Journal of Non-Crystalline Solids 407 (2015) 486–493

Contents lists available at ScienceDirect

Journal of Non-Crystalline Solids

journal homepage: www.elsevier.com/ locate/ jnoncrysol

Dynamics of amorphous and partially crystallized proline solutions

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ARTICLE INFO

Article history:
Received 28 June 2014
Received in revised form 1 August 2014
Available online 12 September 2014

Keywords:
Amino acid;
Water dynamics;
Dielectric spectroscopy;
Proline

ABSTRACT

Dynamics of water in the hydration shells of proteins in the no man's land temperature region is a currently debated topic. Previously [1], we proposed the use of lysine aqueous solutions as a model system to study the dynamics of water in a genuine biological solution at any temperature. We found that lysine can be completely dissolved in water without crystallization, making it possible to access the dynamics of amorphous water in a biological environment. By contrast, in this work we studied proline aqueous solutions, which partially crystallize upon cooling. We found similar results in the two systems: the presence of three water relaxations where the two slowest processes (processes 2 and 3) resemble the behavior of normal glasses. Moreover, we observed the change of a cooperative (α-like) water relaxation to a more local β-like water relaxation with decreasing temperature whereas a faster water relaxation process is also present. In addition, we studied amorphous samples in a restricted temperature interval, and also found three different dynamics (processes 3, 4 and 5). As in the case of the hydrated protein powders, the two faster dynamics (3 and 4) are coupled or slaved.

1. Introduction

Water plays a significant role in many chemical [2], technological [3,4] and biological processes [5,6]. In particular, the dynamics of water in bio-systems has been studied for a long time using several different experimental techniques as well as molecular dynamics simulations [7–17], mainly because these types of studies may provide important insights into biological activity. In addition, studying the dynamics of proteins make it possible to access the no man's land temperature region (150–235 K) where bulk water crystallizes. The dynamics of water coupled with proteins, and in particular the question of whether the dynamics resembles that of bulk water has been extensively debated in the recent literature, but there is still no consensus about the interpretation of the results at low temperatures. Moreover, the biological function of proteins is related to their ability to undergo structural changes and hydration is an important factor in both function and stability. Therefore, the dynamics of hydrated proteins is a subject of major interest because this is one of the factors (together with the protein structure) that controls functionality [18,19].

The dynamics of hydrated proteins at temperatures below their glass transition (T_g, approximately 200 K for several proteins) has been extensively analyzed using dielectric spectroscopy because of the broad frequency range that is accessible using this technique. Hydrated protein powders have shown a rich dynamical behavior with the presence of several dynamical processes in the temperature range from $T_g - 100$ K to $T_g + 50$ K. Below T_g, two relaxation processes related to the solvent were reported, whereas above T_g, three processes were observed and attributed to the reorientation of the protein and/or the water molecules [13,20]. This varied dynamical behavior reflects the permanent motions of proteins [21], which change their conformation to perform a specific function. For this reason, it is difficult to analyze the dynamical behavior of a protein and, in particular, to determine the characteristics of hydration water dynamics. In addition, it is important to note that these studies are only restricted to hydrated protein powders (i.e., low water content) because ice formation cannot be avoided at low temperatures (lower than 235 K). Important and valuable results were obtained using hydrated protein powders but several physiological functions (for instance, protein folding) occur in the cell where proteins are surrounded by a well-diluted solution of water and other components that are absent in hydrated powders [22]. Thus, to study the general characteristics of water dynamics in a genuine biological solution it is necessary to adopt a strategy to avoid crystallization. One route is to mix water with cryo-protectants (sugars or glycerol) [23] or, as recently proposed by Mamontov et al. [15,24], to replace water with lithium chloride solutions that have properties similar to those of water. Another route is to change the protein for a reduced biological system [25–27]. Several researchers have implemented this change in their studies of less complex model systems; these systems include solutions of relatively simple molecules (e.g., amphiphilic peptides [25,26] or short chains of amino acid monomers [27]). This type of simple system reduces the topological disorder and the surface interactions imposed by the protein on the water molecules, and therefore, it allows us to investigate the role of water using a more well-defined local environment. In this sense, we studied the simplest biological system, which is the amino

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http://dx.doi.org/10.1016/j.jnoncrysol.2014.08.049
0022-3093/

acid [1]. Only one amino acid (lysine) from the 20 in animal proteins is sufficiently soluble to be analyzed in a broad concentration range without water crystallization at any temperature. In a previous work [1], we analyzed the dynamics of supercooled water in aqueous solutions of lysine using broadband dielectric spectroscopy. We showed that this system leads to unique water dynamics because, for the first time, a continuation of a cooperative (α-like) water relaxation was observed after a crossover (at the glass transition temperature, T_g) to a more local β-like water relaxation with decreasing temperature. In addition, another faster β-like relaxation was also observed [1].

The present study is concerned with the dynamics of partially crystallized, as well as amorphous, solutions of proline. L-proline is a unique amino acid because it is the only amino acid with a secondary amino group. The amine nitrogen is bound to two alkyl groups to form a pyrrolidine ring. This rigid structure restricts the number of conformations that this amino acid can adopt in a protein [28]. In addition, aqueous proline solutions possess other interesting properties. Plants accumulate proline under adverse conditions, including freezing temperatures [29], and consequently, proline is considered a natural cryoprotectant. Proline–water interactions and proline–proline interactions have been previously studied to clarify the origin of these properties [30]. Some authors have suggested that proline molecules form semi-ordered aggregates in aqueous solutions [31–33], whereas other authors have suggested that proline inhibits the formation of ice crystals via the competitive hydrogen bonding of proline to water [34].

