Energetic Considerations in the Mechanisms of Activation of Rhodopsin-like Receptors

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INTRODUCTION

In the inactive state (R) TM3 and TM6 are held together through electrostatic interactions of the central arginine (R3.50) of the conserved D/ERY motif in TM3, with D3.49 and E6.30 (see Fig. 1). Activation involves the distancing of TM3 and TM6, which is sustained in a ps time scale. We have presented earlier evidence that supports the hypothesis that conformational changes at the level of the aromatic cluster in TM6 and P6.50 account for the separation of TM3 and TM6 triggered by ligand binding. Here we extend those studies to the associated changes in the acidic lock between TM3 and TM6 that can account for the stabilization of the open state, so that such an open state can be sustained in the time scale required for subsequent steps in signaling.

Rhodopsin, activation is associated with the rapid uptake of two protons1,2. The acidic residue at position 3.49 is likely one of two that become protonated during activation3,4. E6.30 seems to be a likely candidate for a second protonation site5. The protonation of the two acidic residues was assumed to destabilize the constraining interaction with R3.50 which then would be free to rearrange to a position compatible with the active state of the receptor6. We explore here the steps of this hypothesis that relate the change in the protonation state of the residue at positions 3.49 and 6.30 to the change in their electrostatic environment caused by the movement of TM6. The inferences from the calculations of the electrostatic potentials in the different states of the receptor were also tested experimentally with mutations that change the character of the electrostatic interactions at the cytoplasmic ends of TM3 and TM6.

COMPUTATIONAL SIMULATIONS OF TM3-TM6 INTERACTIONS

FIGURE 1. Model of H3-H6 proximities in the 5-HT2A receptor. A. Close neighbors of R3.50 viewed from the extracellular side. When H3 and H6 are in close proximity, R3.50 is proposed to interact with both D3.49 and E6.30. Experimental data and simulations have shown that H6 moves away from H3 upon activation1,3, indicated here by an arrow. B. Superposition of 100 representative conformations of H6 obtained by exploration of the conformational space using the biased Monte Carlo method of Conformational Memories. The proline kink in H6 functions as a flexible hinge, but in the presence of an interaction between R3.50 and E6.30 the conformations are restricted to the proximity of TM3. The release of R3.50 facilitates the second protonation at E6.30

FIGURE 2: Molecular Electrostastic Potential displayed in the molecular surface of TM3 and TM6 of the rhodopsin crystal structure7 corresponding to the inactive form of the receptor. A: rendered view; B: mesh view. The position of the atoms of the acidic lock can be seen through the molecular surface in panel B, to clarify their identification in panel A.

FIGURE 3: Molecular Electrostatic potential displayed on the molecular surface of a model of the active form of rhodopsin characterized by a lowered (27 degrees) kink in two different protonation states: A: None of the acidic residues are protonated. R3.50 is caged with E3.49. Note that the MEP on the position of the acidic oxygens of E3.49 and E6.30 has decreased with respect of the values obtained in the inactive state of the receptor (see Figure 2), and thus its proton affinity increased. E3.49 is protonated and R3.50 moves out from its cage.

FIGURE 4: In this panel (B), E3.49 has been protonated and R3.50 moved out from the lock. This caused a new increase in the MEP on E6.30, which supports the proposal of this residue as second protonation site.

FIGURE 5: Constitutive activity of the wild-type and E 6.30Q mutant 5-HT2A receptors. The level of agonist-independent accumulation of [3H]P in the presence of increasing concentrations of the inverse-agonist ketanserin are shown for COS-1 cells expressing WT(•) or E6.30Q (○) receptor. Data are the mean ± S.E of triplicate determinations in a representative experiment performed three times. In this experiment basal accumulation of [3H]P in pcDNA3-transfected cells was 393 dpm/10 µg protein.

FIGURE 6. Normalized constitutive activity of mutant 5-HT2A receptors. Basal [3H]P accumulation in cells expressing mutant receptors was normalized to expression level. Results shown are mean ± S.E of 3-6 experiments for each construct.

EFFECTS OF MUTATION OF E6.30 AND D3.49: The hypothesis that the interaction of R3.50(T73) with both D3.49(S72) and E6.30(S318) favors on inactive receptor conformation in the SH2 domain was evaluated by studying the effects of mutations of these acidic side chains on the binding and functional properties of the receptor. In order to test the role of charge and hydrogen-bonding in this interaction, E8.30(Q318) was substituted with D, N, Q and L.

FINDINGS

1. MEP calculations indicate that the proton affinity of E3.49 increases with the distance between TM3 and TM6, favoring its protonation.
2. Protonation of E3.49 facilitates the release of R3.50 from its cage.
3. The release of R3.50 from its cage facilitates the second protonation at E6.30.
4. The ionic lock between helices TM3 and TM6 stabilizes the inactive state of the receptor, and its weakening or elimination favors the activated state of the receptor.

CONCLUSION

The activation mechanism of rhodopsin is associated with the distancing of TM6 from TM3. The receptor conformation resulting from the initial distancing of the helices is further stabilized (trapped) by protonation of the residues in the acidic lock, so that the distancing of TM3/TM6 is maintained to enable the activated state. The changes in electrostatic properties at the ends of TM3 and TM6 explain how the conformational rearrangement that is triggered by changes in the proline kink and constitutes receptor activation, is sustained in the time scale required for subsequent steps (e.g., interaction with G proteins).