



A functional natural deep eutectic solvent based on trehalose: Structural and physicochemical properties



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ABSTRACT

In this study, the natural deep eutectic solvents (NADESs) based on trehalose and choline chloride have been prepared to enhance the protein thermostability. The results of fourier transform infrared spectroscopy and ¹H nuclear magnetic resonance spectroscopy suggested that there were intensive hydrogen-bonding interactions between trehalose and choline chloride in TCCL3-DES and TCCL3-DES75. The physicochemical properties of TCCL3-DES and TCCL3-DES75 were investigated in the temperature range of 293.15–363.15 K. Our results revealed that the thermostability of lysozyme, a model protein used in this study was dramatically increased in TCCL3-DES75, as evidenced by the disappearance of the denaturing peak from their Differential Scanning Calorimetry (DSC) traces. The results of circular dichroism (CD) experiments further demonstrated that the lysozyme in TCCL3-DES75 unfolded partially at 90 °C and recovered to the initial structure at 20 °C. The study suggests that TCCL3-DES75 might be a potential solvent for stabilizing proteins.

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1. Introduction

Recently, considerable scientific and economic interests have been devoted to extend the range of green solvents for stabilizing protein or enzyme to the health-related areas such as foods, pharmaceuticals, and cosmetics. Natural deep eutectic solvents (NADESs) are inspired by the fact that when certain cell metabolites are mixed together there may be a considerable reduction of

Abbreviations: NADES, natural deep eutectic solvent; T, trehalose; CCL, choline chloride; TCCL-DESs, trehalose and choline chloride based deep eutectic solvents; TCCL3-DES, TCCL-DESs were prepared by mixing trehalose and choline chloride in different molar ratios of 1:3; TCCL3-DES75, TCCL3-DES containing 25% (w/w) water; TCCL3-DES50, TCCL3-DES containing 50% (w/w) water; TCCL3-DES25, TCCL3-DES containing 75% (w/w) water; T_m , melting point of protein; HBDs, hydrogen bonds donors; FTIR, Fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance; DSC, Differential Scanning Calorimetry; V_m , molar volume; E_a , energy barrier based on the Arrhenius equation; f_m , free volumes; n_D , refractive index; α_e , electronic polarizability; R_m , the molar polarizability; a , molar refraction; f_m , free volume; R_m , the hard core molar volume.

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the melting point and the formation of liquids even at very low temperatures (Choi et al., 2011; Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013). The NADESs are distinguished from the conventional solvents because of their excellent properties such as non-volatility, low costs, biodegradability, non-toxicity, sustainability, and simple preparation methods (Dai, Witkamp, Verpoorte, & Choi, 2015). Generally, NADESs were obtained by mixing quaternary ammonium salts with different hydrogen bonds donors (HBDs) (D'Agostino et al., 2015).

A green solvent plays a vital role in health-related industrial processes, such as enzyme biosynthesis of fine chemicals, storage and extraction mediums of functional polypeptides (Yang & Pan, 2005; Zhao, Xia, & Ma, 2007; Sathitsuksanoh et al., 2014; Ventura et al., 2013; Zavrel, Bross, Funke, Büchs, & Spiess, 2009). A key issue is to retain the stability and biological function of proteins in the solvents (Khan, Bisetty, Singh, Permaul, & Hassan, 2015; Khan, Govender, Permaul, Singh, & Bisetty, 2015). However, few studies have been evaluated the functional behavior of NADESs for protein stability. The functional conformation of protein is intrinsically related to the folding process (Esquembre et al., 2013; Khan, Wei, Gu, Hassan, & Tabrez, 2015). The NADESs with

urea and glycerol as HBDs were mostly studied and reversibility of the unfolding process is incomplete. Compared to urea and glycerol, the trehalose (α -D-glucopyranosyl- α -D-glucopyranoside) is a very potential disaccharide as a HBDs. Nowadays, trehalose is an extremely attractive compound widely applied in protecting yeast pyrophosphatase and glucose 6-phosphate dehydrogenase against thermal inactivation (Brewster, Maninder, Simpkins, & Bodor, 1991; Crowe, Crowe, & Chapman, 1984; Eroglu et al., 2000; Sola-Penna, Ferreira-Pereira, Lemos, & Meyer-Fernandes, 1997). The role of trehalose as an effective stabilizer was fairly well-established, and the mechanisms of trehalose resulting in this unique behavior of preservation still remain unclear (Patist & Zoerb, 2005). Some studies suggested that the trehalose-imposed glassy state appearing in organisms enabled them to survive in a variety of stress conditions (Crowe, 2007). Others considered that trehalose kept the structure of proteins and cellular membranes in its native state by binding to polar and charged groups of molecules in ultra-high content states (Hédoux et al., 2009; Timasheff, 2002). The ultra-high trehalose content and glass-forming liquid states may be the underlying mechanism to explain the ability of trehalose to protect protein from denaturation in nature.

