Molecular Modelling of Anesthetic Binding Sites in Ligand-Gated Ion Channels

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Introduction

We are interested in the fundamental molecular mechanisms for inhalational anesthesia. A final common pathway through which many anesthetics create their physiological effects is via an interaction with ligand-gated ion channels[1]. In particular, the gamma amino butyric acid alpha one receptor ($GABA_AR\alpha1$) and the glycine alpha one receptor (GlyRa1) demonstrate differential sensitivities to anesthetics when mutated at key amino acid residues[2]. While it is known that these ion channels are composed of a pentamer of tetrameric subunits[3,4], any further rational understanding of the molecular mechanisms of anesthesia requires a more detailed model of anesthetic interactions. The difficulty is that the target site of action within these receptors is in their transmembrane region. While there do exist several transmembrane proteins whose molecular structures have been worked out in great detail, the majority of transmembrane proteins have characteristics that make them poorly amenable to study by the usual physicochemical techniques required to produce exact atomic coordinates. Therefore, it has been our goal to apply the techniques of molecular modeling and computational chemistry to create a molecular model of the transmembrane anesthetic target region of the ligand-gated ion channels. We suggest that our model is of sufficient accuracy to agree with current experimental findings and to propose experiments that will help us refine the structure[5,6].

Methods / Results

The process of creating a three dimensional model of a protein typically begins with homology modeling. This involves using a variety of standardized algorithms to search a database of proteins with known 3D structure for an amino acid sequence that is similar to the target that is without tertiary structure. Once found, such a similar sequence of known 3D structure can serve as a template onto which the sequence of unknown structure can be threaded. However, conducting such initial searches for the GlyRa1 or $GABA_AR$a1 using sequences alone produced no reasonable results. This technique can be extended, however, by the addition of information regarding the secondary structure of the sequence in question. Since the secondary structure of neither the GlyRa1 nor $GABA_AR$a1 is well defined, this process required that further predictions be made of their secondary structure.

Therefore, our efforts began with the prediction of the secondary structure of the transmembrane regions of GlyRa1 and $GABA_AR$a1. This was accomplished with five transmembrane topology prediction algorithms. The algorithms utilized were HMMTOP from the Hungarian Academy of Sciences[7], TOPPRED2 from Stockholm University[8], TMHMM from the Technical University of Denmark[9], TMPred from EMBnet-CH[10], and PHDhtm from the EMBL)[11]. The resulting predictions were overlapped to analyze for consistency of prediction. All five of the techniques clearly predicted that transmembrane segments 1,3 and 4 are alpha helices, while three of the techniques (HMMTOP, TOPPRED2, PHDhtm) reliably predicted TM 2 to be an alpha helix. Together, these techniques predicted that the transmembrane subunits of the GlyRa1 and $GABA_AR$a1 receptor are tetrameric bundles of alpha helices.

We then went on to incorporate this secondary structure information into our search for a reasonable 3D template for homology modeling. After searching the SCOP and CATH protein fold databases, we found that there exists only one transmembrane tetrameric protein that is of mammalian origin with known 3D structure: chain c subdomain 1 of bovine cytochrome oxidase (1occ.pdb)[12]. A modified version of the annotated SCOP database was also searched using the SeqFold algorithm[13]. This search, using combined sequence and fold information, also produced a high score for this protein. Once obtained, the sequence of our $GABA_AR$a1 could be threaded over our 1occ template. This alignment was then further refined using the mean force potential of Sippl present in the SwissPDB Viewer[14]. With this final correction, we began to see direct correlation of our model with the location of several experimentally mutated residues.

Five copies of the individual tetrameric models were then organized into a pentamer, the middle of which forms the transmembrane ion channel and is lined by five TM2 regions. The pentameric template used was the bacterial mechanosensitive ion channel (1msl.pdb)[15]. This ion channel is thought to be the phylogenetic precursor of many more evolved channels in this class and is also a pentamer of alpha helices. The technique was to align the second transmembrane domain regions (TM2) of each of our previous tetrameric models with the pore-forming alpha helices in the 1msl template, allowing the rest of each tetramer to follow. The process of alignment was performed using the technique of template forcing available within the Discover 3 module of Insight (MSI, San Diego, CA). This allowed for the proper alignment of TM2 from our

tetrameric models with the alpha helices of 1msl while maintaining an appropriate packing of the remaining portions of the five tetramers. This solved a problem that others have had in the past with producing such a model[16]. The stepwise motion of the five tetrameric models onto the pore template avoided the inadvertent and chemically incorrect overlap of amino acid chains from adjacent tetramers. Once formed, this model was subjected to molecular mechanics optimization and sequentially restrained simulated annealing[17].

Having been optimized within its pentameric complex, an individual tetramer could then be removed for further analysis. Upon examining the optimized tetramer more carefully, we found excellent correlation with experimentally derived data regarding the transmembrane region of this receptor as well as its possible characteristics as an anesthetic target site.

Discussion

Previously derived empiric helical packing models of anesthetic binding favor a tetrameric bundle of alpha helices as an anesthetic binding site[18]. Proteolytic cleavage studies have produced data consistent with an all alpha helical transmembrane region[19]. NMR studies of TM2[20] and TM3[21] are consistent with these regions being alpha helices. The tetramer shows the correct orientation of the hydrophilic labeling of TM2 as occurring in a vertical stripe along the pore-lining portion of the helix[22]. Likewise, the hydrophobic labeling of TM4 appears as a vertical stripe on the membrane-facing side of TM4[23]. The hydrophilic and hydrophobic labeling of TM1 and TM3 is reasonable for their given environments, but also highly dependent on the state of the receptor, this model probably being of the closed conformation and not the open/desensitized structures[24]. The resulting effects on anesthetic action of tryptophan scanning mutagenesis studies within TM2 can be explained by this model[25]. Likewise, this model can explain the results of studies on the covalent binding of propanethiol reagents to TM2[26]. In particular for the GABAaRa1, this final refinement allowed for the alignment of Leu232, Ala291, and Ser270 in direct proximity to one another across the core of the tetramer. These are 3 residues whose actual location, or their homologous locations in the glycine receptor, have been mutated and shown to have direct effect on the actions of general anesthetics with the channel[2,27]. These three residues converge on a common binding site that is within the transmembrane core of the tetramer and readily accessible to anesthetics.

Recently, the crystal structure for an acetylcholine binding protein has been worked out in detail[28]. This protein is directly homologous to the extracellular ligand-binding region of the nicotinic acetylcholine receptor. The acetylcholine binding protein is also a pentamer. We have superimposed the crystal structure of the acetylcholine binding protein onto our complete transmembrane model that exists as a pentamer of tetramers (20 alpha helices) and found that their dimensions match almost precisely. That is to say, when the extracellular portion of the protein is placed atop its transmembrane region, a virtually seamless conical channel is formed that has an obvious, continuous ion channel pore spanning the entire length of the protein[29].

Conclusion

Overall, we have utilized techniques of molecular modeling to derive a model of the transmembrane anesthetic target site within a ligand gated ion channel that correlates closely with the majority of physicochemical data regarding this region of the receptor. Such a model will continue to be refined as more data become available and will serve as a tool in understanding the molecular mechanisms of anesthetic action.

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