DOI: 10.1002/cmdc.200800070

Crystal Structures of a Second G Protein-Coupled Receptor: Triumphs and Implications

Abby L. Parrill*[a]

G protein-coupled receptors (GPCR) are integral membrane proteins involved in cellular signal transduction. Genome analysis suggests about 950 distinct human GPCR.[1] GPCR play essential roles in the action of hormones, neurotransmitters, and growth factors and regulate development, immunity, and physiological homeostasis. Consequently, GPCR serve as the biological targets of approximately 25–50% of drugs, depending on whether estimates are derived from total sales, prescriptions, or numbers of drugs.[2,3] These drugs, however, target a relatively small percentage of known GPCR, suggesting that important therapeutics targeting GPCR remain to be discovered. Detailed GPCR structures are therefore of interest to a broad audience. Despite the undeniable significance of GPCR, the first high-resolution GPCR structure was not released until 2000.[4] The first GPCR characterized with atomic detail was rhodopsin, which uses a covalently bound retinal chromophore to sense light. This structure was greeted enthusiastically; however, its atypical covalently bound ligand and complete lack of basal activity left unanswered questions about its suitability as a template for other GPCR structures, particularly for investigations of agonist interactions. Recently reported crystal structures of a second GPCR family member, the β2-adrenergic receptor (Figure 1),[5–7] address some of these questions, and provide a mechanism to triumph over some of the challenges inherent in the characterization of membrane protein structures.

[a] *Prof. A. L. Parrill*
Department of Chemistry
The University of Memphis
Memphis, TN 38152 (USA)
Fax: (+1) 901-678-3447
E-mail: aparrill@memphis.edu

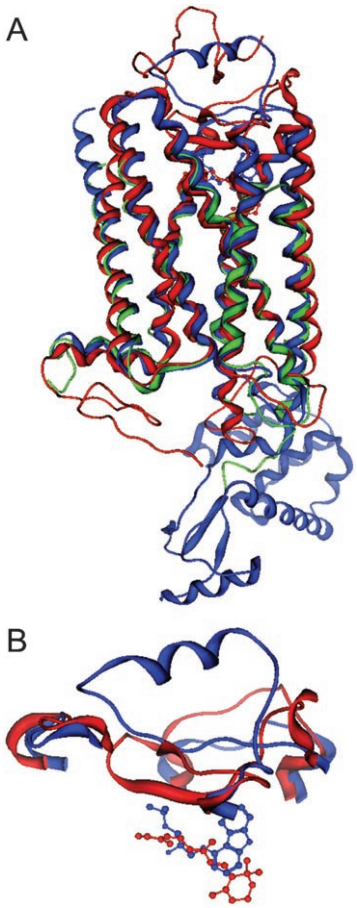

Figure 1. Comparison of β2-adrenergic receptor crystal structures (blue: PDB entry 2RH1, green: PDB entry 2R4R) and two lowest-resolution rhodopsin crystal structures (red: PDB entry 1U19[8]). A) Superposition based on structurally conserved residues gives 1.5 Å root mean squared deviation (RMSD) on α carbon positions of superposed residues (≥13 residues per helix superposed). B) Magnified view of extracellular loops and bound ligands that display a 9.5 Å RMSD.

