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Origin of salt additive effect on solute partitioning in aqueous polyethylene glycol-8000–sodium sulfate two-phase system

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ABSTRACT

Partitioning of a homologous series of dinitrophenylted (DNP-) amino acids with aliphatic side chains was examined in aqueous polyethylene glycol (PEG)-8000–sodium sulfate two-phase systems (ATPS) with the additives NaSCN, $NaClO_4$, and NaH_2PO_4 at concentrations varied from 0.025 M up to 0.54 M. The differences between the relative hydrophobicities and electrostatic properties of the two phases in all ATPS were estimated. Partitioning of adenine, adenosine mono-, di- and tri-phosphates was also examined in all ATPSs, including those with NaCl additive. Partition coefficients for these compounds and for nonionic organic compounds previously reported [L.A. Ferreira, P. Parpot, J.A. Teixeira, L.M. Mikheeva, B.Y. Zaslavsky, J. Chromatogr. A 1220 (2012) 14.] were analyzed in terms of linear solvent regression relationship. The results obtained suggest that the effects of the salts additives are related to their influence on the water structure.

1. Introduction

Aqueous two-phase systems (ATPSs) are formed in mixtures of two (or more) water-soluble polymers or a single polymer and specific salt in an aqueous solution above certain critical concentrations or temperature. Two immiscible aqueous phases are formed in such mixtures. The uniqueness of these systems is that each of the phases contains over 80% water on a molal basis but the phases are immiscible and differ in their solvent properties [1–4]. Therefore they can be used for the discriminating distribution of added solutes. ATPSs have been used for separation of biological macromolecules and particles for over 50 years [5–7] and recently found use in a variety of analytical applications as well [8,9].

The most studied polymer–salt ATPSs are formed by polyethylene glycol (PEG) and inorganic salts, such as sodium sulfate, phosphate, carbonate or citrate. Salt effects on polymers and biopolymers, such as proteins, in water generally follow the well known Hofmeister series [10] with qualitative order of the anions being citrate^{3-} > CO_3^{2-} > SO_4^{2-} > $H_2PO_4^-$ > F^- > OH^- > Cl^- > NO_3^- > Br^- > I^- > ClO_4^- > SCN^-. Anions to the left of chloride are often called kosmotropes and they typically stabilize proteins and salt them out of solution. Anions to the right of chloride are called chaotropes and they commonly destabilize proteins and salt them into solution. The positions of some anions in the series, such as ClO_4^- and SCN^- or SO_4^{2-} and $H_2PO_4^-$ may interchange depending on the particular effect in question and the nature of cation. The Hofmeister effect is commonly explained by the ability of various ions to "make" or "break" water structure in aqueous solution [11,12]. This explanation was put in doubt by many recent experimental and theoretical [13–16] studies showing that the properties of bulk water are not noticeably perturbed by ions in solution. It should be mentioned, however, that some recent experimental data [11,12,17] contradict this conclusion. The mechanism of the ions effects on proteins in aqueous solutions remains debatable.

The anions of sodium salts capable of forming ATPS with PEG (citrate, carbonate, sulfate, phosphate, fluoride, and hydroxide) all belong to the kosmotropes. Attempts to investigate mechanism of phase separation in aqueous polymer–salt mixtures based on thermodynamic analysis of experimental data [18,19] can hardly be viewed as successful and any attempt at considering phase separation in aqueous solution based on deviation from ideality is doomed. This issue, however, is beyond the scope of the present work.

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One of the most interesting aspects of solutes behavior in PEG–salt ATPS is that relatively small amounts of salt additives may significantly affect the solute partitioning [20–22]. The most widely used salt additive in this ATPS is NaCl [20–22]. The data obtained in the studies of protein partition behavior, however, typically leave too much room for interpretation being assigned to the protein–ion specific interactions, protein conformational changes and other often hard to prove (and disprove) possibilities. The data obtained for structurally simple organic compounds generally cannot be interpreted as ambiguously and hence may provide better insight into mechanism of the effect under study.

Recently, effects of different salt additives (NaCl, NaSCN, $NaClO_4$, NaH_2PO_4) over a wide concentration range up to ca. 2 M on partition behavior of small organic compounds in PEG-8000–Na_2SO_4 were reported [23]. Later we examined the effect of NaCl in the concentration range of 0–1.9 M on the phase diagram of this ATPS and characterized solvent properties of the phases [24].

