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Hydration dynamics of collagen in aqueous buffer solution as studied by time domain dielectric spectroscopy

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ABSTRACT

Dielectric relaxation studies of collagen in aqueous buffer solution (pH 3.7) have been carried out at four different temperatures 283, 288, 293 and 298 K as a function of collagen concentration between 0.033 μM and 0.2 μM in the frequency range of 1 GHz to 25 GHz using time domain dielectric spectroscopy technique. Apart from the dominant mode at high frequency around 14 GHz which is assigned to the free water relaxation; we have detected one more pronounced peak close to 1.8 GHz in the δ-dispersion range of the loss spectrum of collagen in aqueous buffer solution. The peak around 1.8 GHz is attributed to hydration water reorientation of the collagen macromolecules and has obtained detailed information on its temperature and concentration dependence. The retardation imposed on the hydration water by collagen is found to be ≈4.7, since collagen can cause long range perturbations beyond the first hydration shell. The activation enthalpy and activation entropy for the dipolar orientation for collagen at different concentrations have been calculated from the Arrhenius plot and is found to be 32 kJ/mol and 4.2 J/mol K respectively. The increase of thermodynamic activation enthalpy and decrease of activation entropy of collagen in buffer in the present study compared to the water have supported the idea that the water associated with collagen is highly ordered.

1. Introduction

Collagen is the most abundant connective tissue protein in multicellular animals including mankind and plays a vital role in maintaining the structural integrity and architecture of several organs. Nature has deployed collagen as the major structural protein to support the growth of a wide variety of human tissues and its metabolism is directly associated with many physiological process of biological adaptations and tissue regeneration. The collagen molecule is a rod about 300 nm long and 1.5 nm in diameter, made up of three polypeptide subunits wrapped around one another in a triple helix. A distinctive feature of collagen is the repeat of three amino acid subunits along the polypeptide chain Gly-X-Y, where X is frequently Proline and Y is hydroxyproline, which accounts for the tendency of three polypeptide strands to form a triple helix, with glycine in the interior of the helix, and the rings of proline and hydroxyproline stacked and pointing outward. It is stabilized by direct inter-chain hydrogen bonds and inter and intra chain water mediated hydrogen bonds [1,2].

Water forms an integral part of collagen molecule and bound water is presented as highly ordered hydration network [3–6]. Crystal structures of model collagen systems with water have been extensively studied *via* X ray diffraction measurements and thermal analysis [7,8]. It has been suggested that the collagen triple-helix structure binds one or several water molecules, the so-called "bound water" per amino acid residue. It has also been reported that such bound water contributes to the stability of the triple-helix structure [8]. A detailed analysis of the hydration structure of the collagen like peptide was presented by Bella et al. [9]. High-resolution X-ray diffraction studies on both native and synthetic collagens showed an extensive water bridge network surrounding the collagen molecule. These and other structural studies [10–12] have provided a molecular model of collagen with at least two categories of water. The most tightly bound consists of one highly immobilized water bridge for every three amino acid residues. A second less immobilized water fraction consists of three additional water molecules per tripeptide unit, residing in the three groove-like depressions between the peptide chains of the triple helix. Fullerton and Maxwell Amurao [13] produced detailed calculations on tendon diameter at different degrees of dehydration and showed that the remaining water on native collagen is in the first monolayer covering the entire surface of the collagen molecule, and not, as others have suggested, in multiple layers. This category of water is less immobilized than the first two categories, but still restricted in motion relative to free water.

Dielectric spectroscopy is a potential tool in study of protein-water interactions. The intimate relationship between the protein surface and hydration water can be analyzed by studying experimental water

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properties measured in protein systems in solution [14–16]. In particular, proteins in solution modify the structure and the dynamics of the free water at the solute-solvent interface. The solution structure of collagen with a large number of polar groups is expected to carry signatures of interactions of water with various polar residues of the protein [17]. In the present work, the dielectric relaxation of collagen in aqueous buffer solution below the denaturation temperature has been examined using time domain dielectric spectroscopy in the GHz regime to understand the subtle aspects of protein-water interactions.

