Membrane Protein Structure II

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Rhodopsin in Bovine Rod Outer Segment Disk Membranes Exhibits Two Thermal Transitions

Arlene Albert, Scott Corley.
University of Connecticut, Storrs, CT, USA.

The photoreceptor, rhodopsin is a GPCR in rod outer segment disk membranes. Activation by light converts the dark-adapted form, rhodopsin is to the bleached form, opsin. Differential scanning calorimetry (DSC) studies showed that rhodopsin and opsin each exhibit an irreversible scan rate dependent endothermic transition (T_m) at approximately 72°C and 55°C respectively. We report here a scan rate dependent exothermic transition. Solubilization was used to examine the contribution of the bilayer. Freeze-thaw cycles were used to examine rhodopsin aggregation in these transitions. Disk membranes were sequentially solubilized in octylglucoside (OG) until rhodopsin was completely delipidated. DSC experiments were performed using a MicroCal VP-DSC microcalorimeter. Samples were scanned at 15, 30, 60 and 90°/hr. Because the protein transitions are irreversible, a second scan was used to determine the baseline. As the OG partitioned into the bilayer the endothermic T_m and E_{act} (activation energy of denaturation) rapidly decreased. Both then remained constant following rhodopsin solubilization. At low detergent concentration the exothermic T_m increased rapidly then remained constant after solubilization. Unlike the endothermic E_{act}, the degree of solubilization had little effect on the exothermic transition E_{act}. Freeze-thaw cycles generated disks in which rhodopsin was aggregated. Increasing aggregation resulted in a decrease in the T_m of the exothermic transition, but had no effect on the endothermic T_m of either rhodopsin or opsin. These results are consistent with earlier cross-linking studies suggesting the exothermic transition results from aggregation. These results indicate that the rhodopsin endothermic transition is due to weakening of the tertiary structure interactions accompanied by changes in the packing of the trans-membrane helices as well as changes in protein-lipid interactions. CD data indicate that rhodopsin secondary structure is almost unchanged.

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NMR Structure of Water-Solubilized Transmembrane Domain of Nicotinic Acetylcholine Receptor

Tanxing Cui[1], Pei Tang[1], Jeffery G. Saven[2], Roderic G. Eckenhoff[2], Yan Xu[1].
[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of Pennsylvania, Philadelphia, PA, USA.

No high-resolution experimental structure of the transmembrane (TM) domains of the nicotinic acetylcholine receptor (nAChR) is currently available. We have addressed some critical issues related to structure determination by strategically mutating 22 hydrophobic, putatively lipid-facing residues to hydrophilic ones, so that the resulting TM domains have improved water solubility while maintaining the native fold. Two different designs based on the nAChR α1 subunit were attempted. The first (TM123-4) consisted of the four TM domains in their natural sequential arrangement, with an artificial linker between TM3 and TM4. The second (TM4-123) has TM4 moved before TM1. TM123-4 failed to be expressed by E. coli, suggesting possible cell toxicity. In contrast, TM4-123 was expressed with high yield and produced stable samples for structure characterization by high-resolution NMR. Monomeric TM4-123 structure was resolved to a backbone RMSD of 1.2 Å. The overall structure of TM4-123 is similar to that of the cryo-EM model, except that the very short TM2-TM3 loop (VPLI) found in the cryo-EM structure was replaced by a significantly longer loop (LIPSTSSA) and shifted by 5 residues towards the TM2 domain. More interestingly, 'TSSA' in this loop exists in two conformations: a rigid helix and a flexible coil, and these two conformations are in slow exchange on the NMR timescale. Given that the interaction between the TM2-TM3 loop and the extracellular domain likely mediates channel gating, the two conformations in this important loop might represent the critical transitions required for the channel to open. The high-resolution structure of water-solubilized nAChR TM domains allows us to better understand the structural basis of channel function (Funded by NIH R37GM049202, R01GM056257, and P01GM055876).

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The Active Conformation Of Opsin (ops*): Role In Signal Transduction And Regeneration

Klaus P. Hofmann.
Institute of Biophysics, Berlin, Germany.

