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Selective protein complexation and coacervation by polyelectrolytes

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ABSTRACT

This review discusses the possible relationship between protein charge anisotropy, protein binding affinity, polymer structure, and selective phase separation. We hope that a fundamental understanding of primarily electrostatically driven protein–polyelectrolyte (PE) interactions can enable the prediction of selective protein binding, and hence selective coacervation through non-specific electrostatics. Such research will partially challenge the assumption that specific binding has to be realized through specific binding sites with a variety of short-range interactions and some geometric match. More specifically, the recent studies on selective binding of proteins by polyelectrolytes were examined from different assemblies in addition to the electrostatic features of proteins and PEs. At the end, the optimization of phase separation based on binding affinity for selective coacervation and some considerations relevant to using PEs for protein purification were also overviewed.

Contents

1. Introduction

The increasing demand for proteins, and especially recombinant proteins [1–3], has increased the pressure for more efficient and low-cost purification techniques [4–6], in addition to scaling up of conventional methods. Current large-scale purification methods have not improved far from the techniques developed many years before, focusing primarily on chromatographic purification [7,8] and membrane separation techniques [9], etc. These techniques have been widely applied as major separation techniques in the industry for several decades. However, they are still limited by apparent drawbacks, e.g. the low throughput, large consumption of solvent for liquid chromatography, or low binding capacity and poor membrane quality for membrane

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separation. New economic, highly selective and large-scale separation techniques hence become more necessary as alternatives to the current methods.

Phase separation has been developed as a promising way for large-scale purification of biomolecules due to its low cost and extremely high throughput [11,12]. In particular, phase separation based on electrostatically driven polyelectrolyte complexation has been recently reported as an efficient replacement for some chromatographic steps (Fig. 1) [10,13–16]. There has been a long recognition that phase separation based on non-specific, electrostatic interactions lacks the high selectivity comparable to affinity chromatography in which epitopes (protein recognition sites) governed by short-range, specific interactions are introduced to achieve highly selective separation [17,18]. However, recent progress has demonstrated that non-specific electrostatic interactions can be properly applied to achieve high protein selectivity. Drawing the analogy with ion exchange chromatography (IEC), small differences in charge can be discriminated by using polyelectrolytes through judicious choice of the proper polymer characteristics and external conditions, as demonstrated in Fig. 2, where charge anisotropy enables discrimination of a variety of polyelectrolyte (PE) assemblies [19–21]. If the segment properties of the polyelectrolyte are complementary to the specific charge feature of a protein, the selectivity can be achieved and the quality of the selectivity depends on the extent of the complementarity [22]. This is different compared with ion exchange methods where eluent conditions were varied, rather than considering the polymer and protein charge properties.

Complex coacervation is a liquid–liquid phase separation which occurs between oppositely charged macromolecules [23], including both polyelectrolytes and charged colloidal particles in solution. Soluble complexes at a critical ionic strength and protein surface charge density are observed before phase separation [24,25]. Phase separation by polyelectrolyte coacervation has been shown to be a relatively "soft method" for protein purification, as the protein–polyelectrolyte complex is fully reversible. Furthermore, proteins are stable during coacervation [26,27], as indicated by only small variations in the protein secondary or tertiary structure, as measured by circular dichroism [28], or more strictly by the absence of any reduction and sometimes even an enhancement in enzyme activity [29].

Selectivity in protein–polyelectrolyte coacervation is usually obtained based on the differences in charge state, as defined by the protein isoelectric point (pI), e.g. a more acidic protein complexes to a polycation more readily than a basic protein at a fixed pH [16]. However, proteins with close pIs can show significant differences in binding affinities as a result of charge anisotropy [30]. The molecular details of this charge anisotropy mean that proteins that appear similar, on average, are able to form complexes that attain charge neutrality with different partner molecules, and hence coacervate at different pH conditions. Consequently, it would seem difficult to predict the feasibility of selective coacervation and to optimize the conditions for attaining it with only a simple consideration of pI. Thus, while charge anisotropy has been shown to affect protein self-aggregation [31,32], protein–protein interactions [33,34], and protein–polyelectrolyte binding, a systematic investigation of whether subtle differences in surface charges can lead to selective binding has not been reported.

