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A green deep eutectic solvent-based aqueous two-phase system for protein extracting

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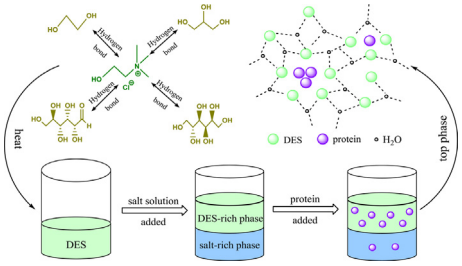
HIGHLIGHTS

- A strategy for the protein purification with a deep eutectic solvent(DES)-based aqueous two-phase system.
- Choline chloride-glycerin DES was selected as the extraction solvent.
- Bovine serum albumin and trypsin were used as the analytes.
- Aggregation phenomenon was detected in the mechanism research.

GRAPHICAL ABSTRACT



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ABSTRACT

As a new type of green solvent, deep eutectic solvent (DES) has been applied for the extraction of proteins with an aqueous two-phase system (ATPS) in this work. Four kinds of choline chloride (ChCl)-based DESs were synthesized to extract bovine serum albumin (BSA), and ChCl-glycerol was selected as the suitable extraction solvent. Single factor experiments have been done to investigate the effects of the extraction process, including the amount of DES, the concentration of salt, the mass of protein, the shaking time, the temperature and PH value. Experimental results show 98.16% of the BSA could be extracted into the DES-rich phase in a single-step extraction under the optimized conditions. A high extraction efficiency of 94.36% was achieved, while the conditions were applied to the extraction of trypsin (Try). Precision, repeatability and stability experiments were studied and the relative standard deviations (RSD) of the extraction efficiency were 0.4246% ($n = 3$), 1.6057% ($n = 3$) and 1.6132% ($n = 3$), respectively. Conformation of BSA was not changed during the extraction process according to the investigation of UV–vis spectra, FT-IR spectra and CD spectra of BSA. The conductivity, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to explore the mechanism of the extraction. It turned out that the formation of DES–protein aggregates play a significant role in the separation process. All the results suggest that ChCl-based DES-ATPS are supposed to have the potential to provide new possibilities in the separation of proteins.

1. Introduction

As a kind of biomolecules which have wide applications in the fields of research, pharmaceuticals and industrials, it is of great importance to prepare pure protein.

The traditional methods for protein purification include ammonium sulfate precipitation, salting out, electrophoresis [1], ion-exchange [2] and affinity chromatography [3]. Because these methods have the drawbacks of complexity, high costs and difficulty to scale up, liquid–liquid extraction (LLE) has emerged as an alternative method [4]. The conventional LLE system consists of water and organic solvent. It is not suitable for proteins purification as proteins are easily denatured or lose their biological activities in organic solvents. Under this circumstance, aqueous two-phase

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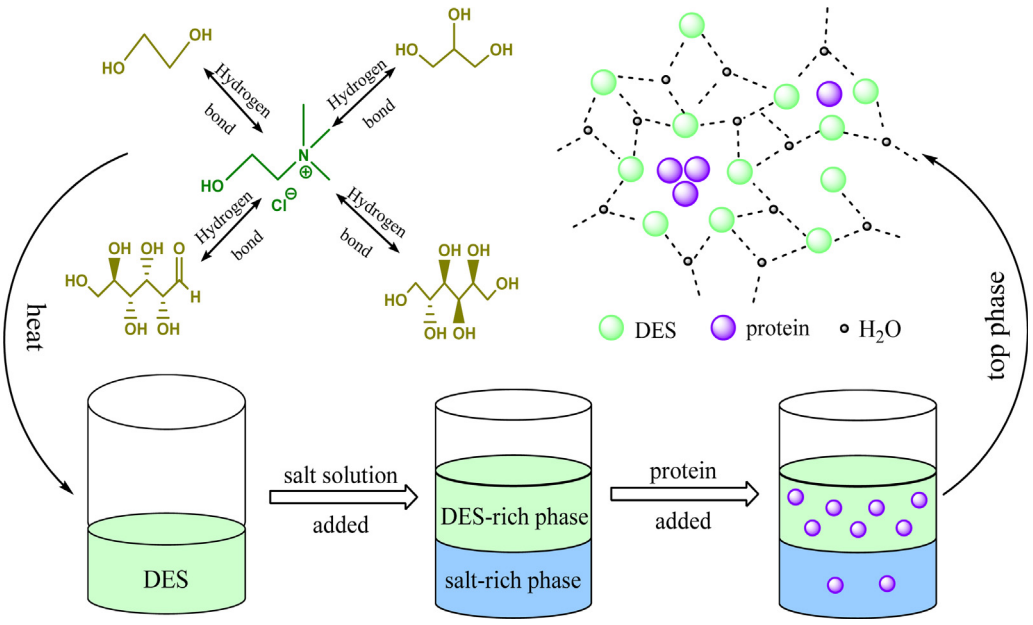

Scheme 1. DES-based ATPS for the extraction of protein.

system (ATPS) [5], which formed when water-soluble polymer and another polymer or certain inorganic salts are mixed above critical concentration, has been widely applied to protein purification owing to its superiority of high water content, moderate extraction environment, short separation time and high biocompatibility [6–8].

