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A general path for large-scale solubilization of cellular proteins: From membrane receptors to multiprotein complexes

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ABSTRACT

Expression of recombinant proteins in bacterial or eukaryotic systems often results in aggregation rendering them unavailable for biochemical or structural studies. Protein aggregation is a costly problem for biomedical research. It forces research laboratories and the biomedical industry to search for alternative, more soluble, non-human proteins and limits the number of potential "druggable" targets. In this study we present a highly reproducible protocol that introduces the systematic use of an extensive number of detergents to solubilize aggregated proteins expressed in bacterial and eukaryotic systems. We validate the usefulness of this protocol by solubilizing traditionally difficult human protein targets to milligram quantities and confirm their biological activity. We use this method to solubilize monomeric or multimeric components of multi-protein complexes and demonstrate its efficacy to reconstitute large cellular machines. This protocol works equally well on cytosolic, nuclear and membrane proteins and can be easily adapted to a high throughput format.

Introduction

The study of protein–protein interactions has contributed critically to development of most biological sciences fields. Yet, one of the most frequently encountered problems in protein chemistry is protein aggregation. To tackle this problem, efforts have been concentrated on development and optimization of protein expression systems [1–8] that have successfully produced folded (recombinantly-expressed) proteins. However, in spite of these advances, and the use of higher eukaryote expression systems, a large number of proteins continue to aggregate inside host cells or upon cell lysis, rendering them unavailable for biochemical and structural studies. Moreover, insoluble proteins are usually of mammalian origin and are frequently critical targets for drug discovery. Protein aggregation has hampered biochemical and drug-discovery studies and has forced structural biologists to opt for shorter versions of full-length proteins or for the more soluble "homologous versions" of the protein found in other species [9–12], particularly thermostable proteins from Archea species.

Several factors can trigger protein aggregation [13,14] some of these include: (1) monomer to oligomer transitions produced by electrostatic or hydrophobic interactions on proteins with complimentary surfaces; or covalent associations due to disulfide bond formation; (2) aggregation initiated by the presence of hydrophobic or highly charged electrostatic patches of partially unfolded intermediates; (3) aggregation of chemically modified products, such as proteolysis fragments and oxidized proteins. Small molecules capable of counteracting aggregating factors, could potentially improve protein solubility. Amphiphilic compounds, such as detergents, containing both hydrophilic "head" and hydrophobic "tail" groups, are great candidates to achieve solubility conditions in non-ideal environments such as protein lysates.

Detergents are classified according to their head group charge as ionic, if they have positive (cationic), negative (anionic), or both, positive and negative (zwitterionic) charges; and non-ionic if they

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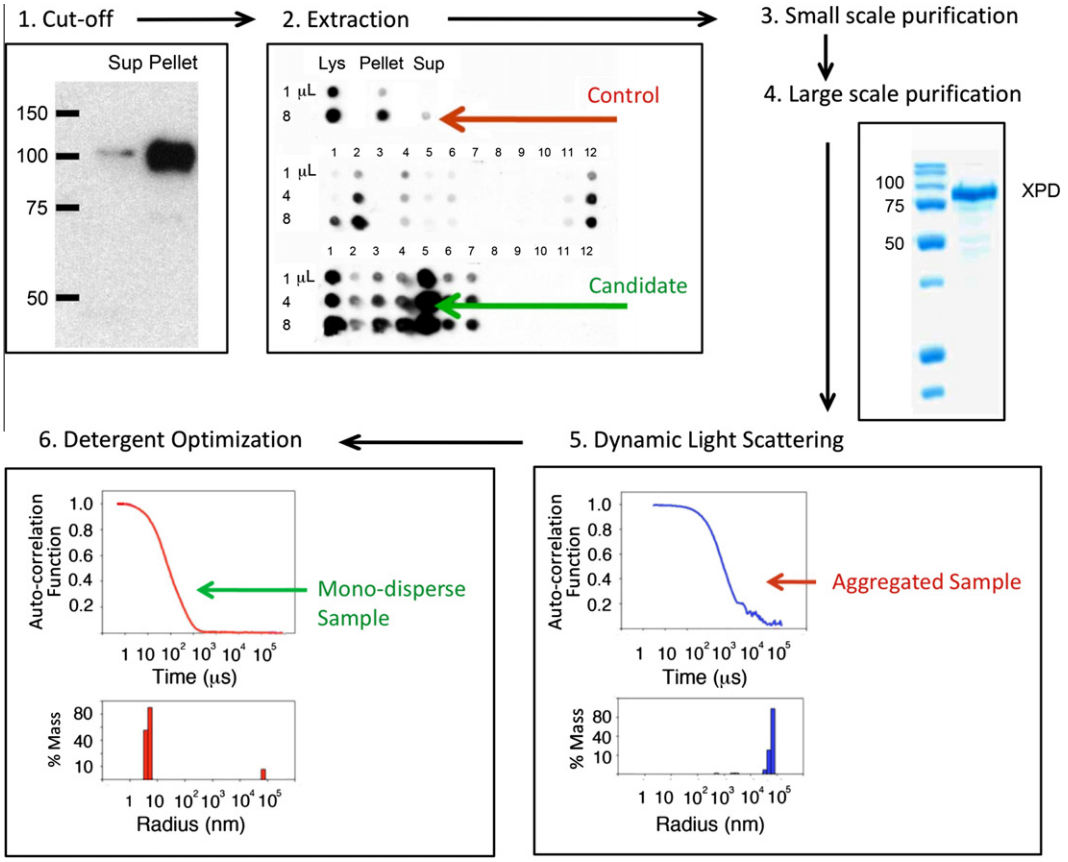

