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The yin and yang of solubilization and stabilization for wild-type and full-length membrane protein

David Hardy [a,b], Elodie Desuzinges Mandon [a], Alice Rothnie [b], Anass Jawhari [a,*]

[a] CALIXAR, 60 Avenue Rockefeller, 69008 Lyon, France
[b] Life & Health Sciences, Aston University, Birmingham B4 7ET, UK

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ABSTRACT

Membrane proteins (MP) are stable in their native lipid environment. To enable structural and functional investigations, MP need to be extracted from the membrane. This is a critical step that represents the main obstacle for MP biochemistry and structural biology. General guidelines and rules for membrane protein solubilization remain difficult to establish. This review aims to provide the reader with a comprehensive overview of the general concepts of MP solubilization and stabilization as well as recent advances in detergents innovation. Understanding how solubilization and stabilization are intimately linked is key to facilitate MP isolation toward fundamental structural and functional research as well as drug discovery applications. How to manage the tour de force of destabilizing the lipid bilayer and stabilizing MP at the same time is the holy grail of successful isolation and investigation of such a delicate and fascinating class of proteins.

Contents

1. Introduction

Membrane proteins (MP) represent 20–30% of human proteins. They are crucial for cellular physiology as they are directly involved in a large spectrum of cellular processes including cell adhesion, cell-cell communication, signal transduction and transport. This may explain why they represent 70% of therapeutic targets [1]. Many of the difficulties associated with MP structure, function and drug discovery have stemmed from the need to solubilize them from the membrane bilayer with detergents. Thus, it is essential to develop tools for membrane protein solubilization and stabilization to unlock structure function details as well as drug discovery.

2. What parameters control stabilized membrane proteins production?

There is not only one parameter to consider for successful MP production. MP production work flow can be improved, at the

* Corresponding author.
E-mail address: ajawhari@calixar.com (A. Jawhari).

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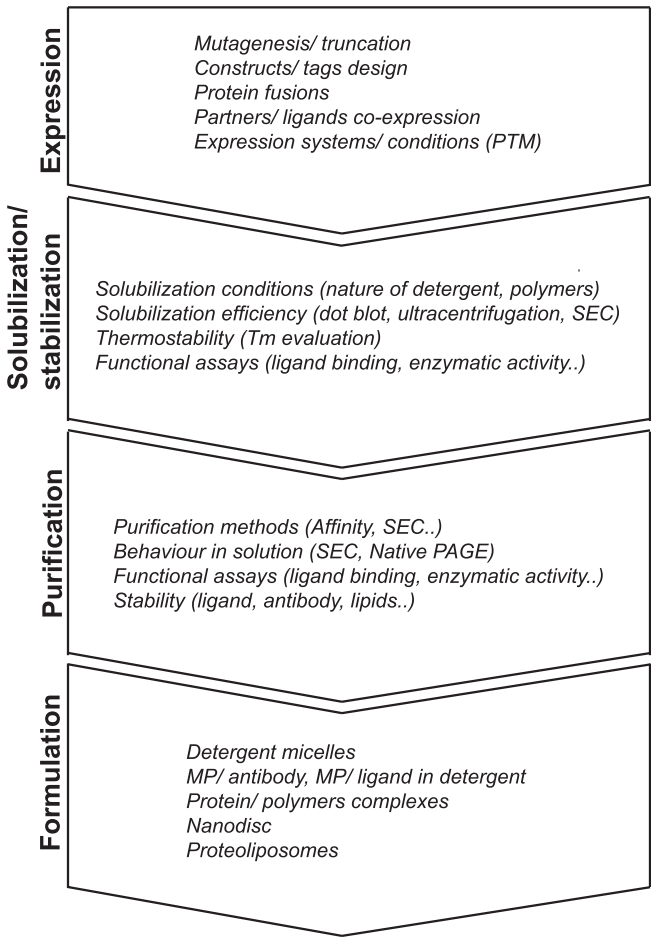

Fig. 1. Parameters to consider for the optimization of membrane protein production including expression, solubilization, purification and formulation. PTM, SEC, Tm, PAGE, MP stand for post-translational modifications, size exclusion chromatography, melting temperature, polyacrylamide gel electrophoresis and membrane protein, respectively.