Inspired by these phenomena, the novel trehalose-based solvents were designed named TCCL-DES. The TCCL-DES was not only an ultra-high trehalose content liquid but displayed a glass-forming state too. In this study, structural property of TCCL-DES and their dilutions were analyzed using Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. The physicochemical properties such as density, viscosity and refractive index of neat and ultra-high content solvents were systematically studied for facilitating their industrial applications. Finally, their ultra-high concentration solvents in protecting proteins against heating were investigated. Results of the structural and physical properties of trehalose-based solvent would be beneficial for further studies of the protective abilities of trehalose. Meanwhile, the remarkable performance of this solvent for stabilizing proteins under heat stress indicated that TCCL3-DES75 might be a good candidate in food and pharmaceutical industry.

2. Materials and methods

2.1. Materials

Trehalose ($\geq 99\%$), choline chloride ($\geq 99\%$) and Deuterium oxide (≥ 99.96 atom% D) were purchased from Aladdin (Aladdin Industrial Corporation, Shanghai, China). Lysozyme from chicken egg white ($\geq 90\%$ mass fraction purity) was purchased from Sigma-Aldrich (Sigma-Aldrich Co. LLC, St. Louis, USA) and used without further purification. Ultrapure grade water (resistivity ≥ 18.2 M Ω cm) was obtained using a Milli-Q filtration system (Res. J scientific instruments CO., LTD, Xiamen, China).

2.2. Sample preparation

NADESs were prepared by mixing trehalose and choline chloride in different molar ratios of 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6, respectively, with continuous stirring while heating at 353.15 K (Abbott, Capper, Davies, Rasheed, & Tambyrajah, 2003). Molar ratios, abbreviations and appearances of TCCL-DESs were shown in Table S1. TCCL-DES dilutions were prepared by diluting with different weigh percentage (w/w) of deuterium oxide prior to the ^1H NMR measurement. For physical properties analysis, NADESs were diluted with 25% (w/w) ultrapure grade water.

2.3. FTIR and ^1H NMR analysis

The spectra of trehalose, choline chloride, TCCL3-DES and TCCL4-DES were performed by the FTIR (Bruker Daltonics, Karlsruhe, Germany). FTIR spectra were collected from 4000 to 500 cm^{-1} at room temperature. The ^1H NMR spectra of the diluted TCCL-DES with a different weigh percentage of deuterium oxide (D_2O) were recorded at room temperature on Bruker AVANCE III HD 600 Hz spectrometer (Bruker Daltonics, Karlsruhe, Germany). A total of 32,768 data points were recorded covering a spectral window of 9615 Hz; 128 scans of a standard one-pulse sequence with 90 flip angle for excitation and pre-saturation during 2.0 s relaxation delay. An exponential window function with a line-broadening factor of 0.3 Hz was applied prior to Fourier transformation. The resulting spectra were manually phased and baseline corrected.

2.4. Densities and viscosities

The dynamic viscosities (η) and densities (ρ) of TCCL3-DES and TCCL3-DES75 were measured using the automated Anton Paar Lovis 2000ME microviscometer (Anton Paar GmbH, Graz, Austria). The density measurement was based on electromagnetically induced oscillations of a U-shaped glass tube. The standard deviations associated with the density measurement were below 0.00005 g cm^{-3} . The measurements were performed at 10 K intervals in the temperature range of 293.15–363.15 K.

The viscosities were measured based on Hoesppler's falling ball principle. The combinations of capillary and ball with different diameters can be selected. A 10 mm long capillary with a diameter of 1.6 mm, 1.8 mm, 3.0 mm or 4.0 mm with a tilting angle of 60° was used. The deviation in dynamic viscosities was below 0.5%.

2.5. Refractive index

The refractive indices (RI) of the TCCL3-DES and TCCL3-DES75 were determined using the automatic Anton paar ABEMAT350 refractometer (Anton Paar GmbH, Graz, Austria) with a resolution of ± 0.00001 and an uncertainty in the experimental measurements of ± 0.0001 . The apparatus was calibrated by measuring the refractive index of ultrapure water before the measurements. The measurements were performed at 10 K intervals in the temperature range of 293.15–363.15 K.

2.6. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) measurements were performed using the NETZSCH DSC 204 F1 (NETZSCH-Gerätebau GmbH, Selb, Germany) in aluminum cells at a heating rate of 10 $^{\circ}\text{C min}^{-1}$. For DSC measurements a 34 mM lysozyme solutions in ultrapure water, TCCL3-DES25, TCCL3-DES50 and TCCL3-DES75 were used. Approximately 4–7 mg of lysozyme samples were weighed into the aluminum liquid pan (E. I. DuPont de Nemours and Company, Wilmington, USA). Samples were scanned from 30 $^{\circ}\text{C}$ to 90 $^{\circ}\text{C}$. The maximum of the melting point of protein (T_m) was registered.

2.7. Circular dichroism

Circular dichroism (CD) measurements were performed on a chirascan Spectrometer (Applied Photophysics, Leatherhead, UK). Lysozyme concentration of 34 mM was examined in the near UV region (310–250 nm) in a 0.1 cm path length quartz cell at a bandwidth and step size of 1 and 0.5 nm, respectively. TCCL3-DES75, TCCL3-DES50 and TCCL3-DES25 were prepared one hour prior to measurements. Each sample was subject to a heating cycle of

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