KP225

Modeling Analysis of Interactions of Bradykinin B_1 and B_2 Receptors with Their Endogenous Ligands

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Introduction

We have been studying on the molecular recognition of G protein-coupled receptors (GPCRs) and their ligands. In particular, peptide receptors and their ligands are our major concern in our modeling studies. Thus, we have modeled a structural model of nociceptin-receptor complex structure [1] and here we demonstrate the model building of bradykinin-receptor complex structures to further understand the peptide-receptor recognition.

Bradykinin (BK) is an endogenous peptide ligand for the bradykinin receptor (BR), a member of GPCRs and affects allergy, pain, edema and hypotension. There are two subtypes in BK receptors, B_1R and B_2R. The activation of the constitutive B_2R causes pain, edema and hypotension, and then the truncation of the C-terminal Arg9 residue to give des-Arg9-bradykinin (DABK), which binds B_1R and causes increase of pain and duration of inflammation. Thus, antagonists of DABK are expected to be anti-inflammatory and analgesic agents.

In this paper, we report the modeling of the complex structures of B_2R and BK and B_1R and DABK [2] using structural models of agonist-bound B_2R which are derived from the photointermediate models in the photoactivation cascade of rhodopsin [3]. GPCRs generally form two functional agonist-bound structures, partial agonist-bound and full agonist- bound structures. Since it is not known that BK or DABK is partial or full agonist, we have examined both possibilities.

Methods

A structural model of a partial agonist-bound B_2R was constructed with homology modeling using the metarhodopsin I_{380} model structure[3] derived from the crystal structure of rhodopsin. The structural model was energy-minimized with Discover3 until the rmsd gradient becomes 0.1 kcal·mol^{-1}·Å^{-1}. The full agonist-bound form was also constructed using the structural model of metarhodopsin II.

We have sequentially docked each amino acid fragment, Arg9 through Arg1. The C-terminal carboxylate of N-acetyl Arg9 was proximally bound to the Ser138(3.33) in TM3 and then the Cα-CO bond (ψ) was rotated to find the plausible binding site for the Arg9 residue. The resulting complex structures were energy-minimized and optimized with molecular mechanics and dynamics calculations. Subsequently, the N-acetyl group of Arg9 residue was replaced with N-aceyl Phe8 to form dipeptide moiety. Again, plausible binding sites were examined by rotating the ψ bond of Phe8. The procedure was continued to have the C-terminal hexapeptide portion of BK.

Results and Discussion

The most vital ligand-binding experiments on the BK mutants [4] showed that a Ser138Lys mutant reduces the binding affinity of BK and suggested an importance of the Thr290(6.55) residue in the sixth transmembrane segment (TM6). A plausible complex model structure of BK and B_2R shown in Figure 1 showed a good agreement with the mutational

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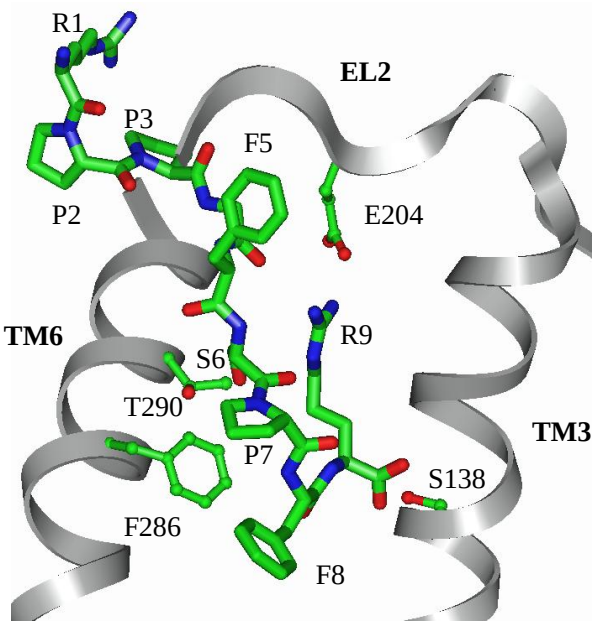

Figure 1. Complex model of the human B_2 receptor with BK in partial agonist-bound form.

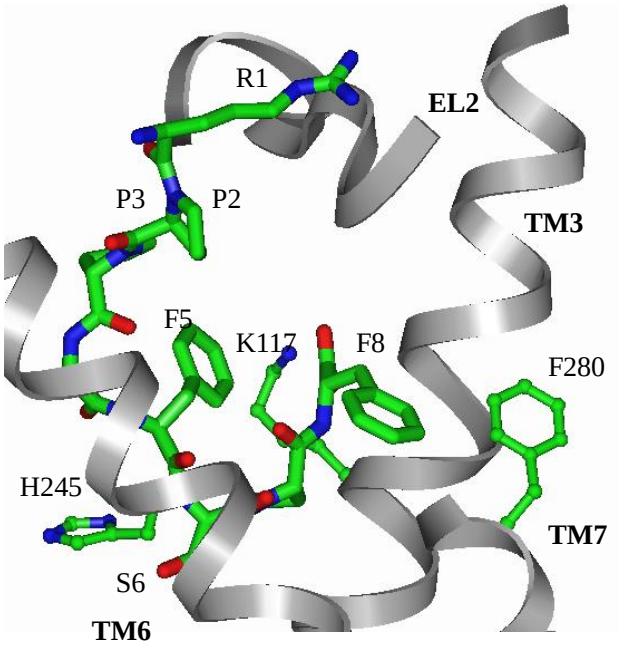

Figure 2. Complex model of the mouse B_1 receptor with DABK in partial agonist-bound form. Phe280(7.43) corresponds to the retinal binding lysine residue of rhodopsin.

experiments. Thus, the carboxylate of Arg9 interacts with Ser138(3.33) and the hydroxyl group of Ser6 with Thr290(6.55). The complex model further suggests that the Arg9 residue interacts with Glu204 in the second extracellular loop (EL2). Although this interaction would be important, there are no available mutational experiments on this residue.

The interaction of the carboxylate group of Arg9 appears to be important for the selective recognition of BK by B_2R, since the Ser residue is replaced by the Lys residue in the B_1R. A larger residue at the 138(3.33) position may not allow the binding of the carboxylate group.

The participation of Thr290 in the ligand recognition is quite important for partial agonist-binding since Thr290 is located at the ligand binding cleft whereas the residue is directed outside of the ligand-binding cleft due to the rigid-body rotation of TM6 in the full agonist-bound form of the receptor. Thus, it is most plausible that BK is recognized by the receptor as a partial agonist. A similar modeling study on the B_1R-DABK complex structure also indicated that DABK binds the partial agonist-bound form of the receptor (Figure 2) [2]. It has been demonstrated that a peptide ligand, nociceptin, binds nociceptin receptor in a partial agonist-bound form. Thus, it should be intriguing that these endogenous peptides may act on their receptors as partial agonists.

The C-terminal hexapeptide portion of BK showed a mostly extended conformation at the binding cleft, while the other portion was not well defined. The conformation of the C-terminal portion of BK will be presented in detail.

References

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