Crystallographic studies of GPCR present numerous obstacles, including difficulties in obtaining suitable amounts of functional protein as well as the flexibility and overall hydrophobicity of these proteins which prevent crystallization. With the exception of rhodopsin, membrane proteins occur at miniscule concentrations in their natural sources. The high concentration of rhodopsin in rod outer segments contributed substantially to successful crystallization of this GPCR family member.[4] Protein yield is most often improved over natural sources through the use of heterologous expression systems. Expression of multiple GPCR family members has been examined in *E. coli*, yeast, insect cells infected with baculovirus, mammalian cells, and cell-free systems with limited success, as recently reviewed.[9] Nevertheless, the recently reported crystallographic characterization of a rhodopsin mutant isolated after heterologous expression in COS cells[10] and the β2-adrenergic receptor after heterologous expression in insect cells[5,6] indicate that GPCR samples isolated from heterologous expression systems can fuel structural characterization studies. Protein concentration, however, is just the first hurdle on the path to a crystal structure. The second obstacle is the development of a purification procedure that produces functional, rather than denatured, receptor. The β2-adrenergic crystal structures both relied on the use of a very high-affinity partial inverse agonist, carazolol, to stabilize the protein structure during purification.[5,6] One of the purification procedures[5] also used the Fab fragment of a monoclonal antibody[11] that selectively recognizes natively structured protein. A third challenge that must be surmounted is the innate conformational heterogeneity of GPCR, which must sample a range of conformations in order to provide temporally controlled signaling. Carazolol decreases the population of receptors in active conformations. However, the β2-adrenergic receptor shows substantial flexibility in its third intracellular loop (IL3) even in complex with carazolol. The method used to solve the crystallization difficulty presented by the intrinsic flexi-

bility of IL3 represents a major difference between the two reported crystal structures. One method used complex formation with the Fab fragment of the aforementioned monoclonal antibody to decrease IL3 flexibility.[5,11] The other method applied protein engineering techniques to replace the sequence of IL3 with a tightly folded, previously crystallized protein that exhibits appropriate inter-terminal distances: T4 lysozyme.[6,7] While different in detail, both methods accomplish the same goal and present guidance for researchers seeking to crystallize additional GPCR family members. A fourth challenge, addressed in concert with the intrinsic flexibility of IL3, is the need for polar surface area that can form crystallographic contacts. Crystallographic contacts were noted in both crystal structures in the modified regions, either between Fab fragments in neighboring cells,[5] or between T4 lysozyme proteins in the dimer.[6] Finally, when all other problems are solved, a method to prepare crystals that diffract at high resolution is needed. Two crystallization methods, lipidic bicelles and lipidic cubic-phase crystallization were used to generate suitable crystals for these studies. The crystals generated using the lipidic cubic-phase crystallization method diffracted to higher resolution (2.4 Å) than those generated from lipid bicelles (anisotropic resolutions of 3.4 and 3.7 Å), resulting in fewer unresolved residues. A summary comparison between the methods used to successfully characterize the β2-adrenergic receptor crystal structures is provided in Table 1.

Table 1. Comparison of β2-adrenergic crystal structures.

Comparison	PDB entries[12]	
	2R4R, 2R4S[5]	2RH1[6]
IL3 alteration	Bound Fab	T4 lysozyme
Crystallization method	Lipid bicelles	Lipidic cubic phase
Resolution [Å]	3.4, 3.7	2.4
Unresolved regions	N terminus, EL1-3, extracellular ends TM2/4–7, ligand	N and C termini (residues 1–28 and 343–365), IL3
Ligand	Carazolol	Carazolol
State	Monomeric	Dimeric

The crystallographic structures of the β2-adrenergic receptor provide at least as much insight as the techniques used to obtain the structures. One compelling feature of the β2-adrenergic receptor structures is their similarity to the crystallographic structures of rhodopsin (Figure 1 A). In particular, at least 13 amino acids in each transmembrane domain of these structures are structurally conserved, with only 1.5 Å RMSD among the α carbon positions. This strong similarity validates numerous studies published using crystallographic structures of rhodopsin as homology modeling templates for other GPCR family members, and the application of such models in drug discovery efforts. A second important characteristic is the substantial difference observed in the extracellular domain of β2-adrenergic receptor relative to rhodopsin (Figure 1 B). While substantial differences in the extracellular domains of GPCR have been anticipated based on sequence comparisons, these structures provide the first atomic-resolution demonstration that this is the case. The observed differences in the extracellular domains indicate that unmodified GPCR homology models are best suited to modeling ligand binding of diffusible ligands that interact within the α-helical bundle, rather than of ligands predominantly recognized by the extracellular loops. Hybrid computational/experimental structures using peptide analogues of extracellular loops may substantially extend the range of GPCR structures that can be effectively applied in lead discovery efforts by providing receptor-specific structural details of the extracellular loops. Another notable feature of the recently reported structures includes their applicability to place accumulated data on conformational changes upon GPCR activation into a structural context. These structures also demonstrate that structural differences between monomeric and dimeric forms are relatively small. This finding gives weight to a role of dimerization, at least for the β2-adrenergic receptor, predominantly in receptor trafficking, rather than function.

