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Effect of excluded-volume and hydrophobic interactions on the partition of proteins in aqueous micellar two-phase systems composed of polymer and nonionic surfactant

Yang Liu*, Yanglong Zhang, Xueyan Wu, Xiaolin Yan

Department of Biology & Guangdong Provincial Key Laboratory of Marine Biotechnology, College of Science, Shantou University, Shantou, Guangdong, 515063, PR China

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ABSTRACT

The partitioning behaviors of several model proteins were investigated in aqueous micellar two-phase systems (AMTPS) composed of polyethylene glycol (PEG) with different molecular weight (4,000, 8000 and 20,000) and nonionic surfactant (TX-100), respectively. The influences of proteins molecular size or hydrophobicity, tie line length (TLL), compositions concentrations and PEG molecular weight on the partition coefficients of proteins were extensively studied. In order to evaluate the contributions of excluded-volume interaction and hydrophobic interaction to proteins partitioning in PEG/TX-100 AMTPS in detail, firstly, the various proteins partition coefficients in PEG/Na_2SO_4 aqueous two-phase systems (ATPS) and TX-100/Na_2SO_4 AMTPS were investigated for the cross-sectional radius fitting in PEG-rich phase and TX-100-rich phase, respectively. The cross-sectional radius fitting results embodied that the order of cross-sectional radius in various phases are PEG4,000-rich phase, PEG8,000-rich phase, TX-100-rich phase and PEG20,000-rich phase. Secondly, the quantitative assessment of excluded-volume and hydrophobic contributions to proteins partitioning in PEG/TX-100 AMTPS were further investigated using the cross-sectional radius in PEG-rich phase and TX-100 phase, respectively. It was indicated that the excluded-volume interaction was stronger than hydrophobic interaction for proteins partitioning in PEG/TX-100 AMTPS. Finally, the effect of hydrophobic interaction on proteins partitioning was also analyzed in detail. The intrinsic hydrophobicity (P_0) and the hydrophobic resolution (R) of PEG/TX-100 AMTPS with various TLL were assessed according to the model proteins partitioning and proteins' surface hydrophobicity. For proteins partitioning in PEG/TX-100 AMTPS, the molecular weight of PEG played an important role in excluded-volume interaction, not in hydrophobic interaction.

1. Introduction

Recently, developments in the polymer/surfactant aqueous micellar two-phase system (AMTPS) have opened up plentiful possibilities for the hydrophobic biomolecules enrichment and purification [1–4] in aqueous solution. The liquid-liquid phase equilibrium in polymer/surfactant AMTPS has the similar characteristic both in traditional polymer/polymer aqueous two-phase system (ATPS) and in surfactant-based AMTPS induced by temperature [5–8]. Namely, the surfactant-rich phase in polymer/surfactant AMTPS can be considered the second polymer phase as polymer/polymer ATPS, and is also regarded as the micelle-rich phase as surfactant-based AMTPS induced by temperature. In polymer/surfactant AMTPS, the abundant micelles structures in surfactant-rich phase not only provide the hydrophobic surrounding for hydrophobic substances in aqueous solution, but also bring the excluded-volume interactions between hydrophilic substances and micelles. Compared with the other types of ATPSs, the polymer/surfactant AMTPS has become an effective ATPS for integral membrane proteins separation and purification [1,9]. The predictability of the proteins partition behavior is important to the wider implementation of polymer/surfactant AMTPS for membrane proteins purification in proteomics research.

For the traditional ATPS such as polymer/polymer or polymer/salt ATPS, there are three main interactions' contributions to proteins partitioning in ATPS including electrostatic interaction,

* Corresponding author. Tel.: +86 754 86503093; fax: +86 754 86502726.
E-mail address: liuyanglft@stu.edu.cn (Y. Liu).

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hydrophobic interaction and excluded-volume interaction [10–12], which rely on ATPS solution conditions (including compositions concentrations, ionic strength, salt type and pH) and the proteins physicochemical properties including isoelectric points, molecular weight and hydrophobicity [13–15]. Thus, the proteins partition coefficient K (defined as the ratio of the protein concentration in the top phase to that of the bottom phase) in ATPS can be expressed in general [13],

$$K = C_T/C_B \tag{1}$$

$$lnK = lnK_{el} + lnK_{hphob} + lnK_{size} \tag{2}$$

where C_T and C_B are the concentrations of proteins in the top and bottom phase, respectively. K_{el}, K_{hphob} and K_{size} are the contribution of the total partition coefficient K by electrostatic, hydrophobic and excluded-volume interactions, respectively. The three contributions are not equally important for the different ATPS. In polymer/polymer ATPS such as polyethylene glycol and dextran (PEG/DEX) ATPS, the hydrophobic interaction is the main contribution to proteins partitioning in ATPS, which is displayed by the dependence of proteins hydrophobicity on proteins partition coefficient [16]. To adjust proteins hydrophobicity by controlling the NaCl concentration in ATPS [17,18], a hydrophobic interaction model was proposed, which can correlate the proteins hydrophobicity with their partition coefficient in PEG/DEX ATPS [19–21]:

