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Artificial membrane-like environments for *in vitro* studies of purified G-protein coupled receptors ☆

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ABSTRACT

Functional reconstitution of transmembrane proteins remains a significant barrier to their biochemical, biophysical, and structural characterization. Studies of seven-transmembrane G-protein coupled receptors (GPCRs) *in vitro* are particularly challenging because, ideally, they require access to the receptor on both sides of the membrane as well as within the plane of the membrane. However, understanding the structure and function of these receptors at the molecular level within a native-like environment will have a large impact both on basic knowledge of cell signaling and on pharmacological research. The goal of this article is to review the main classes of membrane mimics that have been, or could be, used for functional reconstitution of GPCRs. These include the use of micelles, bicelles, lipid vesicles, nanodiscs, lipidic cubic phases, and planar lipid membranes. Each of these approaches is evaluated with respect to its fundamental advantages and limitations and its applications in the field of GPCR research. This article is part of a Special Issue entitled: Membrane protein structure and function.

1. Introduction

The ability to sense environmental cues and respond to them appropriately is critical for the existence of any living cell [1]. A wide variety of receptors has evolved to fulfill this need, and the G-protein coupled receptors (GPCRs) have enjoyed particular evolutionary success. These receptors follow a common mechanism of action. First, the receptor is activated by the binding of a ligand from the extracellular side. Second, the seven transmembrane helices rearrange, transducing the conformational change associated with ligand binding from the extracellular side of the receptor protein to the cytoplasmic side. Finally, the binding of one or more types of heterotrimeric G-proteins on the cytoplasmic side allows downstream signaling cascades within the cell to take effect [2–4]. All GPCRs are metabotropic, *i.e.*, not directly linked to ion channels or pores. The versatility of GPCRs stems from the large variety of ligands that can be accommodated [5] – from photons of light, to ions, neurotransmitters, and even large polypeptide hormones – as well as from the vast possibilities for signal amplification and modulation through downstream signaling cascades [6,7]. At over 800 members, the GPCRs are the largest protein family in the human proteome. From a pharmacological standpoint, they are the target of some 50% of all drugs currently on the market [8]. Understanding molecular mechanisms of GPCR signaling is therefore of both fundamental scientific impact and high medical potential.

However, biophysical studies of these receptors have been particularly challenging because they are transmembrane proteins heavily dependent on the membrane environment for proper function. Purified protein samples are necessary for obtaining quantitative biophysical information on the molecular details of structure and function. Reconstitution into a native-like environment is important in the case of GPCRs to ensure that the sample represents a biologically relevant protein conformation, *i.e.*, as close as possible to the one present in living cells. Reconstitution is also needed to maintain stability. Furthermore, though crystal structures of several GPCRs have been solved [9–19], understanding the kinetics and dynamics of these molecules under physiological conditions remains challenging. Biophysical studies *in vitro*, such as NMR [20–23], infrared [24] and Raman vibrational spectroscopy [25,26], electron paramagnetic resonance (EPR) [27,28], circular dichroism [20–23], and fluorescence spectroscopy [29,30], provide detailed molecular insight into the function of these proteins. All of these functional methods, as well as structural ones like X-ray crystallography [9–19], solid-state NMR [20–23], or neutron scattering [31], require stable, purified GPCR sample reconstituted in a mimic of the native environment.

A fundamental challenge in this research field lies in the basic topology of GPCR signaling. A GPCR can be conceptualized as consisting

Abbreviations: GPCR, G-protein coupled receptor; SUV, small unilamellar vesicle; GUV, giant unilamellar vesicle; MSP, membrane scaffold protein; PLM, planar lipid membrane

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of three parts: an extracellular ligand-binding surface, a transmembrane region, and an intracellular G-protein-binding surface (Fig. 1). Though any given experimental technique imposes its own constraints, a complete molecular-level study of receptor activation and signaling requires each of these surfaces to be accessible to experimental manipulation. Thus, the best reconstitution system for a given investigation permits easy and independent experimental access to the outside and inside surface of the membrane, as well as to the plane of the membrane. While reviews of GPCR structure [4], GPCR dimerization [32], the pharmacological potential of GPCRs [8,33], GPCR purification [34,35], and GPCR–lipid interactions are available [36], reconstitution of purified GPCRs into artificial membrane-like environments has not been reviewed to date. This article reviews various membrane mimics for reconstitution of functional GPCRs *in vitro* for detailed biophysical analysis of their function in relation to their structure and dynamics.

2. Overview of GPCR reconstitution

The general approach to prepare reconstituted samples is to first obtain a sufficient quantity of the GPCR under study, then purify it, and finally reconstitute it into a membrane-like environment. The GPCR must either be expressed recombinantly [34,37] and then purified or purified directly from natural sources [38,39]. For instance, bovine rhodopsin, the first GPCR to be studied *in vitro*, was initially purified from preparations of bovine retinas [38,39]. In general, however, purifying GPCRs from natural sources is difficult due to the low abundance of most GPCRs and the need to raise highly specific antibodies against each for immunopurification. A better strategy is to use an expression system to express a GPCR fused to a tag for purification [34,40]. The choice of expression system depends on the GPCR under study [37]. GPCRs are eukaryotic proteins. A small number of GPCRs can be expressed functionally in bacterial cells [41]; some can be expressed as nonfunctional polypeptides in inclusion bodies and subsequently refolded; others fail to express in a bacterial system at all. Yeast and insect cells [17,42,43] have been used to express some GPCRs, but others require the post-translational modification machinery only found in mammalian cells [44,45]. Robinson and colleagues provide a review of GPCR expression systems [46].

