Integrated recovery-purification strategies of recombinant membrane proteins using alternative solvents

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Abstract: Recombinant proteins have provided important breakthroughs in medical biotechnology, either as biopharmaceuticals in therapeutics or as research agents in structural and functional studies. During the last two decades, recombinant protein production became a prerequisite and essential component of most modern small drug discovery programs [1], among of which membrane proteins are key players. The production of pure, stable and biologically active membrane proteins is often challenged by their highly hydrophobic nature, which need to be circumvented by efficient purification protocols. Aqueous biphasic systems (ABS) are formed when at least two water-soluble components are mixed in water above given concentrations, leading to the formation of a liquid-liquid system. Along with traditional applications as powerful platforms for the purification of high-value biomolecules, it has been recently proposed that the integration of several downstream units (e.g., cell lysis and capture) are key issues for the reduction of costs, also contributing to ensure the stability of the extracted biomolecule. Moreover, the effective implementation of ABS in industrial bioprocessing is dependent on the recyclability, biocompatibility and sustainability (economic and environmental issues) [2]. From the exposed, this work investigates the ability of several surfactants and ionic liquids to solubilize the cell wall and plasma membrane of Pichia pastoris for the recovery of intracellularly produced human cyclooxygenase 2 (COX-2), herein investigated as a model recombinant membrane protein. The ultimate goal is to combine this step with the creation of an ABS able to extract and purify this recombinant protein in a single-step, and thus develop an integrated process. Initially, the solubility behavior of N-acetyl-D-glucosamine (chitin monomers) and ergosterol, representative of structural components of Pichia pastoris cell wall and plasma membrane, was evaluated in silico in a wide range of ionic liquids and surfactants with the aid of conductor like screening model for real solvents (COSMO-RS). After this initial screening, COX-2 extraction was experimentally evaluated under different operational parameters such as biomass concentration (6.98 x 10^8 and 1,4 x 10^9 cells/mL),

incubation period (30 and 60 min), surfactant/ionic liquid concentration (5.4, 27, 50, 150, 350 mM), and temperature (25 and 40 °C). Promising results toward the extraction of biologically active COX-2 were successfully achieved using 350 mM 1-decyl-3-methylimidazolium chloride combined with 162 mM 1-ethyl-3-methylimidazolium acetate during 60 min at 40 °C using 1.4 x 10^9 cells/mL. The creation of an ABS is currently under investigation as well as the ability to reuse and recycle the phase-forming components, guaranteeing that the reproducibility and purity requirements of the original extraction stage are fulfilled. Overall, the approach here reported represents a step forward in the development of efficient processes to overcome the critical demand of high-quality membrane proteins for structural studies while the integration of two downstream units may potentially reinforce the sustainability of the process by decreasing costs and its environmental footprint.

Keywords: Bioprocess integration; aqueous biphasic system; purification; ionic liquid.

References:

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