$$lnK_{hphob} = RlnP - RlnP_0 \tag{3}$$

where P is the protein surface hydrophobicity measured by $(NH_4)_2SO_4$ precipitation in solution, and P_0 is the ATPS intrinsic hydrophobicity. R denotes the hydrophobic resolution and it is the ATPS ability to discriminate the difference of proteins hydrophobicity. In surfactant-based AMTPS induced by temperature, the excluded-volume interaction plays a key role in proteins partitioning due to the cylindrical micellar structure formed by surfactant molecules [22–24]. The excluded-volume interaction model can correlate the hydrophilic proteins size with their partition coefficient in surfactant-based AMTPS as follows [25,26]:

$$lnK_{size} = -\left(\varphi_i^t - \varphi_i^b\right)(1 + R_P/R_0)^2 \tag{4}$$

where φ_i^t and φ_i^b are the volume fraction of surfactant (i composition) in the top phase and the bottom phase of the AMTPS, respectively. R_p denote the protein hydrodynamic radius, and R_0 is the cross-sectional radius of the cylindrical micelles.

In our present work, polyethylene glycol (PEG) with various molecular weight and polyoxyethylene octyl phenyl ether (TX-100) were selected to form polymer/surfactant AMTPS, five model proteins partitioning behaviors with different size and hydrophobicity in polymer/surfactant AMTPS were investigated. For the three contributions for proteins partition in Eq. (2), the contribution of electrostatic interaction between PEG/TX-100 AMTPS and proteins could be ignored at protein isoelectric point. In PEG/TX-100 AMTPS, both the top, polymer-rich phase and the bottom, surfactant-rich phase (namely micelles-rich) have respective aggregation structures and have the balance action for proteins partitioning due to the two excluded-volume interactions in top and bottom phases. Moreover, the hydrophobicity of the PEG/TX-100 AMTPS would be varied by the ATPS compositions type and concentrations. Therefore, the hydrophobic interaction model of polymer/polymer ATPS and the excluded-volume interaction model of surfactant-based AMTPS were combined and applied to correlate the proteins partition coefficients in PEG/TX-100 AMTPS with proteins hydrophobicity, proteins size, polymer molecular weight, polymer concentration and surfactant concentration under different proteins distribution conditions. The results of the work could provide reference data and approach for the researchers whose interest are proteins separation in AMTPS, where this is the example in the field of various model proteins partitioning behavior in AMTPS. Thus, the first aim of the work is to study the correlation between partition coefficient and characteristics of the AMTPS, and model the partition coefficient of the proteins in the systems; the second aim of the work is to develop the application of polymer/surfactant AMTPS in hydrophobic proteins purification and enrichment.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA, CAS 9048-46-8), lysozyme (LYS, EC 3.2.1.17) from chicken egg white, α-lactalbumin (α-LA, CAS 9013-90-5) from bovine milk, ovalbumin (OVA, CAS 9006-50-2) from chicken egg white and catalase (CAT, EC1.11.1.6) from bovine liver have been selected in this study. The five proteins were purchased from Sigma-Aldrich. The proteins physicochemical properties including molecular weights, isoelectric points and the hydrodynamic radii are shown in Table 1.

PEG (polyethylene glycol) with a molecular weight of 4,000Da, 8,000Da and 20,000Da (GR, ≥95% mass purity) were obtained from AMERCO (Shanghai, China). TX-100 (polyoxyethylene octyl phenyl ether, GR, ≥95% mass purity) was also purchased from AMERCO (Shanghai, China). The polymer and surfactants were used without further purification. All other chemicals were of analytical grade from local sources. Aqueous solutions were prepared with deionized and doubly distilled water.

2.2. Proteins partitioning experiments in PEG/TX-100 AMTPS

Partitioning experiments of five proteins were conducted in 2 g ATPS. In order to prepare the ATPS, stock solutions of 40 wt% PEG20,000 and PEG8,000, 50 wt% PEG4,000, 30 wt%TX-100 in respective buffer with different pH value were prepared using a BSM-120.4 analytical balance (Shanghai) with an uncertainty of $\pm 1 \times 10^{-4}$ g. According to the binodal curve and tie-line data from our previous works [4,11,27], the investigated PEG/TX-100 AMTPSs were shown in Table 2 and these systems were formed by PEG and TX-100 stock solutions with protein or without protein, respectively. The AMTPS pH was adjusted by disodium hydrogen phosphate-citric acid buffer (pH 2.2–8.0, 0.01 M) and sodium borate-sodium carbonate buffer (pH 10.8–11.2, 0.01 M) at the isoelectric point of the five distributed proteins in Table 1 within ±0.01, respectively, to eliminate the net charge interferences of proteins in PEG/TX-100 AMTPS. The solid proteins samples were directly added to the AMTPSs with a final protein concentration of 0.5 mg/g, which was generally far lower the mole concentration of the TX-100 micelles in the TX-100-rich phase [1,9]. The mixture were equilibrated by gentle mixing at 298.15 K for 30 min by a QB-128 mixer (Shanghai), and then centrifuged at 6,000 rpm for 30 min by a Universal 320R centrifuge (Germany). The top phase and bottom phase were isolated using a pipette and were diluted as appropriate prior to assay.

2.3. Proteins concentration determination and protein partition coefficient

The proteins concentrations in the top and bottom phases were analyzed by the BCA-assay [28]. The proteins samples were measured at 562 nm using a UVPC2501 UV-VIS spectrophotometer