If polyelectrolyte binding is sensitive to small differences in protein charge domains ("charge patches"), highly selective complexation can occur at appropriate pH conditions as observed in ion exchange chromatography (IEC) [35]. Since coacervation arises from soluble protein–polyelectrolyte complexes, selective complexation might be correlated with selective coacervation, meaning that macroscopic phase separation arises from microscopic short-range electrostatic interactions. Hence, a detailed investigation of protein charge distribution could enable the development of a predictive tool that circumvents the trial-and-error approach of many large-scale protein purification situations. In this review, the correlation among protein charge distribution, polyelectrolyte properties, and external conditions will be systematically reviewed to present a better understanding of selective protein complexation and coacervation at both the molecular and macroscopic levels.

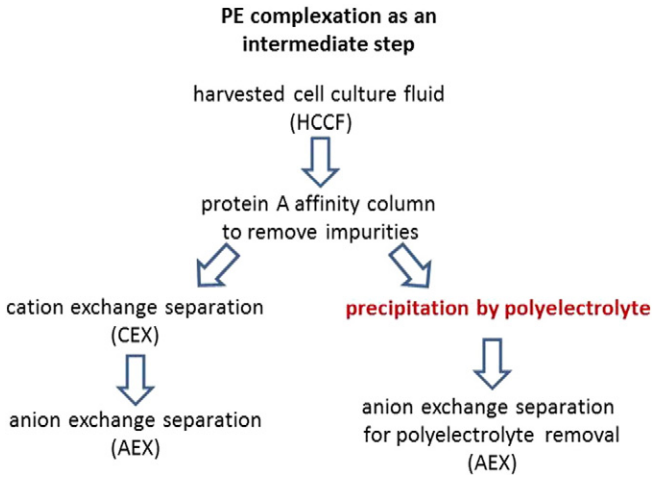

Fig. 1. Conventional monocolonal antibody purification processes for harvested cell culture fluid (HCCF). Polyelectrolyte (PE) phase separation can be used to replace some chromatographic steps, either as an intermediate step or an initial step for impurity removal including host cell proteins (HCP), leached protein A, DNA, antibody aggregates and fragments, and insulin. In both cases, anion exchange (AEX) chromatography is used as a polishing step [10]. Fig. 1 was adapted from ref. [10].

2. Selective protein complexation

2.1. Electrostatically driven selectivity

Several review articles have summarized studies on PE–protein interactions [36–39]. Protein purification by PE complexation is one the most important industrial applications for such electrostatically driven PE–protein interactions. Therefore, this review emphasizes the most typical work on selective protein complexation and coacervation by polyelectrolytes, primarily over the past ten years. We show how the interactions dominated by electrostatics between a protein (i.e. we specifically refer to a "charge patch") and a polyelectrolyte [40,41], lead to selectivity through key variables, e.g. solution conditions, protein charge patterns, polyelectrolyte characters, and even the immobilization of polyelectrolytes as various assemblies.

When a PE interacts with two very similar proteins, parameters defining the difference in protein–PE binding affinities become key parameters to enable the realization of industrial protein purification. Traditional recognition theory believes that the selectivity can only be achieved by specific interactions such as the cases of enzyme–substrate or antigen–antibody interactions [42,43]. Among these specific interactions, the protein "epitope" is the critical site for cooperative geometric fitting and specific short-range interactions including hydrogen bonding, polar–polar interactions, and hydrophobic interactions. To create such a specific interaction, a molecular tag is usually incorporated to substrates or proteins [18]. On the other hand, long-range, non-specific electrostatic interactions are commonly believed to be non-selective, but have also been reported to show prominent results of protein selectivity.

The most common example of electrostatically driven protein separation is ion exchange chromatography in which the charged stationary phase provides sole electrostatically driven selectivity. For example, protein variants BLG-A and BLG-B can be successfully separated on an anion exchange column [44,45]. Thus, it is possible to achieve selective protein separation through the use of non-specific electrostatic interactions, where selectivity can be empirically demonstrated as high binding affinity difference of proteins to PE as discussed later.