We extended this study here and examined the differences between the relative hydrophobicities and electrostatic properties of the phases in PEG-8000–Na_2SO_4 ATPS with additives of NaSCN, $NaClO_4$, and NaH_2PO_4. As reported previously [23], adenosine and guanosine displayed complicated partition behavior in PEG-8000–Na_2SO_4 ATPS with increasing concentration of NaSCN, $NaClO_4$, and NaH_2PO_4 additives. Their partition coefficients increased with salt additive concentration increasing up to ~0.2 M and decreased with further increasing salt concentration. Therefore we also examined here partitioning of adenine and adenosine mono-, di-, and tri-phosphates in all the ATPSs, including those with NaCl additive. We attempted to answer the question of how relatively small amounts of the above salt additives may affect solute partitioning in ATPS formed by exceeding amount of sodium sulfate and PEG.

2. Experimental

2.1. Materials

Polyethylene glycol-8000 (Lot 048K00241) with an average molecular weight (M_w) of 8000 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Adenine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) were purchased from Sigma-Aldrich. Dinitrophenylated (DNP) amino acids—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.

All salts and other chemicals used were of analytical-reagent grade.

2.2. Methods

2.2.1. Aqueous two-phase systems

Stock solutions of PEG 8000 (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 6.70 g $Na_2HPO_4{\cdot}7H_2O$ in 100 ml aqueous solution. A mixture of PEG and salts 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 salt additives solutions were added to give the required ionic and polymer composition of the final system with total weight of 0.5 g (after addition of the solute sample, see below). All aqueous 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 salt additive concentrations as indicated below. Stock solutions of each salt additive (NaCl, $NaClO_4$, NaSCN or NaH_2PO_4) of 0.5 or 5.0 M concentration were prepared and appropriated amounts were added to the two-phase systems to provide the required concentrations from 0.027 M up to ca. 0.5 M.

2.2.2. Partitioning experiments

The aqueous two-phase partitioning experiments were performed using an automated instrument, 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 UV–VIS microplate spectrophotometer (SpectraMax Plus384, Molecular Devices, Sunnyvale, CA, USA). Solutions of all compounds were prepared in water at concentrations of 2–100 mM, depending on the compound solubility. Varied amounts (0, 20, 40, 60, 80, and 100 μl) of a given compound solution and the corresponding amounts (100, 80, 60, 40, 20, and 0 μl) of water were added to a set of the same polymer/salts mixtures. The systems were vortexed in a multi-pulse vortexer and centrifuged for 60 min at 3000 × *g* at 23 °C temperature in a refrigerated centrifuge (Jouan, BR4i) to accelerate phase settling. The upper phase in each system was partially removed, the interface discarded, and aliquots of 15–75 μl from the upper and lower phases were withdrawn in duplicate for analysis. The aliquots were transferred into microplate wells and diluted up to 300 μl. In the cases of considerable difference between the concentrations of a given compound in one phase relative to the other phase, different dilution factors were used for the upper and lower phases. Water was used as a diluent for all compounds. The microplate was sealed, and following moderate shaking for 30 min in an incubator (Vortemp 56EVC, Labnet International, Edison, NJ, USA) at room temperature and short centrifugation (3 min at 1500 rpm), optical absorbance was measured at the wavelength of maximum absorption with the UV–VIS plate reader. The maximum absorption wavelength for each compound was determined in separate experiments by analysis of the absorption spectrum over the 250–500 nm range. In all measurements the correspondingly diluted pure phases were used as blank solutions.

The partition coefficient, *K*, defined as the ratio of the sample concentration in the upper phase to the sample concentration in the lower phase was determined as the slope of the compound concentration in the upper phase plotted as a function of the concentration in the lower phase averaged over the results obtained from two to four partition experiments carried out at the specified polymer and salt composition of the system, taking into consideration the corresponding dilution factors used in the experiment. Deviation from the average *K*-value was consistently below 3% and in most cases lower than 2%.

3. Results and discussion

In order to characterize the differences between the relative hydrophobicities and electrostatic properties of the phases in the ATPSs with varied concentrations of salt additives, the partition coefficients of the homologous series of sodium salts of DNP-amino acids with aliphatic side-chains (alanine, norvaline, norleucine, and α-amino-*n*-caprylic acid) in each ATPS were analyzed as follows. Typical experimental data obtained for sodium salts of the DNP-amino acids are plotted in Fig. 1, and the linear curves observed may be described as:

$$\ln \; K^{(i)}_{\mathrm{DNP-AA}} = C^{(i)} + E^{(i)} N_C \qquad (1)$$

where $K_{\mathrm{DNP\text{-}AA}}$ is the partition coefficient of a DNP-amino acid with aliphatic side-chain; superscript (i) denotes the particular i-th ATPS used for the partition experiments; N_C is equivalent number of CH_2 groups in the aliphatic side-chain of a given DNP-amino acid; E is