expression, solubilization, purification and formulation steps (Fig. 1). A tremendous effort has been made with MP thermostabilization expression approaches using truncation, multiple alanine scan mutagenesis and protein fusion (such as T4 lysozyme or BRIL). Co-expression of membrane protein partners may also help improve stability. It was recently reported that co-expression of mini-protein G helped to stabilize a GPCR [2]. Protein engineering strategies were applied to different GPCRs such as β1 adrenergic or adenosine A_{2A} receptors [3,4] resulting in different atomic structures [5–9]. Although these approaches have proven very successful for generating crystals for structure determination, the modification of the protein sequence may have a significant impact on the protein conformation and can therefore provide misleading information to drug discovery. For example, a study has demonstrated structural deviations of a fused receptor in the crystal due to the protein fusion [10]. Therefore, in this review we will focus on approaches for studying non-mutated full-length proteins which we term wild-type protein. The expression system used may also have an impact on the quality of MP. For some MP, posttranslational modifications can be important for function. Gene optimization as well addition of a variety of tags/fusion at different location may also help improve expression and stability of membrane proteins. Over-expression can also sometimes generate misfolded proteins and a good compromise is to be established. Co-expression with MP partner and/or sometimes in presence of

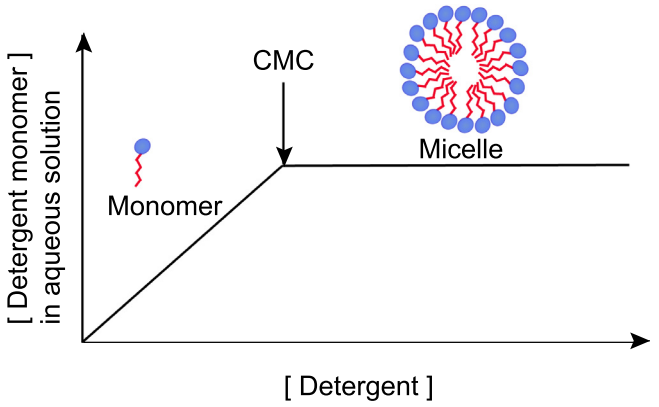

Fig. 2. Equilibrium between detergent monomers and micelles depending on the detergent concentration. Critical micelle concentration (CMC) is defined as the concentration of detergents above which micelles are spontaneously formed.

specific ligands may affect expression yield or stability features. Therefore, the choice of the expression system needs to take into account all the cited parameters. To assess the influence of expression conditions on protein stability in solution you could use fluorescence size exclusion chromatography, utilizing either fluorescence proteins such as GFP fused to the protein of interest or fluorescent multivalent NitriloTriacetic Acid dye that interact with His-tagged proteins [11]. In addition to stabilization at the expression level, the addition of high-affinity ligands, lipids or lipid-like molecules during membrane preparation, solubilization and/or purification can provide conformational or oligomeric stabilization [12–14]. Moreover, the use of antibodies, nanobodies and fusion proteins as chaperones can significantly improve the stability of different classes of membrane proteins [15–17].

3. Solubilizing membrane proteins with detergents

Arguably the most important parameter is to search for the best solubilization conditions that help maintain the structural and functional integrities. Solubilization and stabilization are intimately linked and balancing solubilization efficiency with protein stability can be extremely challenging. To date the majority of studies have utilized detergents for MP solubilization. Detergents are amphiphilic molecules made of hydrophobic and hydrophilic moieties that display monomeric and micellar organization in solution depending on the detergent concentration (Fig. 2). Detergents are commonly defined according to their charge, anionic (eg. sodium dodecyl sulfate; sodium deoxycholate), cationic (eg. cetyltrimethylammonium bromide), zwitterionic (eg. CHAPS for 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; LDAO for lauryldimethylamine-N-oxide or fos-choline) or non-ionic (eg. triton X-100 or DDM for n-dodecyl-b-maltoside) and the nature of hydrophilic (Carboxylate for example) and hydrophobic groups (lipid like structure such as aliphatic chain or alicyclic structures). Differences in detergent features directly influence their Critical Micelle Concentration (CMC), aggregation state, solubility in water, protein solubilization efficiency, protein stabilization ability and pH sensitivity. CMC is specific to each detergent and is defined as the concentration below which only detergent monomers exist in solution and above which detergent micelles start to form. Often biochemists assume that a concentration of 1% (w/v) of detergents is sufficient for comparison of solubilization efficiencies but this is not always true since detergents have very different CMC. Therefore, it is more relevant to rank detergent solubilization efficiency using concentrations related to the CMC. For example, a concentration of 10× CMC is commonly