G-Protein-Coupled Receptors

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Betablockers at Work: The Crystal Structure of the β_2-Adrenergic Receptor**

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G-protein-coupled receptors (GPCR) are transmembrane proteins responsible for the transmission of extracellular signals into cells. With more than 800 members, they are the largest family of signal transduction proteins (approximately 3% of the human genome encode GPCRs),[1] and are of enormous pharmacological importance: over 30% of prescribed drugs target a GPCR.[2] Nevertheless, medicinally relevant GPCRs were only indirectly accessible for a structure-based drug design. High-resolution structures had only been published for a single GPCR, the light-sensitive rhodopsin,[3] which is unique among the GPCR family owing to its covalently linked cofactor retinal. Why is the structural biology of GPCRs so challenging? Apart from the problems typically encountered with eukaryotic transmembrane proteins, such as preparative overexpression and purification, in the case of GPCRs, there is also a lack of polar contact surfaces and a profound conformational heterogeneity.[4]

Cherezov et al.[5] and Rosenbaum et al.,[6] and also Rasmussen et al.[7] and Day et al.[8] have now been able to solve crystal structures of a typical, ligand-activated GPCR. This GPCR, the β_2-adrenergic receptor, is activated by catecholamines, such as adrenaline and noradrenaline, and is the target of betablockers and anti-asthma drugs. In both cases, the structures were obtained as a complex with carazolol, a partial inverse agonist with picomolar affinity, which stabilizes the inactive state of the β_2-adrenergic receptor. Further rigidification of the intracellular domain of the β_2-adrenergic receptor was critical for success in both cases. This was achieved in the first approach by substitution of the flexible, third intracellular loop by the small, well-structured protein T4 lysozyme. In the second case, an antibody was generated that binds to a native, membrane-bound conformation of the third intracellular loop.

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In both approaches, the authors verified that these modifications did not compromise the biochemical properties of the β_2-adrenergic receptor. First, they showed in binding assays that both the T4 lysozyme-fusion construct and the antibody fragment complex bind ligands with similar affinity as the unmodified β_2-adrenergic receptor. Then, fluorescence measurements with labeled β_2-adrenergic receptor constructs were carried out to be sure that the binding of agonists leads to conformational changes similar to those observed for the activated wildtype β_2-adrenergic receptor. These results strongly suggest that the crystal structures obtained correspond to relevant endogenous conformations of the β_2-adrenergic receptor. Furthermore, the fusion with T4 lysozyme or the complexation with the antibody fragment had the desired additional effect of allowing substantially more polar surface contacts. Indeed, the intermolecular contacts observed in the crystal structures are formed to a large degree by T4 lysozyme or by the antibody fragment, respectively.

The crystal structures were refined to a resolution of 2.4 Å for the lysozyme-fusion construct, and to 3.4/3.7 Å for the antibody complex. Both structures show the characteristic heptahelical architecture of a GPCR, and an eighth intracellular helix running parallel to the membrane (Figure 1). Several helices show distinct kinks induced by conserved proline residues, which are presumably important for the function of GPCRs. The ligand carazolol binds between the helices at the end of a negatively charged cleft, which opens up to the extracellular environment. This position superimposes well with retinal covalently bound to rhodopsin; however, unlike the crystal structure of rhodopsin, there is an additional short helix in the second extracellular loop of the β_2-adrenergic receptor, which is stabilized by a second disulfide bridge. This seems to keep the entrance to the binding pocket open to allow access of the diffusible ligand. Another surprising difference to rhodopsin is the orientation of the so-called E/DRY motif at the end of helix III, which is highly conserved in GPCRs.[10] The ionic interactions of these residues with helix VI were generally thought to be responsible for the stabilization of the inactive state of GPCRs.[11] The DRY motif was found to be more distant from helix VI in both β_2-adrenergic receptor structures, irrespective of the constructs used. This argues against an artifact induced by T4 lysozyme, by the antibody fragment, or by crystal-packing effects. Additional structures with alternative ligands or with other GPCRs will be needed to clarify whether the observed

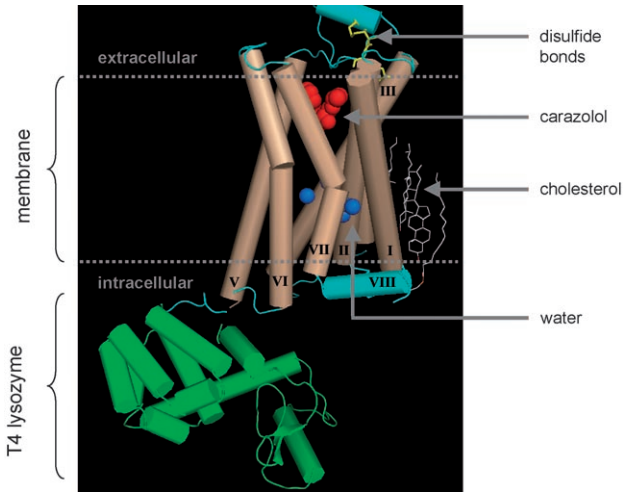

Figure 1. 2.4-Å structure of the β_2-adrenergic receptor.[5] The seven transmembrane helices are labeled with Roman numerals I–VII, with helix IV in the background. Two proline-induced kinks in helix VI and VII are clearly visible; the DRY-motive in helix III is hidden by helix VI. The intra- and extracellular loops and helix VIII are shown in cyan; the fused T4 lysozyme protein replaces the third intracellular loop. The second protein molecule of the β_2-adrenergic receptor dimer is omitted for clarity.[9]

