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Responses of proteins to different ionic environment are linearly interrelated

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ABSTRACT

Protein partitioning in aqueous two-phase systems (ATPS) is widely used as a convenient, inexpensive, and readily scaled-up separation technique. Protein partition behavior in ATPS is known to be readily manipulated by ionic composition. However, the available data on the effects of salts and buffer concentrations on protein partitioning are very limited. To fill this gap, partitioning of 15 proteins was examined in dextran–poly(ethylene glycol) ATPSs with different salt additives (Na_2SO_4, $NaClO_4$, NaSCN, CsCl) in 0.11 M sodium phosphate buffer, pH 7.4. This analysis reveals that there is a linear relationship between the logarithms of the protein partition coefficients determined in the presence of different salts. This relationship suggests that the protein response to ionic environment is determined by the protein structure and type and concentrations of the ions present. Analysis of the differences between protein structures (described in terms of proteins responses to different salts) and that of cytochrome *c* chosen as a reference showed that the peculiarities of the protein surface structure and B-factor used as a measure of the protein flexibility are the determining parameters. Our results provide better insight into the use of different salts in manipulating protein partitioning in aqueous two-phase systems. These data also demonstrate that the protein responses to different ionic environments are interrelated and are determined by the structural peculiarities of protein surface. It is suggested that changes in ionic microenvironment of proteins may regulate protein transport and behavior in biological systems.

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1. Introduction

Protein partitioning in aqueous two-phase systems (ATPSs) is well known as an inexpensive, convenient, and readily scalable protein separation technique [1–3]. It can also serve as a useful analytical tool for protein structural characterization and analysis by offering unique information about changes in protein structure and interactions between proteins and aqueous environment [4–8]. ATPSs are formed in mixtures of two or more water-soluble polymers, such as polyethylene glycol (PEG) and dextran (Dex), or a single polymer and a particular salt in water above certain concentration or temperature thresholds. These systems consist of two coexisting aqueous phases, which are separated by a clear interfacial boundary. Each of the phases is preferentially enriched in one of the two polymers comprising the mixture, while the aqueous media is suitable for biological molecules in both phases [1–4].

These systems are unique, since despite each of the two phases typically containing well over 80% water on a molal basis, they are immiscible and differ in their solvent properties [4,8–14]. In ATPSs, each phase provides a distinct solvent environment for proteins and other solutes. Furthermore, the differences in solute–solvent

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interactions in the two phases tend to result in an unequal solute distribution. This unequal distribution is quantified by a partition coefficient *K*, defined as the ratio of the protein concentrations in the two phases, and it can be exploited for sensitive detection of changes in the solute structure. Therefore, this solvent interaction analysis (SIA), which is based on quantifying the interactions of a protein with two aqueous media of different solvent properties, can be used as an analytical tool in order to obtain useful structural information (see below). It can even provide information regarding changes in the 3D structure of a protein and the differences between 3D structures of closely related proteins, which can be very difficult to gain with conventional biophysical techniques, such as various types of spectroscopy (e.g., circular dichroism, fluorescence, infrared or Raman spectroscopy), HPLC, etc. [15]. In fact, the differences between 3D structures of closely related proteins may be quantitatively characterized by analyzing the partitioning behavior of these proteins in four or more ATPSs using the same polymer but varied ionic compositions [5]. Partition coefficients in several ATPS are used as a signature of the protein structure and the difference between the signatures of proteins is calculated as an Eucledian distance between the normalized partition coefficients for the proteins under comparison in multidimensional space [5]. It was also reported [16] that it is possible to detect specific protein–ion interactions using the so-called Collander linear solvent regression relationship observed between partition coefficients of proteins in ATPS with a given salt additive and those in ATPS without the same salt additive. It is possible that different changes in the protein structure under certain conditions may result in the identical changes in the values of *K*. This issue was addressed in [5], where it was proposed that the *K*-values for the same protein in different multiple ATPS are determined. These *K*-values can then be constructed into a vector, which would serve as a numerical signature of the 3D structure of the protein in question. Such vectors for different proteins can be easily compared using various mathematical tools.

The solvent properties of the aqueous media in the coexisting phases of ATPS formed by two nonionic polymers depend primarily on the polymer and ionic compositions of the phases [9,11,12]. Two practical requirements must be met by ATPS used for protein partitioning with the purpose of description of the protein's 3D structure [17]. First, there must be a lack of protein aggregation and/or precipitation at the liquid–liquid interface. Second, the protein *K*-value should typically be in the 0.1–10 range for robust analytical evaluation in order to ensure that the protein concentrations in the both phases do not differ more than an order of magnitude [5].

