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Dynamics of uncrystallized water and protein in hydrated elastin studied by thermal and dielectric techniques

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ABSTRACT

Dynamics of uncrystallized water and protein was studied in hydrated pellets of the fibrous protein elastin in a wide hydration range (0 to 23 wt.%), by differential scanning calorimetry (DSC), thermally stimulated depolarization current technique (TSDC) and dielectric relaxation spectroscopy (DRS). Additionally, water equilibrium sorption–desorption measurements (ESI) were performed at room temperature. The glass transition of the system was studied by DSC and its complex dependence on hydration water was verified. A critical water fraction of about 18 wt.% was found, associated with a reorganization of water in the material. Three dielectric relaxations, associated to dynamics related to distinct uncrystallized water populations, were recorded by TSDC and DRS. The low temperature secondary relaxation of hydrophilic polar groups on the protein surface triggered by hydration water for almost dry samples contains contributions from water molecules themselves at higher water fractions (ν relaxation). This particular relaxation is attributed to water molecules in the primary and secondary hydration shells of the protein fibers. At higher temperatures and for water fraction values equal to or higher than 10 wt.%, a local relaxation of water molecules condensed within small openings in the interior of the protein fibers was recorded. The evolution of this relaxation (w relaxation) with hydration level results in enhanced cooperativity at high water fraction values, implying the existence of "internal" water confined within the protein structure. At higher temperatures a relaxation associated with water dynamics within clusters between fibers (p relaxation) was also recorded, in the same hydration range.

1. Introduction

In the case of dielectric studies on hydrated proteins, the main relaxation of uncrystallized water is usually attributed to contributions of water molecules in the protein hydration shell [1–8]. The universality of the protein hydration water in terms of temperature dependent characteristic relaxation times, as studied by dielectric relaxation spectroscopy (DRS) and nuclear magnetic resonance (NMR), has been shown for various hydrated proteins, including globular and fibrous proteins [7–10] of varying molecular weights and structural characteristics. Although most dielectric spectroscopy studies are dealing with samples of hydration levels high enough for complete hydration and low enough to prevent crystallization of water (about 30 wt.%), recently efforts are made to study protein dynamics in a broad hydration range, both in partially crystallized solutions [11] and starting at low levels of hydration with hydrated dry samples (powders) [8,10,12–19]. Studies at low levels of hydration have been able to follow the secondary relaxation of water and to establish an additional contribution from the protein surface itself to the dielectric response [7–10,14].

Furthermore, dielectric studies on hydrated BSA in a wide hydration range revealed the existence of a relaxation mode which is slower than the main relaxation of uncrystallized water in the hydration shell and faster than the relaxation associated with the glass transition of the hydrated system [7,15]. This slower relaxation was detected mainly in the intermediate hydration range of about 20–40 wt.%, and was suggested to be attributed to a different form of uncrystallized water (process w) [7], although its origin is not yet fully clear. A relaxation of similar characteristics has been observed in the case of hydrated myoglobin by DRS measurements using insulating thin foils (process IV) [13], and in the case of hydrated elastin (process $\beta 2$) [20]. This relaxation, which has been mainly observed in an intermediate hydration range (20–40 wt.%) has been attributed either to localized or delocalized motions of the protein, or to the region of the protein where tightly bound water dominates ([20] and references therein). The origin, hydration dependence and dynamical characteristics of this relaxation remain open.

Studies of globular proteins in wide ranges of hydration at small steps, from dry protein samples to fully hydrated proteins and partially crystallized samples, combining calorimetric and dielectric studies but also equilibrium water sorption measurements at room temperature [9,14], revealed critical water contents for the gradual formation and completion of the protein hydration shells (primary and secondary) and the formation of ice. In addition, a correspondence was found, between the water coverage of primary sorption sites of the protein

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surface, i.e. hydrophilic polar groups, and the manifestation of a calorimetric glass transition of the hydrated system [9]. This fact supports the idea that the glass transition in hydrated globular proteins is caused by the combined motion of water clusters on the protein surface, which is supported also by theoretical simulation studies [21]. It is worth to mention at this point the original work of Careri, where a correlation was found between the onset of enzymatic activity of globular proteins and a percolation type displacement process of protons on single macromolecules, the prerequisite of both being a minimum water content [22].

In hydrated proteins, the dielectric manifestation of deeply supercooled hydration water exhibits similar characteristics in terms of temperature dependent relaxation times, activation energies and shape parameters of dielectric functions [6,8], when comparing structurally different proteins (in the fully hydrated state for both globular and fibrous [6]) but also at lower hydration levels in the case of globular proteins of varying molecular weights [10]. On the other hand, a main difference exists relevant with the calorimetric glass transition of globular versus fibrous proteins. Thermal and dynamic studies of hydrated globular proteins revealed the presence of a thermal glass transition in the temperature range from about −110 to −70 °C, depending on the protein, the hydration level and the experimental technique employed [7,9,12,14,23–25]. On the contrary, fibrous proteins are more hydrophobic and water insoluble, and exhibit higher values of thermal glass transition when compared to globular proteins, in the range of 0–200 °C, depending on the hydration level [26–28]. A fibrous protein of remarkable structure and properties is elastin [29].