Bridges et al. [9] reported ionic liquids (ILs)-based ATPS for the first time in 2003. ILs are low temperature molten salts that are completely composed of ions [10,11]. ILs have many fascinating properties, including low vapor pressure, wide liquid range, selective dissolution, superior thermal stability and wide structural diversity [12]. ILs–ATPS, which combines the advantages of ionic liquids and aqueous two-phase system, shows unique superiority of short separation time, low viscosity and high extraction efficiency that traditional ATPS cannot match. Based on this, ILs–ATPS is supposed to have significant applications in biological separation. Pei et al. have explored the selective separation of protein and saccharides by $[C_4mim][N(CN)_2]$-based ATPS [13]. Li et al. achieved the efficient and high activity extraction of proteins with ILs–ATPS [14]. Zeng [15] and Ding [16] have investigated the extraction process of proteins based on guanidine ILs–ATPS. However, the synthesis process of IL is complex, high-cost and difficult in purification. In addition, pyridinium or imidazolium-based ionic liquids are not completely "green". Their toxicity is no less than traditional organic solvents, even more than the organic solvents [17,18]. These shortcomings have limited its large-scale industrial applications and development.

To overcome the drawbacks of ILs, deep eutectic solvent (DES) has appeared as a new generation of solvent [19–22]. DES is an eutectic mixture which formed by mixing substituted quaternary ammonium salts and hydrogen bond donors such as amines, alcohols and acids. Choline chloride (ChCl) is one of the most widespread quaternary ammonium salts used for the formation of DES because ChCl is cheap and can be easily extracted from biomass. ChCl-based DESs have attracted considerable attentions in many fields such as electrodeposition [23–26], biocatalytic [27] and organic synthesis [28,29]. Compared to traditional ILs, DESs derived from ChCl are non-toxic, biodegradable and the atom utilization rate is 100% in synthesis process. Besides, the physico-chemical properties of DESs are similar to common ILs, which suggests the DES may be a substitute of ILs.

In this paper, four kinds of ChCl/alcohols-based DESs were synthesized and applied to extract BSA with ATPS for the first time (as shown in Scheme 1). ChCl-glycerol was chosen to study the affecting factors and identify the optimal conditions of the extraction. UV–vis, FT-IR and CD spectra were used to observe the conformation of BSA before and after the extraction process. The mechanism of the separation was determined by conductivity, DLS and TEM.

2. Experimental

2.1. Materials and apparatus

Bovine serum albumin (BSA) and trypsin (Try) were supplied by Sinopharm Chemical Reagent Co., Ltd. Choline chloride (ChCl, 98.0~101.0%, Shanghai Source Biological Technology Co., Ltd.), D-(+)-glucose (>99%, Sinopharm Chemical Reagent Co., Ltd.) and D-sorbitol solution (≥98.0%, Kermel Chemical Reagent Co., Ltd.) were dried under vacuum prior to use. Ethylene glycol (≥99.0%) and glycerol (≥99.0%) were purchased from Sinopharm Chemical Reagent Co., Ltd. and were used without further purification. K_2HPO_4 (≥98.0%) was obtained from Sinopharm Chemical Reagent Co., Ltd.

Materials were dried by a DZF-6051 vacuum drying oven (Shanghai, China). Deep eutectic solvents were heated in a DF-101S heat collection-constant temperature type magnetic stirrer. A QYC 200 incubator shaker was used to mix the two-phase system. A TGL-16C high-speed centrifuge was applied to speed up the phase separation. Ultraviolet spectrum of protein solution was measured by a UV2450 UV–vis spectrophotometer (Shimadzu, Japan). Infrared spectrum of DESs was recorded using a Spectrum One FT-IR spectrometer (PerkinElmer, USA). Secondary structure of protein was determined by a Mos-500 circular dichroism (CD) spectrometer. Aggregation and embrace phenomenon was observed by a Zetasizer Nano-ZS90 dynamic light scattering (Malvern, Britain). Microstructure of sample was examined using a JEM-3010 transmission electron microscope (TEM, JEOL, Japan).

2.2. Synthesis and characterization of DESs

In this study, four kinds of deep eutectic solvents were synthesized in different ratios of quaternary ammonium salts (choline chloride) to hydrogen bond donors (ethylene glycol,