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Analysis of partitioning of organic compounds and proteins in aqueous polyethylene glycol-sodium sulfate aqueous two-phase systems in terms of solute–solvent interactions

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ABSTRACT

Partition behavior of nine small organic compounds and six proteins was examined in poly(ethylene glycol)-8000-sodium sulfate aqueous two-phase systems containing 0.5 M osmolyte (sorbitol, sucrose, trehalose, TMAO) and poly(ethylene glycol)-10000-sodium sulfate system, all in 0.01 M sodium phosphate buffer, pH 6.8. The differences between the solvent properties of the coexisting phases (solvent dipolarity/polarizability, hydrogen bond donor acidity, and hydrogen bond acceptor basicity) were characterized with solvatochromic dyes using the solvatochromic comparison method. Differences between the electrostatic properties of the phases were determined by analysis of partitioning of sodium salts of dinitrophenylated (DNP-) amino acids with aliphatic alkyl side-chain. It was found out that the partition coefficient of all compounds examined (including proteins) may be described in terms of solute–solvent interactions. The results obtained in the study show that solute–solvent interactions of nonionic organic compounds and proteins in polyethylene glycol-sodium sulfate aqueous two-phase system differ from those in polyethylene glycol-dextran system.

1. Introduction

Aqueous two-phase systems (ATPS) are formed in mixtures of two (or more) water-soluble polymers, such as dextran and Ficoll, or a single polymer and specific salt, e.g., polyethylene glycol (PEG) and sodium sulfate, in water above certain critical concentrations. Two immiscible aqueous phases are formed in the mixtures. Solutes from small organic compounds to proteins and nucleic acids distribute unevenly between the phases of an ATPS and may be separated. ATPS formed by polyethylene glycol (PEG) and inorganic salt, such as sodium sulfate, phosphate or citrate, are commonly used for separation of proteins and nucleic acids due to their low cost, good operational characteristics (low viscosity of the phases, high settling speed) and easy scale-up [1–18]. Extraction in ATPS has been demonstrated as an efficient method for large scale recovery and purification of proteins [1–13] and nucleic acids [14,15] as well as various other materials. Design of optimal extraction conditions for any target product remains currently an empirical process, and high throughput methods for screening different separation conditions have been developed [16–18]. For rational design of the optimal separation conditions it is important to understand the mechanisms of solute distribution in polymer-salt ATPS at the molecular level.

We reported [19–21] previously that different salt additives (NaCl, NaH_2PO_4, $NaClO_4$, NaSCN) at the concentrations from 0.027 M up to ca. 1.9 M affect partition behavior of small organic compounds in PEG-sodium sulfate ATPS according to the salt effects on the water structure. Despite broad biotechnological and pharmacological applications of this approach, the molecular mechanism of solute partitioning in PEG-salt ATPS remains unclear, however.

It has been established [22–29] that solute partitioning in two-polymer ATPS is governed by the solute–solvent interactions in

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the coexisting phases. Partition coefficient of a solute in an ATPS is defined as the ratio of the solute concentration in the top phase to the solute concentration in the bottom phase and therefore may be described as [23–28]:

$$\log K = S_s\,\Delta\pi^* + B_s\,\Delta\alpha + A_s\,\Delta\beta + C_s c \quad (1)$$

where K is the solute partition coefficient; $\Delta\pi^*$, $\Delta\alpha$, $\Delta\beta$ and c are the differences between the solvent properties of the top and bottom phases (solvent dipolarity/polarizability, hydrogen-bond donor acidity, hydrogen-bond acceptor basicity, and electrostatic interactions, respectively; S_s, B_s, A_s, and C_s are constants (solute-specific coefficients) that describe the complementary interactions of the solute with the solvent media in the coexisting phases; the subscript 's' designates the solute.

The differences between the solvent dipolarity/polarizability, $\Delta\pi^*$, hydrogen-bond donor acidity, $\Delta\alpha$, hydrogen-bond acceptor basicity, $\Delta\beta$, may be quantified using a set of solvatochromic dyes [23–29] (see below). The difference between the electrostatic properties of the phases may be determined from the analysis of the partition coefficients of a homologous series of sodium salts of dinitrophenylated (DNP-) amino acids with aliphatic alkyl side-chains [22–28] (see below). It has been shown that for a given compound (including proteins) the solute-specific coefficients may be determined by multiple linear regression analysis of the partition coefficients of the compound in multiple two polymer ATPSs formed by different polymers but with the same ionic composition [23–28]. It was also shown [23,26] that the partition coefficients of compounds with pre-determined solute specific coefficients in new ATPS with established solvent properties of the phases could be predicted with the 90–95% accuracy.

Huddleston et al. [30,31] examined the solvent properties of the coexisting phases in PEG-2000-K_3PO_4 and PEG-2000-$(NH_4)_2SO_4$ ATPSs and found negligible differences between the solvent dipolarity/polarizability, $\Delta\pi^*$, and hydrogen bond acceptor basicity, $\Delta\beta$, of the phases. The authors [30,31] also reported on the challenges regarding accurate determination of the solvent hydrogen bond donor acidity, α, in such ATPSs due to effects of high salt concentrations on the solvatochromic probe used. We assumed that the challenges encountered by Huddleston et al. [30,31] might be related to the low molecular weight of PEG used in the studies requiring using high total salt concentration (9–10 wt.% K_3PO_4) for ATPS formation. Hence in this work we used PEG-8000 and PEG-10000 enabling us to decrease the salt concentration necessary for phase separation down to 6.3 wt.% Na_2SO_4.