Elastin is a hydrophobic, insoluble protein which is a main part of several connective tissues such as lung, arteries and skin. It exhibits quite complex structure and hydration dependent functional properties, which are not yet clearly understood, despite the extensive experimental and theoretical studies throughout the previous decades [30]. A variety of structural studies have demonstrated that the elastin molecule consists of tropoelastin subunits physically crosslinked by the enzyme lysyl oxidase [31]. By this way a three dimensional network is formed, which obtains different conformational states depending on the hydration level and the stress–strain applied. It has been viewed as a random network devoid of any organization [32], constituted of an aggregate of tropoelastin globules [33], considered as a regular arrangement of successive β turns forming a β spiral [34] or composed of isolated and dynamic β turns providing classical entropic elasticity [35,36]. The dynamical entropy of the system significantly decreases in the stretched state, and the classical theory of rubber elasticity could be applied to the hydrophobic domains [37,38]. The biological function of elastin is manifested mainly through the mechanism of elasticity, which depends inextricably on a complex interaction with the solvent, i.e. hydration water. Elastin is elastic only when hydrated [39]. In thermal analysis, elastin undergoes a glass transition phenomenon, dependent on hydration. The glass transition temperature varies from 200 °C in the freeze–dried state to approximately 20 °C in physiological conditions and is associated with the amorphous phase [26,40,41]. Another well known characteristic of elastin is that it undergoes an inverse temperature transition at approximately 20–40 °C, a process that results in a reduction of the protein radius of gyration, expulsion of water, and formation of a complex network of hydrogen bonds [42]. Recent studies have shown also that this inverse temperature transition depends on the amino acid sequence in the chains, pointing out that the structuring of water in elastin exists as an inhomogeneous distribution [43]. Studies by deuterium Double Quantum filtered NMR [44] indicated an increase in order in the surrounding water molecules of the hydration shell in elastin, upon increasing the temperature above temperatures characteristic of the inverse temperature transition. Furthermore, results obtained by T_2–T_2 exchange NMR measurements provide information on the exchange rate of distinct hydration water populations (water inside fibers, water on the fiber surface, water between fibers and more free water) [45]. Recent dielectric studies on hydrated elastin powders at subzero temperatures and at different levels of hydration in the hydrated and immersed state have shown the existence of three dielectric relaxations connected to hydration water [20]. Two relaxations ($\beta1$, $\beta2$) are attributed to distinct water populations of the hydration shell, and a third slower relaxation ($p1$) is attributed to intrafibrillar water clusters (water between fibers).

In this study we employ a combination of ESI, DSC, DRS and TSDC measurements in hydrated elastin pellets at a relatively low hydration level range, i.e. excluding the case of elastin immersed in water. The results of the abovementioned techniques are discussed in analogy to similar results on globular proteins and to recent results on elastin by various techniques, aiming in deeper understanding of the water–protein interactions in the protein hydration shell. The evolution of hydration levels at small steps together with the combination of dielectric techniques with calorimetry and ESI measurements proves to be highlighting on the hydration dependence of water dynamics in the system. It becomes clear that even small variations in hydration level may cause important changes in the dynamics and water organization. Finally, the knowledge of the structural differences between elastin and globular proteins proves to be very helpful in order to assign the dielectric processes to particular molecular modes in the protein–water system.

2. Materials and methods

2.1. Sample preparation

Elastin from bovine neck ligament in form of powder (Sigma E1625) was purchased from Sigma-Aldrich and used as received. Water with 10 μS/cm conductivity was used in the sample hydration procedure.

DSC, TSDC and DRS measurements, to be described in the following, were performed at various levels of water content h, calculated on the dry and the wet basis, h_d and h_w, respectively, by

$$h_d = \frac{m_{water}}{m_{dry}} \tag{1}$$

$$h_w = \frac{m_{water}}{m}. \tag{2}$$

In these equations m is the mass of the hydrated protein sample, m_{dry} the mass of the dry sample and $m_{water} = m - m_{dry}$ the mass of water inside the sample. The dry mass m_{dry} was determined by drying the protein sample in vacuum for 72 h at room temperature.

Both h values are used throughout the paper, depending on the method of data analysis. The term water content is used for h_d, while the term water fraction is used for h_w.

They are related to each other by

$$h_d = \frac{h_w}{1-h_w}. \tag{3}$$

2.2. Water equilibrium sorption isotherms

For water ESI measurements solid samples were used, prepared by compressing an amount of protein powder ~ 100 mg to a cylindrical pellet of thickness 0.6–0.8 mm and diameter of about 14 mm. ESI measurements were performed at room temperature by exposing the samples to various controlled water vapor atmospheres in sealed jars above saturated aqueous salt solutions [46]. By this way, the water activity α_w (relative humidity, rh) was systematically varied between about 0.20 and 0.97. The attainment of equilibrium and final weights was determined via continuous monitoring of sample weight using a Bosch SAE 200 balance with 10^{-4} g sensitivity.