

FUNCTIONAL BIOASSAY ON G PROTEIN-COUPLED RECEPTORS USING TOTAL INTERNAL REFLECTION FLUORESCENCE

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INTRODUCTION: The ability of organisms, or individual cells to sense and react to different external signals (light, hormones, odorants, taste) is crucial for their survival. External signals interact with cell surface receptors which transduce the signals across the membrane and activate a variety of intracellular processes leading to the cellular response. G protein-coupled receptors (GPCRs) constitute a major class of membrane receptors¹. They transmit extracellular stimuli by activating intracellular heterotrimeric GTP-binding proteins, called G proteins. In their resting state, G proteins bind to guanosin diphosphate (GDP). The exchange of GDP by GTP is promoted by the formation of a trimeric ligand-receptor-G protein complex, which is induced by ligand binding to the receptor. Binding to G protein in turn triggers dissociation of the G protein heterotrimer into its functional units: the GTP-complexed alpha and the betagamma subunit. The intracellular concentration of small signalling molecules (cAMP, cGMP, diacylglycerol, inositol-trisphosphate, Ca²⁺, K⁺) is then modulated by the conetration of both parts with the respective enzymes or ion channels. The GPCRs are therefore the target in the human body for the majority of clinical used drugs.

Today, functional investigations of GPCRs are mostly based on detection of indirect events occurring upon activation of the receptors. There is a high demand for both fundamental research and for different screening purposes in the pharmaceutical research and industry to detect the ligand binding events and the subsequent molecular interactions on the target membrane directly. Since optical evanescent wave techniques offer on-line detection of molecular interactions without additional separation steps, they are of great interest in this context. Here we propose to develop a generally applicable procedure for assessing G protein activation by its receptor using Total Internal Reflection Fluorescence (TIRF) spectroscopy²⁻³.

RESULTS: We choose the neurokinin (tachykinin)-1 receptor (NK1R) as a model of a GPCR. It is activated by the natural tachykinin peptide Substance P. The immobilization of this

member of the superfamily was performed using the high specificity of biotin-streptavidin interaction. The quartz surface was first covered with biotinylated BSA and then with streptavidin. Native membrane fragments containing mutant NK1R protein, genetically modified with a biotin tag⁴ were finally added to the surface. The immobilization of these fragments on several surfaces was shown to be reproducible.

The binding of a fluorescent agonist (substance P labelled with fluorescein) to the NK1R at different ligand concentrations was followed. The specificity of the binding was controlled using a competitive antagonist non fluorescent. It allowed the establishment of the binding curve of the fluorescent agonist, which is in good agreement with the pharmacological features of the receptor in its native environment and in solution, suggesting that the immobilization of the protein doesn't affect its binding features.

It was thus possible to perform a pharmacological study on few amounts of protein, coming from crude cellular extracts, without further purification.

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