We previously reported on the effects of different salt additives (NaCl, CsCl, Na_2SO_4, $NaClO_4$, and NaSCN) on the partition behavior of 15 proteins in the dextran-70–polyethylene glycol (PEG)-8000 aqueous two-phase systems (ATPSs) in the presence of 0.01 M sodium phosphate buffer, pH 7.4 [18]. The results of these analyses showed that the presence and concentration of salt additives affected the protein partitioning in the protein- and salt-specific manner [18]. The examination of different descriptors of the protein structures revealed that the partition behavior of proteins is determined by the peculiarities of their surfaces (e.g., the number of water-filled cavities and the averaged hydrophobicity of the surface residues) and by the intrinsic flexibility of the protein structure measured in terms of the B-factor (or temperature factor) [18].

We reported recently on the new finding that different aspects of polar organic compounds behavior in aqueous solutions of different ionic compositions are linearly interrelated in three- or more dimensional space [19]. This behavior includes compound solubility represented by Setschenow's salting-in and salting-out constants [19], optical rotation of enantiomeric amino acids and sugars in the presence of different salts [20,21], partition coefficients of polar compounds in PEG-sodium sulfate ATPS with different salt additives [22,23], and partition coefficients of organic compounds in octanol–water biphasic systems of different ionic compositions (manuscript in preparation). It was suggested [19] that different polar organic compounds respond to different ionic environment by changing their solute–water interactions in the structure specific manner. The purpose of this study was to explore if proteins would demonstrate behavior similar to that of polar organic compounds; i.e., if the changes in the protein partition coefficients in the presence of different salt additives would be linearly interrelated. It was suggested by Ninham et al. (see in [24]) that while electrostatic protein–ion interactions dominate at the low salt concentrations of 0.1–0.2 M, at the higher salt concentrations the dispersion forces dominate the solute–ion interactions. In order to explore if the ionic responsiveness of proteins would confirm this model the previous study [18] is extended here by analysis of the proteins partitioning in the dextran-70–polyethylene glycol (PEG)-8000 ATPSs containing 0.11 M sodium phosphate buffer, pH 7.4 with a number of different salts additives. Phosphate buffer of 0.11 M concentration was used here since this buffer provides isotonic physiological conditions similar to those provided by 0.15 M NaCl in 0.01 M phosphate buffer. The role of the buffer concentration in the effects of salts on the protein partition behavior in ATPS is examined as well.

Table 1
Proteins used in this study.

Protein[a]	Abbreviation	Molecular weight (kDa)	p*I*
Albumin fatty acid and globulin free	HSA	66.4	4.7
α-Chymotrypsin	CHY	25.0	8.75
α-Chymotrypsinogen A	CHTG	25.7	8.97
Concanavalin A	ConA	104.0	4.5–5.5
Cytochrome c	Cyt c	12.4	9.1
Hemoglobin bovine	BHb	66.0	6.8
Hemoglobin human	HHb	64.5	6.8
β-Lactoglobulin A	bLGA	18.3	5.3
β-Lactoglobulin B	bLGB	18.3	5.1
Lysozyme	HEL	14.3	11.0
Papain	Pap	23.4	8.75–9.55
Ribonuclease A	RNase A	13.7	9.63
Ribonuclease B	RNase B	17.0	8.88
Subtilisin A	SubA	27.0	9.4
Trypsinogen	TRY	24.0	8.7; 9.3

[a] All proteins from Sigma–Aldrich, details see Section 2.

2. Materials and methods

2.1. Materials

Polyethylene glycols PEG-8000 (Lot 048K00241) with an average molecular weight (MW) of 8000 Da and Dextran-70 (Lot 106H0841) with an average MW of 69,000 Da as evaluated by light scattering were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Albumin from human serum (fatty acid and globulin free (~99%)), α-chymotrypsin from bovine pancreas, α-chymotrypsinogen A from bovine pancreas, concanavalin A from *Canavalia ensiformis* (jack beans), cytochrome c from equine heart (>95%), hemoglobin human, hemoglobin bovine, β-lactoglobulin A from bovine milk (>90%), β-lactoglobulin B from bovine milk (>90%), lysozyme from chicken egg white, papain from papaya latex, ribonuclease A from bovine pancreas, ribonuclease B from bovine pancreas, subtilisin A from *Bacillus licheniformis*, and trypsinogen from bovine pancreas were purchased from Sigma–Aldrich. All proteins and the abbreviations used throughout the text are listed in Table 1.

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