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

Thesis by

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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 - elucidating long-range functional coupling in allosteric receptors (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

Acknowledgements iii
Abstract vi
List of Figures x
List of Tables xii

Chapter 1: Introduction to Chemical-Scale Neuroscience I-1
1.1 Toward a Chemical-Scale Understanding of the Brain I-1
1.2 The Nicotinic Acetylcholine Receptor: The Prototypical Cys-Loop Receptor I-2
1.3 Heterologous Expression of Synaptic Proteins and Electrophysiological Characterization I-4
1.4 Unnatural Amino Acid Incorporation I-7
1.5 α-Hydroxy Acid Incorporation for Probing the Peptide Backbone I-10
1.6 Dissertation Research I-11
1.7 References I-13

Chapter 2: An Intersubunit Hydrogen Bond in the Nicotinic Acetylcholine Receptor Contributes to Channel Gating. II-1
2.1 Abstract II-1
2.2 Introduction II-1
2.3 Results II-4
2.4 Discussion II-9
2.5 Materials and Methods II-13
2.6 References II-17

Chapter 3: Probing the Role of Backbone Hydrogen Bonding in a Critical β-Sheet of the Extracellular Domain of a Cys-Loop Receptor. III-1
3.1 Abstract III-1
3.2 Introduction III-1
3.3 Results III-5
3.4 Discussion III-11
3.5 Materials and Methods III-14
3.6 References III-17

Chapter 4: Long-Range Coupling in an Allosteric Receptor Revealed by Mutant Cycle Analysis. IV-1
4.1 Abstract IV-1
4.2 Introduction IV-1
4.3 Results IV-5
4.4 Discussion IV-16
4.5 Materials and Methods IV-24
4.6 References IV-28

Chapter 5: Probing the Role of the Peptide Backbone in the Aromatic Binding Box of the nAChR V-1
5.1 Introduction V-1
5.2 Results and Discussion V-3
5.2.1 α-Hydroxy Incorporation at Aromatic Box Residues in the Primary Subunit V-3
5.2.2 Biphasic Behavior of Complementary Binding Subunit Ester Mutations V-6
  5.2.2.1 Whole-cell Electrophysiological Characterization of the Biphasic Phenotype V-6
      5.2.2.2 Biphasic Behavior is Theoretically Possible from a Single Receptor Population V-8
  5.2.2.3 Anomalous Receptors Display Normal Subunit Stochiometry V-11
  5.2.2.4 Biphasic Behavior: Novel Phenomenon or Experimental Artifact? V-14
5.3 Materials and Methods V-17
5.4 References V-20

Chapter 6: Studies of α-Hydroxy Acid Incorporation in silico VI-1
6.1 Introduction VI-1
6.2 Results and Discussion VI-1
  6.2.1 Homology Modeling VI-2
  6.2.2 Ester Parameterization VI-3
  6.2.3 Molecular Dynamics Simulations VI-5
    6.2.3.1 Structural Analysis of MD Trajectories VI-5
    6.2.3.2 Correlated Motion in MD Trajectories VI-11
6.3 Conclusions and Future Work VI-13
6.4 Materials and Methods VI-14
6.5 References VI-29
6.6 Appendices VI-31
  6.6.1 Constructing a Basic Homology Model using Modeller VI-31
  6.6.2 Backbone Ester Parameters for GROMACS VI-35
  6.6.3 Minimization and Molecular Dynamics Parameter Files VI-38

Chapter 7: Investigations into the Role of the Unusual Disulfide in the nAChR Agonist Binding Site. VII-1
7.1 Introduction VII-1
7.2 Results and Discussion VII-5
7.3 Conclusions and Future Directions VII-10
7.4 Materials and Methods VII-10
7.5 References VII-12
List of Figures

