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Relative hydrophobicity between the phases and partition of cytochrome-c in glycine ionic liquids aqueous two-phase systems

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ABSTRACT

In this work, glycine ionic liquids tetramethylammonium glycine ([N_{1111}][Gly]), tetraethylammonium glycine ([N_{2222}][Gly]), tetra-n-butylammonium glycine ([N_{4444}][Gly]), tetra-n-butylphosphonium glycine ([P_{4444}][Gly]) and tetra-n-pentylammonium glycine ([N_{5555}][Gly]) were synthesized and used to prepare aqueous two-phase systems (ATPSs) in the presence of K_2HPO_4. Binodal curves of such ATPSs and partition coefficients of a series of dinitrophenylated (DNP) amino acids in these ATPSs were determined at 298.15 K to understand the effect of cationic structure of the ionic liquids on the phase-forming ability of glycine ionic liquids, relative hydrophobicity between the phases in the ionic liquids ATPSs, and polarity of the ionic liquids-rich phases. With the attempt to correlate the relative hydrophobicity of the phases in the ATPSs with their extraction capability for proteins, partition coefficients of cytochrome-c in the ATPSs were also determined. It was shown that partition coefficients of cytochrome-c were in the range from 2.83 to 20.7 under the studied pH conditions. Then, hydrophobic interactions between cytochrome-c and the ionic liquid are suggested to be the main driving force for the preferential partition of cytochrome-c in the glycine ionic liquid-rich phases of the ATPSs. Result derived from polarity of the ionic liquids-rich phases supports this mechanism.

1. Introduction

Ionic liquids (ILs) are one of the hot materials today in chemistry, materials and environmental sciences [1]. They are organic salts with melting points below 100 °C. As a new class of non-molecular liquid materials, ILs have many unique and fascinating properties, such as negligible volatility, non-flammability, high chemical and thermal stability, tunable chemical structures and physicochemical properties. This makes ILs attractive as novel extractants for various compounds, such as amino acids, proteins and other biomaterials [2–4]. However, hydrophobic ILs have to be used in order to create IL/water biphasic systems for the liquid–liquid extraction. Compared with hydrophilic ILs, hydrophobic ILs are more expensive and only limited number of them are available.

In 2003, it was found that ATPS could be formed by the addition of inorganic salts such as K_3PO_4 into a concentrated aqueous solution of a hydrophilic IL and utilized to extract a series of short chain alcohols [5]. These novel ATPSs show many advantages, including low viscosity, little emulsion formation and quick phase separation, high extraction efficiency, and gentle biocompatible environment [6,7]. Thus, they are potentially more efficient solution for liquid–liquid extractions. In recent years, significant progress has been made in the research and application of the ILs-based ATPSs [6–8], especially in the field of extraction and purification of biomolecules and/or drugs, such as amino acids [9,10], alkaloids [11], testosterone and epitestosterone [12], penicillin G [13], bovine serum albumin [14,15], lipase [16], and antibiotics. [17] However, it is shown that among a large number of ILs that have been investigated, the ILs consisting of imidazolium (or pyridinium) cations and halide-containing anions are highly toxic [18]. Therefore, widespread application of ILs would inevitably result in the loss of the ILs into water ecosystems, leading to the aquatic environmental pollution. An alternative approach for overcoming these drawbacks is the development of ILs from their components which have well characterized toxicological properties. Therefore, using ILs with better biodegradability and low toxicity in ATPSs is the best way to avoid ILs pollution. It is known that ammonium and phosphonium-based ILs have lower toxicity than imidazolium- and pyridinium-based ILs [19]. In addition, amino acids are biodegradable and non toxic, and using them as anion or cation would add advantage of these ILs [20,21].

In this work, tetramethylammonium glycine ([N_{1111}][Gly]), tetraethylammonium glycine ([N_{2222}][Gly]), tetra-n-butylammonium glycine ([N_{4444}][Gly]), tetra-n-butylphosphonium

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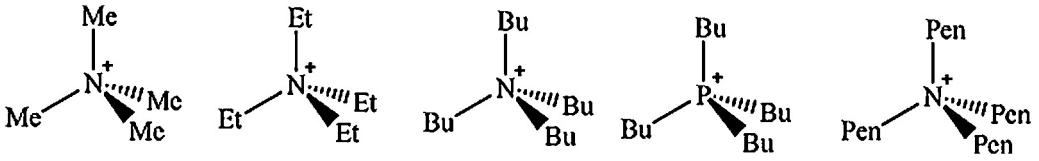

Fig. 1. The chemical structure of cations of the glycine ILs.

glycine ([P_{4444}][Gly]) and tetra-n-pentylammonium glycine ([N_{5555}][Gly]) were synthesized and utilized to prepare ILs ATPSs in the presence of K_2HPO_4. It was found that DNP-amino acids and cytochrome-c can be efficiently partitioned in the IL-rich phases. Therefore, partition coefficients of DNP-amino acids and the protein in these ATPSs and polarity of the IL-rich phases have been determined at 298.15 K. Of these experimental data, partition coefficients of DNP-amino acids were used to calculate the Gibbs energies for the transfer of a CH_2 group ($\Delta G_T(CH_2)$) from the salt-rich to the IL-rich phases which characterize the relative hydrophobicity of the IL-rich phase to the salt-rich phase, while partition coefficients of cytochrome-c were utilized to study the liquid–liquid extraction mechanism of the model protein in the ATPSs. Therefore, the current work allows us to investigate the effect of cationic structure of the ILs on their phase-forming ability, relative hydrophobicity between the equilibrium two phases, partition behavior of cytochrome-c in the ATPSs and polarity of the ILs-rich phases. Such results would be helpful to understand the partition process of biomacromolecules in amino acid ILs-based ATPSs and to improve their extraction efficiency by modulating the relative hydrophobicity between the two phases and polarity of the IL-rich phases.

