Chemical-Scale Investigations of Cys-Loop Neurotransmitter Gated Ion Channels

Thesis by

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The most exciting phrase to hear in science,
the one that heralds the most discoveries, is
not "Eureka," but "That's funny…"
-Isaac Asimov

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Abstract

Cys-loop ligand gated ion channels mediate rapid synaptic transmission in the mammalian central and peripheral nervous system. Proper functioning of this superfamily of receptors is critical to brain function and as such the proteins are implicated in a number of neuropathies and are a target for many pharmaceuticals. A central concern is how these receptors recognize and bind their neurotransmitter agonists as well as how these binding events lead to a conformational change spanning a distance of at least 50 Å. Using the nonsense suppression methodology, we are able to incorporate unnatural amino acids into these proteins and identify the precise molecular interactions involved in neurotransmitter binding and the conformational changes that take place during channel activation.

In chapters two through four we investigate the role of the nicotinic acetylcholine receptor (nAChR) α_1 loop 2 residues in channel activation. Using conventional mutagenesis, we have identified several residues that are part of a global electrostatic network. This is the first study to present an element of activation that is universal to the entire Cys-loop superfamily. Using unnatural amino acids, we identify the pro-S methyl group of αVal46 as a critical element in the activation pathway of the muscle type nicotinic acetylcholine receptor, thereby validating a proposed the pin-into-socket mechanism for this residue.

We switch our focus from the excitatory nAChR to the inhibitory glycine (Gly) and γ-aminobutyric acid type A ($GABA_A$) receptors in chapter 5. By incorporating successively fluorinated phenylalanine analogs into the binding site of both the GlyR and $GABA_AR$ we were able to identify a cation-π interaction at α_1Phe159 of the GlyR and

β_2Tyr97 of the $GABA_AR$, providing further evidence that the cation-π interaction is conserved across the superfamily.

Finally we investigate the mechanisms of GABA activation and flurazepam (FLZM) potentiation in the $GABA_AR$. Incorporation of a photo-activated backbone cleaving unnatural amino acid reveals that an unstructured linker connecting loops A and E of the $GABA_AR$ α_1 subunit is critical to GABA but not pentobarbital activation. We further investigate this region of the receptor and its role in GABA activation and flurazepam potentiation using conventional mutagenesis and incorporation of α-hydroxy acids. The data indicate that GABA activation and FLZM potentiation are differentially affected by side chain mutations in this region, but not by backbone mutations. Loss-of-function due to incorporation of α-hydroxy acids strongly suggests the unstructured linker becomes more structured during channel activation.

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