Figure 1.1  General topology of the muscle nAChR  I-4
Figure 1.2  Basics of an electrophysiology assay  I-6
Figure 1.3  Natural and unnatural amino acids  I-7
Figure 1.4  Overview of unnatural amino acid incorporation  I-8
Figure 1.5  Implementation of the suppression methodology in *Xenopus laevis* oocytes  I-9
Figure 1.6  Amide-to-ester mutations  I-11
Figure 2.1  Structure of AChBP with agonist bound, highlighting the potential interaction partners of the αS191 backbone NH  II-3
Figure 2.2  Natural and unnatural amino acids and their EC_{50} ratios  II-4
Figure 2.3  Mutant cycle analysis between αS191 and γD174/δD180  II-7
Figure 2.4  Coupling energies between αS191 and its potential interaction partners  II-8
Figure 3.1  The outer β-sheet of the nAChR  III-2
Figure 3.2  Schematic of the backbone amide versus backbone ester bond in the context of a β-sheet  III-4
Figure 3.3  Characteristics of nAChR with backbone mutations in β-strands 7 and 10  III-6
Figure 3.4  Analysis of nonfunctional nAChRs containing two amide-to-ester mutations by TIRF microscopy  III-10
Figure 4.1  Residues in the nAChR that do and do not exhibit long-range coupling with the pore domain  IV-3
Figure 4.2  The relationship between EC_{50} and Θ  IV-7
Figure 4.3  Scheme for double mutant cycle analysis  IV-10
Figure 4.4  Values of Ω for mutations in the extracellular domain  IV-10
Figure 4.5  Single-channel currents for select mutants  IV-12
Figure 4.6  Values of Ω for various reporter mutations  IV-13
Figure 4.7  Variation in I_{max} in response to introduction of a βL9'S reporter mutation  IV-15
Figure 5.1  Overall structure of the muscle nAChR  V-1
Figure 5.2  EC_{50} shifts and Ω-values for α-hydroxy mutations in the binding box of the primary subunit  V-3
Figure 5.3  Hydrogen bond existence maps for αL199  V-5
Figure 5.4  Dose-response relations for the wild-type and backbone mutants at γ55/δ57  V-7
Figure 5.5  Basic kinetic scheme for the muscle nAChR  V-9
Figure 5.6  Photobleaching histograms  V-13
Figure 5.7  Dose-response curve for partial agonists  V-16
Figure 6.1  DOPE profiles for selected homology models  VI-3
Figure 6.2  RMSD and energy minimization profiles for model peptides  VI-4
Figure 6.3  RMSF profiles and B-factors of the WT and ester-containing proteins  VI-6
Figure 6.4  RMSD profiles  VI-7
Figure 6.5  Side chain plane angle fluctuations of the aromatic box residues  VI-8
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 6.6</td>
<td>Movement of the C-loop at the α/γ interface</td>
<td>VI-9</td>
</tr>
<tr>
<td>Figure 6.7</td>
<td>Hydrogen bond existence maps between the $\alpha_1$ C-loop and the γ-subunit</td>
<td>VI-10</td>
</tr>
<tr>
<td>Figure 6.8</td>
<td>Positions of the C-loops in the first and second MD simulations on the ester-containing protein at 2650 ps</td>
<td>VI-11</td>
</tr>
<tr>
<td>Figure 6.9</td>
<td>Correlated fluctuations of the C$^\alpha$ atoms</td>
<td>VI-13</td>
</tr>
<tr>
<td>Figure 6.10</td>
<td>Sequence alignment between <em>Lymnaea stagnalis</em> AChBP and the muscle nAChR</td>
<td>VI-15</td>
</tr>
<tr>
<td>Figure 7.1</td>
<td>Conformation of vicinal disulfide in various crystal structures</td>
<td>VII-3</td>
</tr>
<tr>
<td>Figure 7.2</td>
<td>Cysteine analogues used in this study</td>
<td>VII-5</td>
</tr>
<tr>
<td>Figure 7.3</td>
<td>Geometry optimized structures</td>
<td>VII-6</td>
</tr>
<tr>
<td>Figure 7.4</td>
<td>Selected conformational parameters</td>
<td>VII-8</td>
</tr>
<tr>
<td>Figure 7.5</td>
<td>Relative energy differences between amide, ester, and N-methyl model compounds</td>
<td>VII-8</td>
</tr>
</tbody>
</table>
List of Tables

| Table 2.1 | EC_{50} values ± standard error for mutations made in this study | II-5 |
| Table 3.1 | EC_{50} and Hill coefficient (±SEM) values for mutations made in this study | III-7 |
| Table 3.2 | Puncta densities and corresponding estimated current sizes from TIRF microscopy experiments | III-9 |
| Table 4.1 | EC_{50} values with and without βL9'S reporter mutation for coupled and non-coupled residues | III-9 |
| Table 4.2 | Coupling parameters, Ω, and I_{max} ratio from whole-cell data | IV-23 |
| Table 5.1 | Fold shifts in EC_{50} values and Ω-values for backbone ester mutations in the α-subunit | IV-24 |
| Table 5.2 | Parameters used in STOIC simulations | V-4 |
| Table 7.1 | Relevant parameters for the geometry-optimized structures in this study | V-11 |
| Table 7.1 | Relevant parameters for the geometry-optimized structures in this study | VII-7 |