Functional modules are specific subsets of the proteome, which fulfil an autonomous function in living cells. Functional modules in retinal photoreceptors include those devoted to phototransduction and regeneration [1]. In signal transduction, rhodopsin is kept inactive in the dark by a set of structural elements and residues conserved in the E(D)RY and NPxxY(x)5,6F regions of the receptor protein. On light activation, the same residues are crucial determinants of the Meta II state [2], with the hallmark of a motion of the sixth of the seven transmembrane helices [3]. The same elements stabilize the ligand free active form of the opsin apoprotein, Ops* [4], and build up new interactions of Ops* with the G-protein α-subunit C-terminal interaction site [5]. The Ops* state can thus act as the key element in both functional modules of the rod cell. In signal transduction, the Ops* conformation is present in the highly active Meta II photoproduct. In rhodopsin regeneration, Ops* facilitates the uptake of 11-*cis*-retinal into the binding site [6] and causes the visual system to behave as if it is experiencing an ''equivalent background'' [7].

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Agonist and Inverse agonist Induced Conformation Changes in G-protein Coupled Receptors

Nagarajan Vaidehi.
Beckman Research Institute of City of Hope, Duarte, CA, USA.

Conformational flexibility in G-protein coupled receptors and the effect of ligand binding on this flexibility is important for an understanding of functional specificity of ligands. Ligands of varied efficacies such as agonists, partial agonists, inverse agonists, and antagonists have different effects on the receptor conformation depending on its binding affinity and efficacy. 3D structural information of these ensemble of conformations stabilized by various ligands would be very critical to understand the molecular basis of ligand efficacy. To this end, we have developed a computational method, Ligand Induced Transmembrane Conformational changes (LITiCon), based on simultaneous optimization of the ligand induced movement of the seven transmembrane helices, followed by molecular dynamics simulations in explicit lipid and water for predicting the conformational changes that occur on ligand binding. We have studied the conformational changes resulting from the activation of bovine rhodopsin starting from the crystal structure of the inactive (dark) state. The predicted active state of rhodopsin satisfies all of the 30 known experimental distance constraints and also correlates well with the G-transducin peptide bound opsin crystal structure. Details of these results will be presented. Results for agonist, partial agonist and inverse agonist bound to β2-adrenergic receptor and β1-adrenergic receptor will also be presented. We find that the agonist induced larger conformational changes in the transmembrane region as compared to the inverse agonist. Our calculations for the chemokine receptor CCR3 shows that the small molecule antagonist UCB35625 binds in a different location in two similar receptors CCR1 and CCR3 whereas the small molecule agonist CH0076989 binds only to CCR3 and not to CCR1.

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Probing the Dynamic Structure and Function of Intracellular Loop 2 in Structurally Cognate GPCRs

Jufang Shan[1], Ernest L. Mehler[1], Harel Weinstein[1,2].
[1]Department of Physiology and Biophysics, Weill Medical College of Cornell University, New York, NY, USA, [2]HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Medical College of Cornell University, New York, NY, USA.

The increasingly frequent identification of functional attributes of loops connecting TMS in transmembrane proteins such as GPCRs, prompted us to address the structures of loops in the context of specific mechanistic hypotheses about their role in protein-protein interaction underlying signaling processes. Experimentally and computationally-derived evidence suggest that these functional properties of the loops are directly dependent on their specialized structure and positioning relative to the rest of the protein. The intracellular loop 2 (IL2) of class A GPCRs is such an example. It is known to be involved both in G-protein-coupling and in regulating β-arrestin binding and β-arrestin-mediated internalization. Structural information about IL2 reveals differences in conformation even between nearly identical short loops in different GPCRs (e.g., IL2 has different secondary structure in β1AR and β2AR: helical v.s. non-helical), as well as roles for key residues (e.g., replacing of the well conserved Pro3.57 by Ala in β2AR and 5HT2CR decreases β-arrestin binding and internalization (Marion et al, JBC 2006)). We have used a recently described *ab initio* method for loop structure prediction (Mehler et al, Protein 2006) for