It has been shown previously [32–35] that the osmolytes additives change the solvent properties of the phases under fixed salt composition of the system, while not being engaged in direct interactions with compounds (including proteins) being partitioned. Therefore these additives enable one to vary solvent properties of the polymer-salt ATPS without changing overall polymer and salt composition of the particular system.

The purpose of the present work was to explore if partitioning of different solutes in PEG-Na_2SO_4 ATPS is governed by the factors similar to those established in the ATPSs formed by two polymers. To this end, partitioning of several different organic compounds and proteins was examined in several PEG-Na_2SO_4 ATPSs in the presence of different nonionic additives.

2. Experimental

2.1. Materials

Polyethylene glycol-8000 (Lot 091M01372 V) with an average molecular weight (M_w) of 8000 and polyethylene glycol-10000 (Lot 043K2522) with an average molecular weight (M_w) of 10000 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The solvatochromic probes 4-nitrophenol (reagent grade, >98%) was purchased from Aldrich (Milwaukee, WI, USA) and 4-nitroanisole (>97%, GC) was received from Acros Organics. Reichardt's carboxylated betaine dye, 2,6-diphenyl-4-[2,6-diphenyl-4-(4-carboxyphenyl)-1-pyridino]phenolate, sodium salt was kindly provided by Professor C. Reichardt (Philipps University, Marburg, Germany).

Sorbitol, trimethylamine N-oxide (TMAO), and trehalose were purchased from Sigma-Aldrich, and sucrose was received from USB (Cleveland, OH, USA). 4-Aminophenol, benzyl alcohol, caffeine; coumarin, methylanthranilate, 4-nitrophenyl-α-D-glucopyranoside, phenol, 2-phenylethanol, vanillin, and o-phthaldialdehyde (OPA) reagent (complete) were purchased from Sigma–Aldrich. All compounds were of 98–99% purity and used as received without further purification. All salts and other chemicals used were of analytical-reagent grade.

2.1.1. Dinitrophenylated amino acids

Dinitrophenylated (DNP) amino acids—DNP-glycine, DNP-alanine, DNP-norvaline, DNP-norleucine, and DNP-α-amino-*n*-octanoic acid, were purchased from Sigma–Aldrich. The sodium salts of the DNP-amino acids were prepared by titration.

2.1.2. Proteins

α-Chymotrypsin from bovine pancreas, α-chymotrypsinogen A from bovine pancreas, concanavalin A from Canavalia ensiformis (jack beans), lysozyme from chicken egg white, and papain from papaya latex were purchased from Sigma–Aldrich. Porcine pancreatic lipase was purchased from USB Corp. (Solon, OH, USA). All protein samples were characterized by SDS-PAGE electrophoresis in a microfluidic chip using Experion automated electrophoresis station (Bio-Rad, USA) under non-reduced conditions. All proteins were observed as single bands in the electrophoregrams.

2.2. Methods

2.2.1. Aqueous two-phase systems

Stock solutions of PEG 8000 (50 wt.%), PEG-10000 (50 wt.%) and Na_2SO_4 (20.3 wt.%) were prepared in water. Sodium phosphate buffer (NaPB; 0.5 M, pH 6.8) was prepared by mixing 3.45 g of $NaH_2PO_4{\cdot}H_2O$ and 3.55 g Na_2HPO_4 in 100 mL aqueous solution. Stock solutions of osmolytes: sorbitol (2 M), sucrose (1.8 M), trehalose (1.4 M), and TMAO (1.8 M) were prepared in water. A mixture of PEG-8000 or PEG-10000 and buffer was prepared by dispensing appropriate amounts of the aqueous stock PEG-8000, Na_2SO_4 and NaPB solutions into a 1.2 mL microtube using a Hamilton (Reno, NV, USA) ML-4000 four-probe liquid-handling workstation. Appropriate amounts of water and/or stock osmolytes solutions were added to give the required ionic, polymer, and osmolyte composition of the final system with total weight of 0.5 g (after addition of the solute sample, see below). All aqueous PEG-8000-Na_2SO_4 two-phase systems had a fixed composition of 11.10 wt.% PEG-8000, 6.33 wt.% Na_2SO_4 and 0.01 M NaPB, pH 6.8, with different 0.5 M osmolyte additive. The aqueous PEG-10000-Na_2SO_4 two-phase system had the same composition of 11.10 wt.% PEG-10000, 6.33 wt.% Na_2SO_4 and 0.01 M NaPB, pH 6.8.

2.2.2. Partitioning experiments

The aqueous two-phase partitioning experiments were performed using an Automated Signature Workstation, ASW (Analiza, Cleveland, OH, USA). The ASW system is based on the ML-4000 liquid-handling workstation (Hamilton Company) integrated with a FL600 fluorescence microplate reader (Bio-Tek Instruments, Winooski, VT, USA) and a UV–VIS microplate spectrophotometer (SpectraMax Plus384, Molecular Devices, Sunnyvale, CA, USA).