5 - 77 Insights into the Molecular Mechanism of Positive Cooperativity Between Partial Agonist MK-8666 and Full Allosteric Agonist AP8 of hGPR40 by Gaussian Accelerated Molecular Dynamics (GaMD) Simulations

An Xiaoli and Yao Xiaojun

The free fatty acid receptor 1 (FFA1, also called GPR40) has been proposed as a therapeutic target of type 2 diabetes mellitus[1]. And synthetic agonists of GPR40 have been reported[2,3]. Radioligand-binding interaction studies demonstrate that multiple ligand-binding sites on GPR40, present positive binding cooperativity between the full agonists (AgoPAMs) and partial agonists. Herein, we performed long-time GaMD simulations on GPR40 to shed light on the mechanism of positive binding cooperativity between the partial agonist MK-8666 and AgoPAM AP8[4]. Our results reveal the bidirectional induced-fit conformational coupling between the partial agonist and AgoPAM binding sites. Re-docking and the molecular mechanics/generalized born surface area (MM/GBSA) energy estimate confirm that there is positive binding cooperativity between the two sites. The trans-membrane (TM) helices TM3, TM4, TM5 and TM6 make up the two sites. Upon alternative ligand or both ligands binding, the extracellular sides of TM4 and TM5 shift toward TM6, and TM6 shifts toward outside of helical bundle (Fig. 1). Simultaneously, the TM4 shifts downward (toward the intracellular side), and the TM5 shifts upward (toward the extracellular side) (Fig. 1(c)). That is to say, alternative ligand binding, the conformational changes of residues in corresponding site induce rearrangement of these helices. That leads to rearrangement of residues in other site, making the remote site suitable for ligand banding. Because of presenting Gly139$^{4.58}$ (the Ballesteros-Weinstein numbering scheme for GPCRs)[5]on TM4 and prolines on TM3, TM5 and TM6 (Fig. 1(c)), which give inherent flexibility of these helices. And gear-like movements of Leu138$^{4.57}$, Leu186$^{5.42}$ and Leu190$^{5.46}$ between the two sites coordinate the conformational rearrangement of residues in the two sites (Fig. 2). These results not only clarify the mechanism of cooperativity between the two sites, but also provide detailed structural information in structure-based compound screening or design. And by considering the positive binding cooperativity between different sites, it is maybe more reasonable with considering the effect of other site bound in structure-based compounds screening.

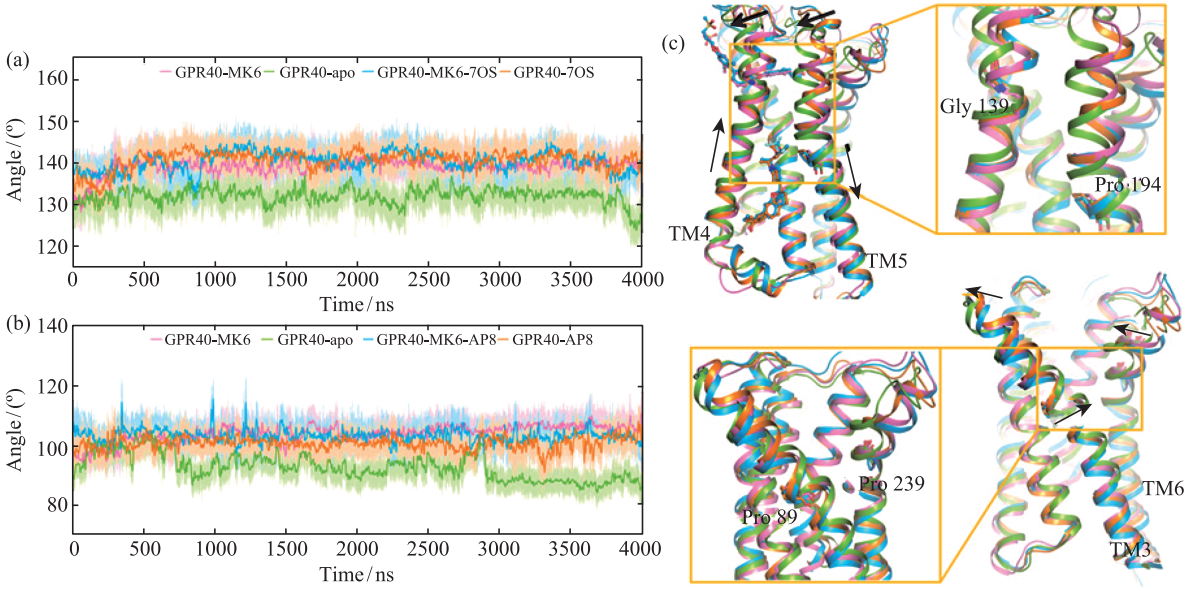

Fig. 1 (color online) (a) The angle α formed by TM2-TM3-TM4 evolves with time, (b) The angle β formed by TM6-TM3 evolves with time, (c) The conformational differences of TM3, TM4, TM5 and TM6 among the GPR40-MK6 (pink), GPR40-apo (green), GPR40-MK6-AP8 (blue) and GPR40-AP8 (orange).

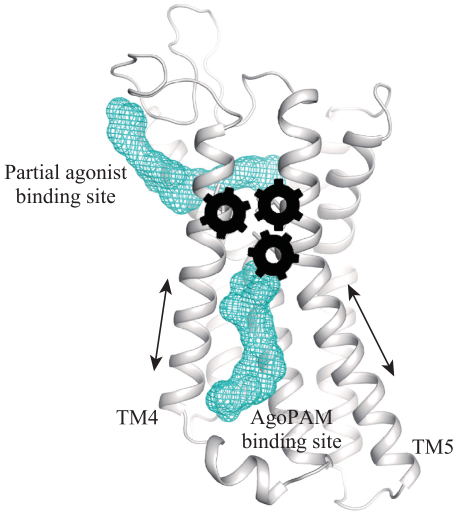

Fig. 2 (color online) The mechanism of bidirectional induced-fit conformational coupling between partial agonist MK-8666 and AgoPAM AP8 binding sites of hGPR40.

References

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