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Influence of surface groups of proteins on water studied by freezing/thawing hysteresis and infrared spectroscopy

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ABSTRACT

The influence of proteins and solutes on hysteresis of freezing and melting of water was measured by infrared (IR) spectroscopy. Of the solutes examined, poly-L-arginine and flounder antifreeze protein produced the largest freezing point depression of water, with little effect on the melting temperature. Poly-L-lysine, poly-L-glutamate, cytochrome *c* and bovine serum albumin had less effect on the freezing of water. Small compounds used to mimic non-polar (trimethylamine N-oxide, methanol), positively charged (guanidinium chloride, NH_4Cl, urea) and negatively charged (Na acetate) groups on protein surfaces were also examined. These molecules and ions depress water's freezing point and the melting profiles became broad. Since infrared absorption measures both bulk solvent and solvent bound to the solutes, this result is consistent with solutes interacting with liquid water. The amide I absorption bands of antifreeze protein and poly-L-arginine do not detectably change with the phase transition of water. An interpretation is that the antifreeze protein and poly-L-arginine order liquid water such that the water around the group is ice-like.

1. Introduction

The nature of water has implications to the role of hydrophobicity in the collapse of proteins, the influence of water on the dynamics of proteins, and the specificity of substrate binding. The characteristics of water at the surface of proteins have been debated for a long time [1–4].

Hydrophobic and hydrophilic groups at the surface of proteins both play roles in maintaining structure and function [5,6]. Hydrophilic groups interact with water, and they are required for the specificity of function of the protein, but the surface of proteins also contain up to approximately 50% hydrophobic areas. Although hydrophobic patches on protein surfaces do not H-bond to water, they can also change the water around them; for instance, simulation of water around apolar groups indicates that the neighboring water molecules are more tetrahedral than in bulk [7]. The existence of the resulting structured water is invoked to explain the action of antifreeze proteins or thermal hysteresis proteins (THP) [8–12]. Mutation studies suggest that binding to ice is specific [13–15], but details of the binding remain a subject of investigation [9,16].

In this study the effects of common groups of protein surfaces on water structure are studied using infrared (IR) spectroscopy and the thermal phase transition of water. Addition of solutes depresses the freezing point of water in a concentration dependent manner [17], and the empirical relationship for the colligative contribution to freezing point depression is:

$$\Delta T_F = -k_f C \quad (1)$$

where C is the total concentration of solute entities (molecules and all ions) in molal units, and k_f is 1.85 °C/(mol/kg) for water [18]. However, under typical experimental conditions, the liquid to solid phase transition of water shows hysteresis. Although the equilibrium H_2O phase transition occurs at 0 °C, the temperature of pure water can be reduced to ~−40 °C before crystallization occurs [19]. This necessarily means that at temperatures between melted (T_M) and frozen (T_F) states the system is not in Gibbs equilibration. If a solute molecule preferentially binds to ice surfaces, it could inhibit the growth of the crystal, and then the observed freezing point will be depressed below that produced by the colligative effect. The freezing point transition in the presence of solutes usually remains sharp, indicating a cooperative transition, or at least a complete transition across the dimensions of the sample within a very narrow temperature range, providing a well defined measure of T_F. In contrast, solutes often broaden the melting transition of water several degrees or more. This broadening may result from local non-homogeneous sample composition due to demixing of solute/solvent at the liquid–solid phase boundary, making the measurement and interpretation of ΔT_M more difficult. However, if the solute alters the H-bonding of liquid water, information from the melting profile may be extracted by IR spectroscopy. IR spectra can sensitively detect both freezing and melting phase transitions of water since its IR absorption bands are sensitive to

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H-bonding. As H-bonding strength between OH and an H-bond acceptor increases, the OH stretching goes to lower frequency and the water bending band goes to higher frequency [20].

If the solute contains peptide groups, or other identifiable IR active groups, IR spectroscopy may also be used to directly probe the water–solute interaction through the freezing and melting phase transitions. The amide I bands of peptides, composed predominantly of a C=O stretch, when acting as an H-bond acceptor to water, also shifts to lower frequency as H-bonding increases [20–22]. Thus, IR spectra report on all the water molecules, including those that are bonded to the solute. For instance, in previous work using water's IR absorption, Nucci et al. examined the H-bonding effects of the Hofmeister ions and showed that changes in ion-bound water, as measured by IR, reflects the overall distribution of charge on the ions [23].

Three proteins are examined in this paper: flounder antifreeze protein, cytochrome *c* (representative of a cationic protein) and albumin (representative of an anionic protein) and three polypeptides are studied: anionic polyaspartate and cationic polylysine and polyarginine. As model compounds, trimethylamine N-oxide (TMAO) and methanol serve as references for aliphatic groups. The effect of negatively charged carboxyl group is studied using Na acetate; amino and arginino-groups are examined using NH_4Cl, urea, arginine and guanidine. We show that ice melting is no longer sharp in the presence of most of these solutes. In one case, for poly-L-arginine the freezing point was depressed with retention of a sharp melting point. For proteins, the freezing point of water is depressed, with the largest depression observed for antifreeze protein; the freezing point depression was larger than predicted by Eq. (1).

