eastwood set a high bar for desk management and order, a talent I hope at least in part rubbed off on me. Erik Rodriguez is a very capable scientist who always impressed me with his technical skills. Joanne Xiu, Kiowa Bower, Katie McMenimen, and Ariele Hanek have been welcome additions to the lab and I am sure will continue the Dougherty group tradition in developing time-wasting technologies.

I have made several good friends (apart from Dougherty group members) who have contributed significant enjoyment to my time at Caltech. Oren Scherman, my first roommate at Caltech, is a loyal friend and always has a unique (some say slightly crazy) take on things. Chris Thomas, my second roommate at Caltech, is an impressive chemist and wise beyond his years. Greg Drummond and I made several memorable treks to the Sierras, and I appreciate his adventurous and self-reliant spirit.

I save my final thanks for family. I am deeply thankful to my parents. Their passion for learning and adventure set a continual example of a well-lived life. My sister has a very kind soul and is always ready to help. I am infinitely grateful to my wife and kids. Maxwell and Beckett have brought more joy to my life than I could have ever imagined and put simply, Wendy makes my world spin.

Abstract

The Cys loop receptors, a family of ligand-gated ion channels, mediate fast synaptic transmission throughout the peripheral and central nervous systems. These are large multisubunit proteins, whose primary function is to transduce a chemical signal, binding of a neurotransmitter, into an electrical signal, ion flux across the cell membrane. These receptors have been implicated in several disease states and represent major therapeutic targets. The work presented in this thesis focuses on the chemical-scale elucidation of Cys loop receptors. The main approach of this work is the structure-function study using *in vivo* nonsense suppression methods. This technique allows for the site-specific incorporation of an unnatural amino acid into a protein expressed in a living cell.

Nonsense suppression methods were used to incorporate a series of fluorinated tryptophan derivatives into the binding site of the 5-HT_3R. This study identified a cation-π interaction between Trp 183 and the neurotransmitter, serotonin. A similar study using fluorinated phenylalanine derivatives identified a cation-π binding site at Tyr 198 in the $GABA_C$ receptor. These studies build on previous work from our research group and provide further evidence that the cation-π interaction is a common feature in ligand recognition by Cys loop receptors.

Nonsense suppression was also used to examine the role of several tyrosine residues in the 5-HT_3R. Here the findings demonstrated that the side chains of Tyr 143 and 153 make functionally important hydrogen bonds. These data were used to refine several computational models of serotonin docked into the binding site.

Structure-function studies of two conserved prolines in the M2-M3 loop showed that this region of the receptor is involved in the conformational changes associated with

receptor activation. The data also provide preliminary evidence that Pro 308 may serve as hinge for the gating movement of the M2 helix.

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