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Research review paper

Expression, stabilization and purification of membrane proteins via diverse protein synthesis systems and detergents involving cell-free associated with self-assembly peptide surfactants

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ABSTRACT

G-protein coupled receptors (GPCRs) are involved in regulating most of physiological actions and metabolism in the bodies, which have become most frequently addressed therapeutic targets for various disorders and diseases. Purified GPCR-based drug discoveries have become routine that approaches to structural study, novel biophysical and biochemical function analyses. However, several bottlenecks that GPCR-directed drugs need to conquer the problems including overexpression, solubilization, and purification as well as stabilization. The breakthroughs are to obtain efficient protein yield and stabilize their functional conformation which are both urgently requiring of effective protein synthesis system methods and optimal surfactants. Cell-free protein synthesis system is superior to the high yields and post-translation modifications, and early signs of self-assembly peptide detergents also emerged to superiority in purification of membrane proteins. We herein focus several predominant protein synthesis systems and surfactants involving the novel peptide detergents, and uncover the advantages of cell-free protein synthesis system with self-assembling peptide detergents in purification of functional GPCRs. This review is useful to further study in membrane proteins as well as the new drug exploration.

Contents

Abbreviations: ac-A_6K-CONH2, AAAAAAK-$CONH_2$; ConA, lectin concanavalin A; Dcytb, cytochrome b; DDM, n-dodecyl-β-D-maltoside; *E. coli*, *Escherichia coli*; FSEC-TS, fluorescence-detection size-exclusion chromatography-based thermostability assay; GPCRs, G-protein coupled receptors; hFPR3, human formyl peptide receptor; hPOMGnT1, human protein of O-linked mannose β-1,2-N-acetylglucosaminyltransferase; hTAAR5, human trace-amine receptor; LCP, lipid cubic phase; MBCD, methyl-beta-cyclodextrin; MBP, maltose-binding protein; NAPols, nonionic amphiphols; OG, n-octyl-ß-D-gluco-pyranoside; ORs, olfactory receptors; PS-I, photosystem I; SDC, sample displacement chromatography; Sf21, *Spodoptera frugiperda* 21; TAAR, human trace amine-associated receptor; TF, trigger factor; T4L, T4 lysozyme; VNRs, human vomeronasal receptors.

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Introduction

Functionally diverse group of membrane proteins anchored in cellular membrane, like G protein-coupled receptors (GPCRs), are critical for signal transduction in cell physiological actions (Ballesteros and Palczewski, 2001; Stefanovic and Hegde, 2007). The receptors receive primary stimuli such as hormone, odorant, light and neurotransmitter, producing vision, smell, taste and pain by an exogenous signal (Premont and Gainetdinov, 2007; Rosenbaum et al., 2009), associated with the extracellular and intracellular signal transmissions. GPCRs represent the most important family of drug targets, being responsible for more than 60% of the current pharmaceutical drugs for a variety of diseases (Gainetdinov et al., 2004; Lagerstrom and Schioth, 2008; McCusker et al., 2007). However, their structure/function is still difficult to investigate due to low abundance of GPCRs on cell surface. Heterogeneous protein synthesis systems, including *Escherichia coli* (*E. coli*) system, yeast system, mammalian or insect cells with virus vector expression system, cell-free system and zebrafish system (Ghaemmaghami et al., 2003; Sarramegna et al., 2003), contribute to high yields of GPCRs from micrograms to milligrams, but are still with the post-translation modification problems such as phosphorylation, glycosylation and N-myristoylation (Carlson et al., 2011; Gerngross, 2004; Prinster et al., 2005).

Compared with those various expression systems, cell-free system has some advantages to produce some notorious proteins (Katzen et al., 2005; Nirenberg and Matthaei, 1961; Spirin, 2004) or construct proteins at industrial scale, but still are limited to the low protein production rate, expensive reagent costs and short reaction durations of protein synthesis (Carlson et al., 2011; Zawada et al., 2011).