To access the dynamics of proline–water solutions, we used broadband dielectric spectroscopy (BDS) for samples with three water concentrations (40, 50 and 60 wt.%). First, we focused on the low temperature range (below T_g), where the solutions partially crystallize. We found three dielectric processes related to water, with the same general characteristics as those in lysine solutions. Then, we analyzed the behavior of amorphous samples in a restricted temperature interval. We also found three dynamical processes and, similar to the hydrated protein powders, the two faster processes were coupled or slaved. Finally, we compared lysine and proline solutions to define the difference between these two systems.

2. Experimental section

2.1. Materials

L-proline (M_n = 115.13 g/mol) and ultrapure water from Sigma Aldrich Chemical Co. Inc, were used to prepare samples in a water concentration (c_w) range from 40 wt.% to 60 wt.% (in weight). In these concentration ranges, transparent solutions were obtained at room temperature. Table 1 shows the general characteristics of all the samples analyzed in this work.

2.2. Experimental techniques

The complex dielectric permittivity, $\varepsilon''(\omega) = \varepsilon(\omega) - i\varepsilon''(\omega)$, was measured by combining two different dielectric techniques to obtain a wide spectral range (0.05 Hz to 10^8 Hz). For the frequency range from 5×10^{-2} Hz to 10^6 Hz, we used a Novocontrol Alpha-A -Analyzer, whereas for the frequency range from 10^6 Hz to 4×10^8 Hz, an Agilent rf impedance analyzer 4192B was used. For all measurements, the sample thickness was 0.1 mm, and the sample diameter was 30 mm and 10 mm for the low and high frequency measurements, respectively. For the low frequencies (10^{-1} Hz to 10^6 Hz), each sample was placed in a sample holder, quenched at 130 K and then reheated to 300 K while isothermal (± 0.1 K) scans were performed every fifth degree for the temperature range from 130 to 200 K and every third degree over the temperature range from 202 to 250 K. Measurements in the high frequency range (10^6–4×10^8 Hz) were performed every fifth degree from 300 K to 250 K.

Table 1
General characteristics of the samples. c_w is the weight fraction of water, h is the grams of water per gram of dry proline, $T_{g,\,DSC}$ is the calorimetric glass transition temperature, and N is the number of water molecules per proline molecule. For the samples P means proline, W means water, and the number represents the content in weight of either proline or water respectively.

Sample	c_w [wt%]	h	$T_{g,\,DSC}$ [K]	pH	N
P60–W40	40	0.66	194	7.0	4.3
P50–W50	50	1.00	176	6.9	6.4
P40–W60	60	1.50	196	7.0	9.4

To analyze the complex permittivity (ε^*), simultaneous fitting of both the real (ε') and imaginary (ε') components was performed using the Cole–Cole (CC) function [35]

$$\varepsilon^*(\omega) = \frac{\Delta\varepsilon}{1 + (i\omega\tau)^{\alpha}} \tag{1}$$

where $\Delta\varepsilon$ is the dielectric strength ($\Delta\varepsilon = \varepsilon_s - \varepsilon_\infty$, ε_∞ and ε_s are the unrelaxed and relaxed values of the dielectric constant, respectively), τ is the relaxation time, α is the stretching parameter of each relaxation process and $\omega = 2\pi f$ is the angular frequency. The total fit function was given by

$$\varepsilon^*(\omega) = \varepsilon_\infty + \sum_{j=1}^{N} \frac{\Delta\varepsilon_j}{1 + \left(i\omega\tau_j\right)^{\alpha_j}} - i\left(\frac{\sigma}{\varepsilon_0\omega}\right)^N \tag{2}$$

where N represents the total number of relaxation processes at each temperature. In addition, at low frequencies, conductivity effects dominate; to account for these effects, a power law term was added (last term in Eq. (2)), where ε_0 denotes the vacuum permittivity and σ is the static ionic conductivity.

Differential scanning calorimetry measurements were performed using a DSC Q-2000 from TA Instruments with cooling and heating rates of 10 K/min whereas Fourier transform infrared spectroscopy (FTIR) was conducted using a JASCO 6500 and the attenuated total reflectance method (ATR) in the range of 4000–500 cm^{-1}. The sample was cooled to 163 K and then heated back to room temperature using liquid nitrogen as a refrigerant at a cooling/heating rate of 30 K/min.

3. Results and discussion

3.1. Thermal response

Fig. 1 shows the calorimetric scans for all the analyzed samples. For cooling scans, an exothermal feature is observed at different

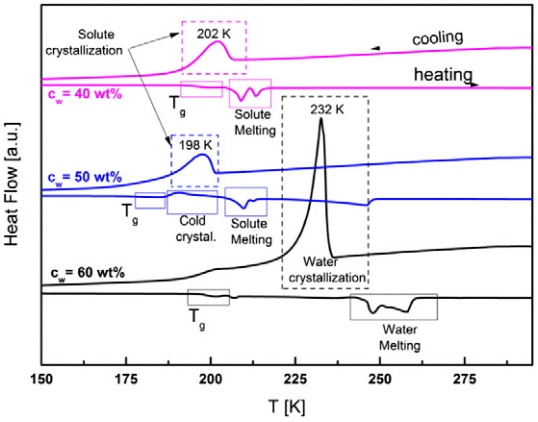

Fig. 1. Cooling and heating scans of aqueous proline solutions. Different thermal events such as the glass transition (T_g), solute and water crystallization, or solute and water melting, are depicted in the plot.