Fig. 1. Overall strategy. See text for details. (1) Cut-off: Proteins whose soluble fraction is less than 30% are subject to a detergent screen as illustrated for the human nucleotide excision repair protein (NER) XPD expressed in a baculovirus-infected Sf9 system. (2) Detergent extraction candidates: recombinant protein is extracted from insoluble pellets using a panel of detergents and the efficiency of each detergent to solubilize sample is quantified using immuno dot blots. (3) Small scale purification allows us to fine-tune salt and detergent concentration for sample binding to affinity beads and tag removal. (4) Large-scale purification with best detergent candidate. (5) Dynamic Light Scattering (DLS) and Multi Angle Light Scattering (MALS) experiments to test monodispersity. Aggregated samples (defined here as proteins whose measured radius (by DLS) is three times larger that it's predicted one) will be subject to additional detergent screening. (6) Detergent optimization: purified samples with large particle size are re-screened and analyzed in batch form using DLS. Detergents that can successfully produce monodisperse samples (measured radius <3× predicted radius) are selected.

lack head group charges. Hydrocarbon tails can be saturated alkanes (with different chain lengths), branched non-saturated alkenes or aromatic. With over a hundred detergents commercially available, a particular combination of hydrophilic head group and hydrocarbon tail length can interact favorably with charged surface residues and shield hydrophobic patches on subunits of multi-protein complexes or partially unfolded intermediates. Detergents have played critical roles in solubilization of membrane proteins; however their use as solubilizing agents for protoplasmic proteins has never been explored methodically and has been limited to the empiric use of few ionic and non-ionic detergents.

In this study we present a general method that features the systematic use of detergents to solubilize and purify – biomedically-relevant – human proteins to homogeneity, and apply this strategy to solubilize monomeric and multimeric components of multi-protein complexes (MPCs)[3] towards their reconstitution.

Methods and results

Extraction of target proteins from insoluble pellets

Our approach towards protein solubilization involves the following steps (Fig. 1).

Selecting a target for detergent extraction

To decide whether a protein will be subject to a solubilization protocol, we first perform immunoblot experiments of the supernatant and pellet after cell lysis to identify the fraction of insoluble protein. Visual inspection of western blots allows determination of the soluble and insoluble fractions (Fig. 1, panel: cut-off). We routinely perform detergent extraction and solubilization protocols for those samples whose soluble fraction is less than 30–50% of the total lysate. This is based on the observation that proteins with borderline or low solubility are not generally monodisperse during dynamic light scattering experiments.

Detergent extraction

The following is a general approach that we have taken for a large number of aggregated yeast and human proteins purified from *Escherichia coli* (*E. coli*), *Saccharomyces cerevisiae* or baculovirus-infected *Spodoptera frugiperda* (Sf9) cells. Protein samples with soluble fractions below 30–50% are subject to detergent extraction. Since it is hard to predict which detergent will have a positive effect on protein solubilization a wide range of surfactants including ionic and non-ionic and zwitterionic species are used. There are several commercially available detergent kits that provide a good starting point for the screen; among them is a 96-well block format screen from Hampton Research (detergent screen HT catalog number HR2-406). Approximately 0.5 g of cells (*E. coli* or Sf9) expressing a target protein are re-suspended in 9.5 mL of buffer containing 200 mM NaCl, 50 mM Hepes pH 7.5, and 2 mM β-mercaptoethanol

[3] *Abbreviations used*: MPCs, multi-protein complexes; BME, β-mercaptoethanol; cTFIIH, core TFIIH; GPCRs, G-protein coupled receptors; Fzd4, frizzled-4; PTHR1, parathormone receptor-1; CBP, calmodulin binding peptide; dsDNA, double-stranded DNA.