2. Experimental

2.1. Materials

Tetra-n-pentylammonium bromide [N_{5555}]Br (>99%), tetramethylammonium bromide [N_{1111}][Br] (>99%), tetraethylammonium bromide [N_{2222}][Br] (>99%) and tetra-n-butylammonium bromide [N_{4444}][Br] (>99%) were purchased from Sigma-Aldrich. Tetra-n-butylphosphonium bromide [P_{4444}][Br] (>98%) was obtained from Aladdin Chem. Co. Ltd., and Ambersep 900(OH) ion exchange resin was purchased from Alfa. Glycine (98.5%) and cytochrome-c (95%) were from Beijing Jingke Hongda Biotech. Co. and Aladdin Chem. Co. Ltd., respectively. Dinitrophenylated (DNP) amino acids including N-(2,4-dinitrophenyl)glycine (DNP-Gly), N-(2,4-dinitrophenyl)-D,L-n-valine (DNP-Val), N-(2,4-dinitrophenyl)-D,L-n-leucine (DNP-Leu) and N-(2,4-dinitrophenyl)-D,L-amino-n-caprylic acid (DNP-AOc) were purchased from Sigma–Aldrich. N-(2,4-dinitrophenyl)-L-alanine (DNP-Ala) was obtained from Tokyo Chem. Ind. Co. and 2,6-dichloro-4-(2,4,6-triphenyl-N-pyridino) phenolate (HPLC Grade, 98.5%) was obtained from Sigma–Aldrich. Methanol (HPLC, >99.8%) was obtained from Sinopharm Chem. Reagent Co., Ltd. The above materials were used without further purification unless otherwise stated. The double distilled deionized water was used in the experiments.

2.2. Preparation of the glycine ionic liquids

The glycine ILs [N_{1111}][Gly], [N_{2222}][Gly], [N_{4444}][Gly], [P_{4444}][Gly] and [N_{5555}][Gly] (see Fig. 1 for their structures) were prepared by neutralization [22]. Firstly, [N_{1111}][Br], [N_{2222}][Br], [N_{4444}][Br], [N_{5555}][Br] and [P_{4444}][Br] were transformed into [N_{1111}][OH], [N_{2222}][OH], [N_{4444}][OH], [P_{4444}][OH] and [N_{5555}][OH] by Ambersep 900(OH) ion exchange resin. Then, their aqueous solutions were respectively added dropwise to a slight excess of aqueous glycine solution at room temperature under stirring until no AgBr precipitate could be detected with 0.5 M $AgNO_3$. Then water was evaporated with rotary evaporator, and mixed solvent of acetonitrile/methanol (volumetric ratio: 7/3) was added. After filtration, the solvent was removed by evaporation, and the products were dried at 333 K under vacuum for at least 2 days. All the purified glycine ILs were analyzed by ^{1}H NMR (Bruker, AV-400) to confirm the absence of any major impurities, and the purity of these ILs was found to be greater than 99% in mass fraction.

2.3. Phase diagram determination of the ATPSs

The binodal curves were determined by turbidimetric titration method [5]. The tie lines, which describe the concentrations of salt and IL in the two phases, were measured with the procedure outlined in our previous work [23]. The system temperature was maintained at 298.15 ± 0.05 K in the experimental process. Concentration of the phase components was calculated by mass quantification of all the components added within an uncertainty of $\pm 1 \times 10^{-4}$ g.

2.4. Measurements of partition coefficients of DNP-amino acids and cytochrome-c

The partition coefficients of DNP-amino acids (see Fig. 2 for their structures) were determined in the graduated glass tubes according to the method described by Macedo and co-workers [23]. The used total composition of the IL ATPSs was 20.28% (w/w) of K_2HPO_4 and 20.25% (w/w) of glycine ILs. The system temperature was controlled at 298.15 ± 0.05 K. Solution pH was adjusted by the addition of small amount of H_3PO_4 or KOH, and the pH values were determined by a 720 pH meter (Orion, USA) with a combination pH

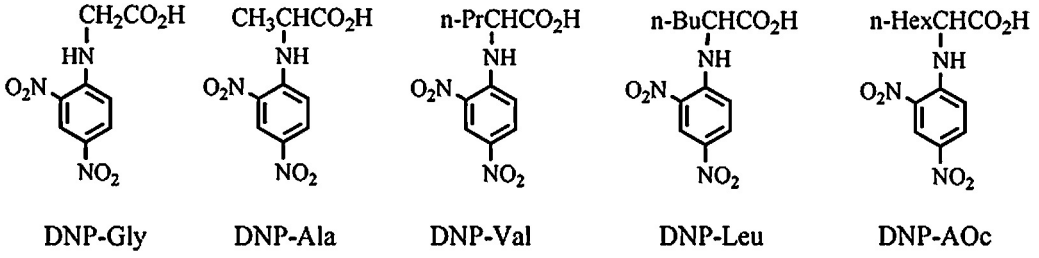

Fig. 2. The chemical structure of the DNP-amino acids.