Purification of Interferon α-2b using ionic liquid-based technologies

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Abstract

The development of protein-based biopharmaceuticals and their introduction on modern medicine brought enormous benefits to the treatment of life-threatening diseases such as cancer, diabetes and neurodegenerative disorders. However, their current high-cost still limits their widespread use. While significant advances at the upstream level have occurred, current purification strategies are unable to cope with high product concentrations resorting to cost-effective approaches. This work entails the development of sustainable downstream processing methodologies for recombinant interferon (IFN) α-2b derived from *Escherichia coli* using ionic liquids, either by exploring their application as adjuvants in polymer-polymer aqueous two-phase systems (ATPS) or as chromatographic ligands covalently attached in silica (supported ionic liquids - SILs). Overall, our results demonstrate the high potential exhibited by ionic liquids toward the preparative purification of the recombinant IFN α-2b biopharmaceutical.

Author Keywords. Biopharmaceuticals, purification, supported ionic liquids, chromatography, aqueous two-phase systems, IFNα-2b,

1. Introduction

Over the last decades, society has been facing an increment of chronic diseases due to the higher human life expectancy and the lack of efficient treatments for several human pathologies. In this regard, biopharmaceuticals have become one of the most effective clinical treatment for a broad range of diseases, including cancer, metabolic and neurodegenerative disorders (Kesik-Brodacka 2018). Most of these biotherapeutics are biological products obtained using recombinant DNA technology, and have been commercialized since 1982, with the approval of the first recombinant therapeutic insulin by the Food and Drug Administration. Since then, a massive growth has been witnessed in the biopharmaceuticals' market with global sales reaching $188 billion in 2017, being expected a further dynamic growth due to the tremendous demand for these drugs (Kesik-Brodacka 2018; Walsh 2018). Among biopharmaceuticals, the role of interferons, particularly interferon α-2b (IFNα-2b), should be underlined, as they have been marketed for over 30 years with a considerable impact on the global therapeutic proteins market (Castro et al. 2021).

Usually based on the recombinant DNA technology, the manufacturing process of biopharmaceuticals encompasses two main stages: the upstream and downstream stages. Typically, the upstream phase includes recombinant protein production processes in a suitable host microorganism, such as *Escherichia coli* (Jozala et al. 2016), while the general downstream processing of biopharmaceuticals comprises four stages - recovery, isolation, purification and polishing -, which are responsible for the majority of the production costs of

biopharmaceuticals (50–90%) (Jozala et al. 2016). From the exposed, the development of cost-effective purification processes is in crucial demand, and for which ionic liquids (ILs) have allowed for promising results when employed in the purification of biomolecules. In this field, ILs have been used in aqueous two-phase systems (ATPS) (Castro et al. 2019) and in supported ionic liquids (SILs) as alternative ligands (Neves et al. 2020). In this work these two IL-based strategies are investigated for the purification of IFNα-2b recombinantly produced from *E. coli*.

2. Materials and Methods

ATPS composed of polyethylene glycol with a molecular weight of 600 g/mol (PEG 600, 30 wt%), polypropylene glycol with a molecular weight of 400 g/mol (PPG 400, 30 wt%) and ionic liquids as adjuvants (5 wt%) at pH 8 were investigated to purify IFNα-2b obtained from *E. coli* lysates. To this end, ILs with different chemical structures, namely imidazolium, cholinium, phosphonium and ammonium cations and anions with different hydrogen bond basicities were evaluated. The total amount of protein was quantified using Pierce™ BCA Protein Assay Kit and the amount of IFNα-2b was determined using enzyme linked immunosorbent assay (ELISA) in order to determine IFNα-2b purification factors and extraction efficiencies. On the other hand, SILs were synthesized, by the covalent attachment of different IL cationic (imidazolium and ammonium chloride-based ionic liquids) moieties to silica (Neves et al. 2020) and characterized. In this approach, different elution conditions were applied to increase the purity of the target protein, either by exploring ionic interactions (Tris 10mM pH 8 and an increasing NaCl gradient) or hydrophobic interactions (Decreasing $(NH_4)_2SO_4$ gradients in Tris 10 mM pH 8). In this step, the elution pattern of proteins was evaluated using UV spectrophotometry (280 nm) and sodium dodecyl-sulfate polyacrylamide gel electrophoresis.

3. Discussion

Partition experiments of IFNα-2b with PEG 600-PPG 400 ATPS at a fixed mixture point (30 wt% - 30 wt%) demonstrated that the target protein is extracted to the PEG-rich phase, while most impurities precipitate at the interface of the system. In comparison with the system without IL adjuvant, an increment on the IFNα-2b purification factor of 3.5 was achieved in the presence of the 1-butyl-3-methylimidazolium acetate ionic liquid. The enrichment of IFNα-2b in the PEG phase seems to be driven by electrostatic interactions and aromatic interactions between the aromatic residues of IFNα-2b and IL cations, as well as hydrogen bonding interactions between IL anions and IFNα-2b.

In the approach using SILs, eight different modified-materials were synthesized, characterized and applied as chromatographic stationary phases for IFNα-2b purification. In general, the SIL modified with the imidazolium-based ionic liquid demonstrated the highest IFNα-2b purification ability, either in conditions favoring electrostatic interactions or hydrophobic interactions. Also, it was demonstrated that the IL chemical structure is responsible for the observed enhanced purification ability.

4. Conclusions

Overall, this work demonstrates the potential of two IL-based processes for the purification of the IFNα-2b biopharmaceutical. It is demonstrated the ability of ILs acting as adjuvants to tune the characteristics of the ATPS coexisting phases towards an improved purification. On the

other hand, it is demonstrated that ILs as ligands in solid supports allow the establishment of a multitude of different molecular interactions, which can be explored to enhance the purification of recombinant therapeutic proteins.

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