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Conformational changes of G-protein coupled receptors can be monitored using dyes based on GFP-core

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G-protein coupled receptors (GPCR) are very important class of proteins that take part in many important processes in the human body. About 60% of all drugs have an effect on GPCR. GPCRs are transmembrane proteins with seven alpha-helices. Our protein regulates the myocardial oxygen demand and increases coronary blood circulation due to vasodilation. In addition, this receptor can suppress the activity of immune cells, thereby protecting tissues from inflammation [1].

To study the structure of the receptor, search for ligands, many biophysical methods were previously used, including NMR, crystallography [2], cryo-electron microscopy [3], fluorescence microscopy [4], molecular dynamics methods, binding of radiogands. Fluorescence microscopy can be singled out separately. Solvatochromic fluorescent dyes are able to change their fluorescence spectrum depending on their environment. Thus, it is possible to monitor various protein states and check whether the studied proteins interact with the proposed ligands. Since the protein conformation changes during ligand binding, the local environment of the dye will also change, which will entail changes in the emission and excitation spectra of fluorescence. Most proteins have cysteine in the structure, which allows you to hang one label using, for example, thiol-maleimide interaction. In the case of our labels, one spot for labeling will be enough to track all the main characteristics used in fluorescence microscopy: the lifetime of the fluorophore, emission spectra and fluorescence excitation.

The aim of this work is to search for solvatochromic fluorescent dyes that would be suitable for determining the conformational state of the receptor. Since each ligand changes the conformation of the protein in different ways, such dyes will allow finding new ligands and determining what state the protein is in at a particular time. The discovery of new ligands gives an understanding of what processes the protein is involved in in the body, and this gives new opportunities in therapy. We investigated 2 dyes based on the GFP chromophore nucleus that interact with cysteines in the protein via the maleimide-thiol interaction. We have shown the binding of the receptor to the dye and changes in photophysical properties when an agonist and antagonist are added to the protein.

References

1) Ohta A., Sitkovsky M., "Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage", Nature. 2001. Vol. 414, № 6866. P. 916–920.
2) Doré A.S. [et al.], "Structure of the adenosine A 2A receptor in complex with ZM241385 and the xanthines XAC and caffeine", Structure. 2011. Vol. 19, № 9. P. 1283–1293.
3) García-Nafría J. [et al.], "Cryo-EM structure of the adenosine A2A receptor coupled to an engineered heterotrimeric G protein", Elife. 2018. Vol. 7. P. 1–19.
4) Maslov I. [et al.], "Structural dynamics of the A 2A adenosine receptor revealed by single-molecule FRET", FASEB J. 2021. Vol. 35, № S1.