INFLUENCE OF MEMBRANE LIPID COMPOSITION AND POINT MUTATIONS ON CONFORMATIONAL EQUILIBRIUM OF B2-ADRENOCEPTOR: MOLECULAR DYNAMICS STUDY

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One of the important external factors directly affecting signaling cascades mediated by functionality of ligand-activated G-protein coupled receptors is related to the excess or deficiency of the various molecular components of plasmatic membranes. In the case of β2-adrenoreceptor experimental studies have shown that expression of the receptor within membrane-environment saturated with cholesterol resulted in the increase of thermal stability of the receptor. On other hand by means of molecular dynamics simulation of the adenosine A2A receptor in explicitly modeled lipids it was shown that presence of the cholesterol in complex with the receptor resulted in the stabilization of specific conformation of its intramembrane helices, eventually leading to change of the activity of the receptor. According to following studies in case of the β2-adrenergic receptor and number of related receptors from rhodopsin-like subfamily, it was found conserved amino acid motif which is responsible for the interaction of these proteins with cholesterol. However, the functional significance of individual amino acid residues of the receptor in its interactions with cholesterol, as well as its allosteric effect on the macromolecular dynamics, are still lack of full clarity. Investigation of the influence of these factors on the functional behavior of β2-adrenergic receptor is possible by means of amino-acid substitutions (point mutations) of such functionally-relevant residues and subsequent molecular dynamics simulation of the mutated protein.

In scope of this paper, the method of classical molecular dynamic simulation is used for investigation of the effect of the membrane composition (the presence or absence of cholesterol) on the conformational dynamics of β2-adrenergic receptor. Additionally, we examined the effect of the point mutation within the conserved amino acid sequence which is responsible for interaction of the receptor with cholesterol by means of redistribution of its conformational equilibrium. Biomolecular simulations were carried out for the β2-adrenergic receptor inserted within modeled membrane using supercomputer "Lomonosov" of Computer Center of Moscow State University by Lomonosov. MD software package GROMACS, force field CHARMM36 were used for the calculations, the length of the molecular dynamics trajectories were 700 ns.

It was found that presence of the cholesterol stabilizes specified inactive state of the receptor, eventually facilitating transition of the protein towards its active form. The presence of the point mutation S74A made in conservative amino acid sequences of the receptor's cholesterol binding site subsequently promoted shift of its dynamic equilibrium towards the active state. In all of the modeled systems these results were obtained for the system with artificially lowered basal activity due to the presence of the inverse agonist within receptor's orthosteric binding site. Such findings clearly demonstrate effect of the cholesterol binding as the potential allosteric modulator of receptor's molecular eficasy by means of redistribution of its conformational equilibrium. This work was supported by the Russian Foundation for Basic Research (project № 14-04-01198-a).