The value of the recently reported crystal structures of the β2-adrenergic receptor is exceptional, both for the structural insight they provide and for the methodological concepts applicable to crystallization of additional GPCR, and in fact the broader group of all membrane proteins. They do not, however, provide the final pieces for our efforts to understand the puzzle of GPCR function. One question remaining to be answered is the true difference between a fully inactive and fully active GPCR structure. This will require characterization of a GPCR structure bound at least to a very high-affinity agonist, and potentially with G protein partners. This information will be particularly relevant to the structure-based discovery of novel agonists, which preferentially interact with the active GPCR conformations. A second open avenue for investigations is the nature of the dynamic changes in GPCR equilibria in response to ligands, effector coupling, and membrane composition. Even with these remaining questions, β2-adrenergic receptor crystal structures represent a substantial triumph over numerous challenges, and offer substantial value to researchers interested in GPCR structure, function, and therapeutic lead discovery.

Acknowledgements

Research supported by the National Institutes of Health award R01 HL 084007.

Keywords: crystallography · diffusible ligands · drug design · GPCRs · receptors

[1] S. Takeda, S. Kadowaki, T. Haga, H. Takaesu, S. Mitaku, *FEBS Lett.* **2002**, *520*, 97–101.
[2] K. Lundstrom, *Curr. Protein Pept. Sci.* **2006**, *7*, 465–470.
[3] T. A. Esbenshade, *Drug Discovery Ser.* **2006**, *4*, 15–36.
[4] K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739–745.
[5] S. G. Rasmussen, H. J. Choi, D. M. Rosenbaum, T. S. Kobilka, F. S. Thian, P. C. Edwards, M. Burghammer, V. R. Ratnala, R. Sa-

nishvili, R. F. Fischetti, G. F. Schertler, W. I. Weis, B. K. Kobilka, *Nature* **2007**, *450*, 383–387.

[6] V. Cherezov, D. M. Rosenbaum, M. A. Hanson, S. G. Rasmussen, F. S. Thian, T. S. Kobilka, H. J. Choi, P. Kuhn, W. I. Weis, B. K. Kobilka, R. C. Stevens, *Science* **2007**, *318*, 1258–1265.

[7] D. M. Rosenbaum, V. Cherezov, M. A. Hanson, S. G. Rasmussen, F. S. Thian, T. S. Kobilka, H. J. Choi, X. J. Yao, W. I. Weis, R. C. Stevens, B. K. Kobilka, *Science* **2007**, *318*, 1266–1273.

[8] T. Okada, M. Sugihara, A. N. Bondar, M. Elstner, P. Entel, V. Buss, *J. Mol. Biol.* **2004**, *342*, 571–583.

[9] E. C. McCusker, S. E. Bane, M. A. O'Malley, A. S. Robinson, *Biotechnol. Prog.* **2007**, *23*, 540–547.

[10] J. Standfuss, G. Xie, P. C. Edwards, M. Burghammer, D. D. Oprian, G. F. Schertler, *J. Mol. Biol.* **2007**, *372*, 1179–1188.

[11] P. W. Day, S. G. Rasmussen, C. Parnot, J. J. Fung, A. Masood, T. S. Kobilka, X. J. Yao, H. J. Choi, W. I. Weis, D. K. Rohrer, B. K. Kobilka, *Nat. Methods* **2007**, *4*, 927–929.

[12] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic Acids Res.* **2000**, *28*, 235–242.

Received: March 3, 2008
Published online on April 11, 2008