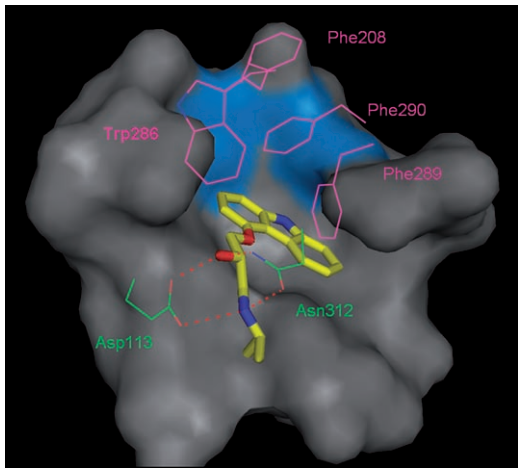

Figure 2. Binding pocket of the β_2-adrenergic receptor,[6] showing the partial inverse agonist carazolol (yellow), the four hydrogen bonds of Asp113 and Asn312 to the ligand (dotted, red), and the aromatic network around Trp286 and Phe290 (pink). The solvent-accessible surface of the remaining binding pocket is colored gray, and interaction partners (Ser204/Ser207) for potential catechol-agonists are indicated in blue.

alternative arrangement of the DRY motif is a special property of the carazolol-β_2-adrenergic receptor complex or whether it is a more general characteristic of class A GPCRs.

An interesting finding of the T4 lysozyme fusion structure is a relatively close, parallel, and pairwise packing of two β_2-adrenergic receptor molecules. This arrangement is compatible with the dimerization of two receptor molecules in the plasma membrane of cells, a phenomenon which has been discussed intensively for GPCRs in recent years.[12] The dimerization of the β_2-adrenergic receptor in the crystal is mediated by four well-resolved cholesterol molecules. Notably, cholesterol was an essential component for the crystallization, and is also known to be important for the function of the β_2-adrenergic receptor in vivo.

Several water molecules could be localized in the 2.4-Å structure between the transmembrane helices, an initially surprising finding which had also been observed in the previous rhodopsin structures. These water molecules form an extensive network of hydrogen bonds, often with highly conserved amino acid residues at the inside of the helices. Recently, Pardo et al. suggested that these water molecules are a conserved characteristic of class A GPCRs. This model now gains considerable experimental support by the present β_2-adrenergic receptor structure. [13]

The higher resolution cocrystal structure of the β_2-adrenergic receptor will have substantial impact on the medicinal chemistry of GPCRs. It shows the detailed binding mode of carazolol (Figure 2), a representative of the class of betablockers. Betablockers inhibit β-adrenergic receptors, and are used to treat cardiovascular disorders. The crystal structure clearly demonstrates how the vicinal amino alcohol, a privileged motif derived from endogenous catecholamine ligand ligands like adrenaline and noradrenaline, and found in many adrenergic drugs, is recognized by the β_2-adrenergic receptor. The amino alcohol is locked by asparagine 312 and by aspartate 113 by two hydrogen bonds each. Both residues are known to be essential for binding of ligands to the receptors.

The heterocycle of carazolol is embedded in a hydrophobic pocket formed in part by phenylalanines 289 and 290. Phe290 is additionally fixed by Phe208 and Trp286. Trp286 is a highly conserved residue, and thought to play a crucial role in the mechanism of receptor activation, the so-called "toggle switch".[14] Cherezov et al. postulate that inverse antagonists, such as carazolol, stabilize the aromatic network around Trp286 in an inactive conformation.

An analysis of the binding pocket also allowed Rosenbaum et al. to propose a hypothesis for the binding mode of agonists. Thus, a binding model of the prototypical agonist isoproterenol was generated based on the assumption that the alkylamino alcohol unit would interact with Asn312 and Asp113 in a similar way as it was found for carazolol. From structure–activity relationships and mutagenesis studies, it was already known that the aromatic hydroxy groups of catechol agonists interact with two serine residues (Ser204 and Ser207). In the cocrystal structure with carazolol, these residues are too far away from the modeled isoproterenol. Rosenbaum et al. therefore suggest that upon binding of agonists, the residues Asp113 (helix III) and Asn312 (helix VII) and the residues Ser204 and Ser207 (helix V) move closer together, enabling both the interactions with the alkylamino alcohol moiety and with the catechol group. This conformational change could be part of the ligand-induced signal transduction.

Currently, one of the most important questions in GPCR research is how these conformational changes around the ligand binding pocket are transferred to the intracellular part of the receptor. Here, too, the present crystal structures give a

first indication, e.g., by localizing the exact positions and interaction patterns of constitutively activating or deactivating mutation. These residues are thought to be important for the stabilization of the inactive and active state of the β_2-adrenergic receptor, respectively. The best insight into the molecular mechanisms of the activation would, however, be a high-resolution structure that could be assigned to an activated state of the receptor. A key step towards this goal will be the stabilization of a structurally homogeneous activated state of the GPCR from an ensemble of possible conformations. Closely linked to this issue is the question of how the activated receptor binds to its cognate heterotrimeric G protein complex.[15] Detailed information on this question can only be given by the structure of a ternary agonist-GPCR-G-protein complex.

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