Stabilization of the GPCRs is often a vital issue. The traditional detergents SDS (Sodium dodecyl sulfate), DDM (n-dodecyl-β-D-maltoside) and Triton X-100 have been widely used in these studies. Self-assembly peptide surfactants as a new kind of detergent, coupled with cell-free systems, might also be with potential ability to stabilize membrane proteins (Heerklotz et al., 2009; Le Maire et al., 2008), like olfactory receptors (ORs), human trace amine-associated receptor (TAAR) and human vomeronasal receptors (VNRs) (Corin et al., 2011a,b; Luo and Zhang, 2012). In this review, we will focus on the protein synthesis systems, the surfactants of stabilizing membrane proteins and some typical GPCRs using cell-free system with self-assembling peptide detergent to review their progress in recently years.

Expression, stabilization and purification of GPCRs

Expression

The available sufficient quantities of purified proteins are one important factor to obtain high-resolution 3D structures of GPCRs. Impractical rendering of their direct purification from the poorly abundance natural sources (Sarramegna et al., 2003), recombination heterologous expression systems with the development of protein synthesis technologies has circumvented the problem, including *E. coli* expression system, yeast expression system, mammalian or insect cells with virus vector expression system, cell free protein synthesis system and zebrafish embryo expression system.

Escherichia coli (E .coli) expression system

The product of human insulin synthesized by *E. coli* system was a major shock and breakthrough in the pharmaceutical industry. This technology has been successfully utilized for protein production in medicine, commercial pharmaceutical industry, or basic scientific research (Falzon et al., 2006). With the improvement as well as simple operation processes, such as the opening up of possibilities to express low copy number proteins (Cai et al., 2006), *E. coli* as a microorganism host is widely used in recombinant protein production (Fig. 1A).

With important significance in the protein synthesis fields, *E. coli* has many advantages, including short generation time, high-yields, simple process scale up, fast mass production and less cost (Demain and Vaishnav, 2009). The proteins are usually expressed as insoluble inclusion bodies in *E. coli*, and the controlled of the cellular milieu are potential to the reverse of inactive inclusion bodies into a soluble and functional fraction (Sarramegna et al., 2003). The inclusion bodies also owe the property to protect the synthesized proteins from proteolytic degradation in cytoplasm, membranous or periplasmic proteases, increasing the yields of recombinant proteins and making them easy to be purified (Sahdev et al., 2008). Cleaved proteins, obtained from the fusions like Fh8 fusion and maltose-binding protein (MBP) fusion, were in higher scales (Costa et al., 2012; Hewitt et al., 2011). However, the lack of endogenous G proteins and the diffidence of lipid construction can affect the binding properties and high-affinity agonists of the recombinant receptors. The insufficient post-translation modifications with products of inactive proteins offer the greatest challenges in the bacterial expression system (Sarramegna et al., 2003). For promoting the proper refolding and keeping of biological activity of GPCRs, optimized reaction steps and novel artificial chaperone-assisted molecules are necessary (Attrill et al., 2009; Michalke et al., 2010). Vectors with the potential for overproduction of recombinant proteins include pCold-PDI vector, pIGPZ vector, DO-controlled nar promoter expression vector and co-expression vector of pHEX, most of which even can regulate to express membrane proteins with toxicity to host cells. (Kim et al., 2012; Subedi et al., 2012; Zaleski et al., 2012; Zeng and He, 2012). Liu W and colleagues found that optimizing the bacterial strain, growth conditions, and the cDNA codon usage result in a yield of ~26 mg of purified human duodenal cytochrome b (Dcytb),offering ~7-fold improvement in yields (Liu et al., 2011). Strained metabolic conditions such as temperature, minimal media, oxidizing environment, carbon dioxide evolution rate and induction time appear to be essential for high-level, full-length protein expression (Natarajan et al., 2011; Tait and Straus, 2011).

Yeast expression system

Yeast exp-ression system is the most efficient producers, playing an important role in eukaryotic systems for protein synthesis (Lamping et al., 2007). Despite its insufficiency of post-translation processing to eukaryotic proteins, the property with stable cell lines, time-consuming to generate and less expensive has made it increasingly popular (Fig. 1B) (Idiris et al., 2010; Newstead et al., 2007). Complimentarily, using the