Regardless of the choice of expression system, three steps need be carried out to reconstitute GPCRs. The first step is to solubilize the plasma membranes of the expressing cells, typically with the help of detergents [35,40,47]. Afterward, affinity purification can separate the target GPCR from all other membrane components of the expressing cells. Finally, detergent is removed by dialysis [48,49], gel filtration [50], or adsorption onto a material such as Biobeads® [51]. At that time, the GPCR has been reconstituted and is available for quantitative biophysical investigations.

3. Membrane-like environments

Six basic types of membrane mimics will be considered in this review. These are (1) micelles, (2) unilamellar lipid vesicles, (3) bicelles, (4) nanodiscs, (5) planar lipid membranes, and (6) lipidic cubic phases. Conceptual considerations and examples of applications for each are discussed below.

3.1. Detergent and mixed micelles

The most basic strategy for *in vitro* studies of purified GPCRs is the use of detergent or mixed micelles [35,40]. Mixed micelles are prepared from a mixture of detergents or a mixture of detergent and lipid. Since the protein contains both hydrophobic and hydrophilic regions, amphipathic molecules such as detergents provide an environment that lends some stability to the GPCR molecules and prevents their precipitation (Fig. 2).

A variety of detergents is available for studies of transmembrane proteins, but the choice must fall on a detergent that is gentle enough to avoid immediate denaturation of the protein. For example, sodium dodecyl sulfate (SDS), a very common but highly denaturing detergent, is not suitable for this reason. Detergents such as cholate, dodecyl-β-D-maltoside (DDM) [52], and *n*-octyl-β-D-glucopyranoside (OG) [53], as well as mixed micelles have been used with greater success. For instance, the first crystals of rhodopsin that yielded a high-resolution structure were obtained from solutions of mixed micelles of nonyl-β-D-glucoside and heptanetriol [18,54].

Newer, rationally designed detergents include amphipols [55,56], which can wrap around the hydrophobic region of a GPCR while exposing their hydrophilic side chains to the solvent. This feature is especially useful since detergent concentration does not have to exceed the critical micelle concentration (CMC), and therefore the interference with molecular conformation or spectroscopic measurements due to free detergent in solution is lower. Zhang and colleagues reported that addition of peptide surfactants to traditional detergents like DDM and OG enhances thermal stability of rhodopsin compared to what is attainable with detergent alone [57]. Recently, Welte and coworkers implemented a modified detergent system, using maltoside detergents with cyclohexyl or aromatic groups in place of the alkyl chains. This modification resulted in increased thermal stability for at least two human GPCRs compared to DDM [58]. A detailed review of the variety of detergents used to stabilize transmembrane proteins is available to the interested reader [49].

In spite of these successes, it is clear that a true lipid bilayer environment is best suited for biophysical studies of GPCRs in a functional and stable form. For instance, the photochemical properties of rhodopsin are sensitive to the composition of its lipid environment [59]. Indeed, a crystal structure of bovine rhodopsin retains tightly bound phospholipid molecules [19]. This tight binding suggests an important role for these molecules in maintaining the proper structure of the receptor.

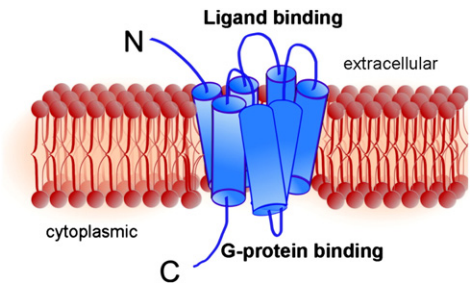

Fig. 1. Schematic overview of a GPCR (blue) embedded in the plasma membrane (red).

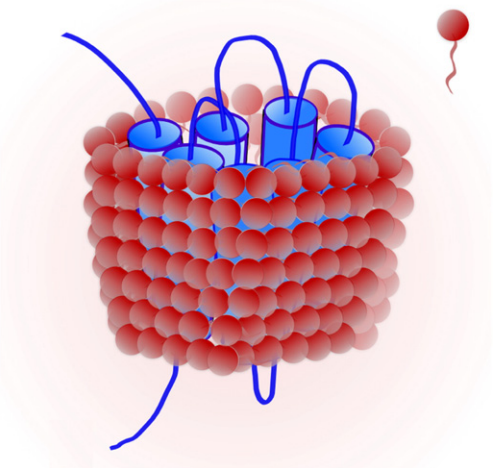

Fig. 2. A GPCR (blue) stabilized by a detergent micelle (red).