2. Materials and methods

2.1. Source of chemicals

Sigma Chemical Co (St. Louis MO) supplied TMAO, sodium acetate, poly-L-aspartic acid sodium salt (5000–15,000 D), sodium chloride, guanidine hydrochloride, poly-L-lysine hydrochloride (15,000–30,000 D), poly-L-arginine hydrochloride (5000–15,000 D), horse heart cytochrome *c* and bovine serum albumin. Methanol was certified A.C.S. spectro-analyzed from Fisher Scientific Inc. and antifreeze protein THP type I from winter founder was from A/F Protein, Inc. (Waltham, MA). Deuterium oxide (D 99.9%) was supplied by Cambridge Isotope Laboratories, Inc. (Andover, MA). H_2O was obtained from tap water that was Millipore ion exchanged and then distilled.

Solutions were prepared by weighing the solutes and adding a known volume of water. From weights and volumes, we calculated molar, molal and molecular or ionic ratios of solute to molecular water (s/w). The small solutes were prepared without buffer, but the pH was approximately neutral as measured with glass electrodes using Accumet AB15

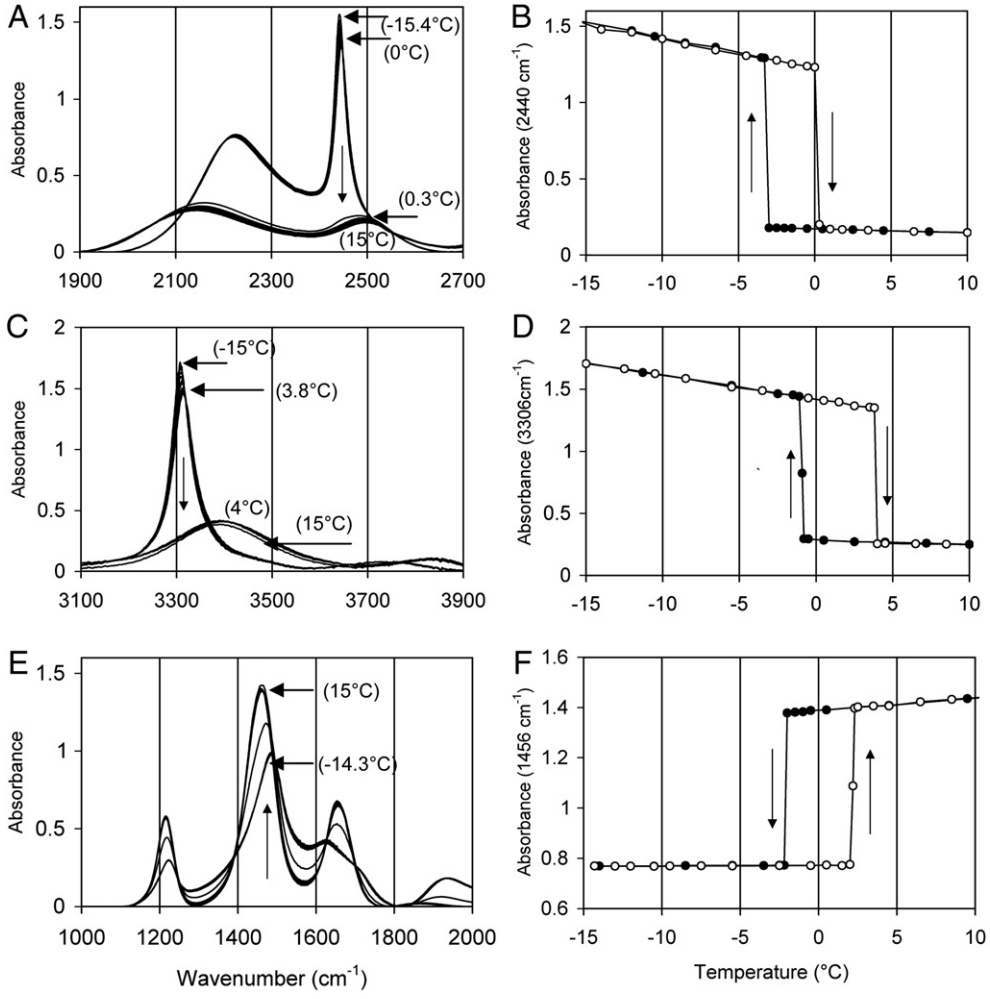

Fig. 1. Temperature dependence of IR spectra. (A) IR spectra of D_2O (1% D_2O in H_2O) in the temperature range of −15.4 to 15 °C. (B) O–D stretching absorption at 2440 cm^{-1}. Liquid to solid phase transition (closed circles), and solid to liquid phase transition (open circles, data taken from (A). (C) IR spectra of H_2O (1% H_2O in D_2O) in the temperature range of −15 to 15 °C. (D) O–H stretching absorption at 3306 cm^{-1}. Liquid to solid phase transition (closed circles), and solid to liquid phase transition (open circles) Data taken from (C). (E) IR spectra of H_2O/D_2O (1/1 v/v) in the temperature range of −14.3 to 15 °C. (F) Thermal hysteresis of H_2O/D_2O using H–O–D bending absorption at 1456 cm^{-1}. Liquid to solid phase transition (closed circles), and solid to liquid phase transition (open circles). Data taken from E.