

Stabilization of $\alpha 4$, $\beta 2$, $\alpha 7$ nAChRs and Prediction of the Intracellular Loop Structure of $\alpha 7$ Through Use of Computational Methods

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Introduction

The central nervous system is responsible for coordinating the various processes of the body. Acetylcholine plays an important role in the central nervous system as a neurotransmitter that elicits a variety of cellular responses.¹ The main activity of the acetylcholine neurotransmitter is the excitation of muscle tissue.¹ Acetylcholine interacts with its target cells by binding to acetylcholine receptors in cellular membranes. Nicotinic acetylcholine receptors (nAChRs) are an example of membrane-bound acetylcholine receptor proteins. These receptors are also putative targets of interaction with anesthetic drugs.²

Most study of the interaction between receptor proteins and anesthetic drugs is technically challenging due to the difficulty of isolating membrane proteins and limitations when using nuclear magnetic resonance (NMR) to elucidate receptor structure at a high resolution.³ Within the membrane, both neurotransmitter ion-gated channels and cys-loop regions of membranous proteins have been implicated as sites of interaction with general anesthetics.^{3,4} Studies have thus far demonstrated that nAChRs are composed of various protein subunits which span the membrane and extend extracellularly while the pore of the receptor is located inside the membrane with a

surrounding frame that separates it from the lipids within the membrane.⁵ Neuronal nAChRs can be composed of different subunits, such as a heteromeric nAChR of two $\alpha 4$ and three $\beta 2$ subunits, or a homomeric nAChR containing five $\alpha 7$ subunits.⁶

Aims of Study

As mentioned previously, difficulties arise when attempts are made to determine the structure of membrane-bound proteins. X-ray crystallography, a popular method of protein structure determination, could not be performed on a membrane protein due to its inherent instability when isolated from the membrane. NMR would likewise provide data of little value because of high pH solution necessary to stabilize the protein, causing the presence of Hydrogen peaks from water to increase. Our ultimate goal is to gain an understanding of the mechanism by which anesthetic drugs affect the nervous system. In order to reach this goal, computational methods will be used in an attempt to find mutations of the $\beta 2$ and $\alpha 7$ neuronal nAChRs and the $\alpha 1$ glycine receptor that are more stable at a lower pH. This will allow NMR spectroscopy to be performed to further elucidate subunit structure. The native sequence of the transmembrane domain of $\alpha 4$ and $\beta 2$ subunits expressed in isolation for NMR study has a high pI (isoelectric pH) and a tendency to form aggregates in low pH solution. Mutations of particular residues lowered the pI of the protein subunits, making them more stable at pH 4.6, an ideal pH for NMR spectroscopy. However, the mutations of $\beta 2$ have not been as successful as those in $\alpha 4$ and it may still form aggregates under certain conditions. Therefore, a comparison of the two sequences should indicate potential residue changes that could increase the stability of the $\beta 2$ subunit at a lower pH. Residues will be targeted on the basis of location within

the protein in an effort to avoid destabilizing the protein by disrupting beneficial intramolecular interactions and charge.

Fig. 1: Subunit sequences (residues of interest highlighted)

1) α 4-5:

SNAEELPLFY TINLIIPC LLISCLTVLVFYLPSECGEKITLCISVLLSLTVFLLLITEIIP
STSLVIPLIGEYLLFTMIFVTL SIVITVFVLNVHHRSPETH TGGGGGIDRIFLWMFII
VCLLGT VGLFLPPWLAGE

2) β 2-5:

SNAEE EPLFY TINLIIPC VLITSLAILVFYLP SDCGEKMTLCISVLLALTVFLLLS KI
VPPTSLDVPLVG KYLMFTMVLVTF SIVT SVC VLNVHHRSPETH TGGGGGIDRLFL
WIFVFCVFGTIGMFL QPLF QEE

3) α 7:

SNARRTLYYGLNLLIPC VLISALALLVFLLPADSGEKISLGITVLLSLTVFMLLVAE
IMPATSDSVPLIAQYFASTMIIVGLSVVVTVIVLQYHHHDPDGGKGGGGGIDRLC
LMAFSVFTIICTIGILMSAPNFV

Furthermore, computational software will also be used in order to model the intracellular cytoplasmic loop of the α 7 nAChR as this region of the protein is a likely candidate for anesthetic interaction.³ The final aim of our study is to study both α 4 and β 2 subunit structure separately before applying NMR spectroscopy to the study of the structure and dynamics of the nAChR pentamer composed of two α 4 and three β 2 subunits. Ideally, these studies will be usable to design a new version of the subunits for testing and study in the lab.

Methods & Materials

Computational methods of simulation and structural determination will be used in an effort to stabilize the α 4, β 2, and α 7 proteins of the transmembrane domain of the nAChRs. The α 1 glycine receptor will also be studied similarly. The structure of the

intracellular loop of the $\alpha 7$ nAChR will also be modeled using computational methods. Programs used will likely include MOE (Molecular Operating Environment), VMD (Visual Molecular Dynamics), and NAMD. In addition to the computational study of these proteins, NMR studies will then be performed to verify model accuracy. NMR spectroscopy will also be performed to further our understanding of $\alpha 4$ and $\beta 2$ subunit structure and dynamics within the nAChR pentamer.

Expected Results

The mutation of specific residues within $\beta 2$ should increase its stability in solution with a pH of approximately 4.6. Stability could be observed in pH 4.6 solution used for NMR as low stability proteins would more frequently precipitate in solution than proteins with optimal stability. As for the NMR studies of $\alpha 4$ and $\beta 2$, these tests should indicate chemical shifts corresponding to interaction between residues of $\alpha 4$ and $\beta 2$ and an administered anesthetic. The NMR spectra should contain information regarding the overall structure of both $\alpha 4$ and $\beta 2$ and the structure of the pentameric nAChR of which they are subunits.

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