2. Materials and methods

The dielectric spectra of collagen in aqueous buffer solution were obtained by the time domain reflectometry technique. A digital serial analyzer sampling mainframe (Tektronix, model no. DSA8200) along with the sampling module (model 80E08) has been used for the time domain reflectometer. Fig. 1 shows experimental setup for time domain reflectometer (TDR). A repetitive fast rising 250 mV voltage pulse of 200 kHz with 18 ps incident rise time was fed through coaxial line system of impedance 50 Ω. The co-axial cable semi-rigid, copper, EZ_86/M17 (Huber + Suhner Electronics Pvt. Ltd.) with flat end was used. The detailed analysis and procedure for time domain reflectometer system has been explained previously [18,19]. All measurements were carried out in open load condition. Sampling oscilloscope monitors changes in step pulse after reflection from the end of line. Reflected pulses without sample R_1 (t) and with sample R_x (t) were recorded in time window of 5 ns and digitized in 2000 points. These pulses are added [q (t) = R_1 (t) + R_x (t)] and subtracted [p (t) = R_1 (t) − R_x (t)]. Further the time domain data have been converted to frequency domain using Fourier transformation. Shannon [20] and Samulon [21] methods were used to obtain the Fourier transformation of p(t) and q(t) respectively, up to 25 GHz. The frequency dependent complex reflection coefficient spectra $\rho^*(\omega)$ were calculated using the following equation:

$$\rho^*(\omega) = \left(\frac{c}{j\omega d}\right)\left[\frac{p(\omega)}{q(\omega)}\right]$$

where $p(\omega)$ and $q(\omega)$ are Fourier transforms of p(t) and q (t) respectively, ω is the angular frequency, d is the effective pin length, c is the velocity of light and $j = \sqrt{-1}$. Complex permittivity spectra $\varepsilon^*(\omega)$ were obtained from reflection coefficient spectra $\rho^*(\omega)$ by applying the bilinear calibration method [18,22]. The conductivity of the sample was computed using the expression [22].

$$\sigma = \frac{c\varepsilon}{d}\left[\frac{R_1(t) - R_x(t)}{R_1(t) + R_x(t)}\right]$$

where R_1 (t) and R_x (t) are values of reflected pulses at time t = ∞ without and with sample respectively, and ε is the permittivity of free space. The statistical average of 15 points were taken to determine the base line values of R_1 (t) and R_x (t) at t = ∞.

Rat tail tendon (Type 1) collagen used was obtained from Sigma-Aldrich Chemical Co. Ltd. and used without any further purification. The average molecular weight of collagen was 300 kDa, on the basis of which the molar concentration was determined. The HPLC grade double distilled and deionized water was used in the preparation of acetic acid – sodium acetate buffer solution. The temperature of sample under test has been maintained using calibrated temperature controller system with accuracy of ±0.15 K. A reference spectrum containing weak acetate buffer (pH 3.7) was also recorded along with the spectrum of pure water and shown in Fig. 2. The dielectric spectrum of weak acetate buffer solution is exactly similar in nature as that of the pure water spectrum except with downshift in the γ relaxation frequency by 2 GHz and reduction in permittivity. Further reduction of 2 GHz in the γ relaxation frequency is noticed for collagen in buffer and appeared at around 14 GHz. Dielectric measurements for collagen in buffer were repeated two times and the results obtained are the same for each measurement.

3. Results and discussion

The complex dielectric permittivity spectra of collagen in acetate buffer solution (pH 3.7) covering the frequency range from 1 GHz to 25 GHz at four temperatures (283, 288, 293 and 298 K) were recorded using time domain reflectometer. Dielectric permittivity (ε′) and loss (ε″) spectra as a function of Log frequency (GHz) for collagen in acetate buffer solution at different concentrations measured at 298 K is shown in the Fig. 4. Dielectric permittivity (ε′) and loss (ε″) spectra (ε″) as a function of Log frequency (GHz) for 0.2 μM collagen in acetate buffer solution measured at different temperatures is shown in Fig. 6. In general, dielectric spectra of aqueous protein solutions show at least three dispersion regions [23–26], revealing the typical signatures of relaxation processes, namely a step in the dielectric constant (ε′) and a peak in the dielectric loss (ε″). In the biophysics community, they are often termed as β, δ and γ relaxation (Fig. 3). The β relaxation in the low

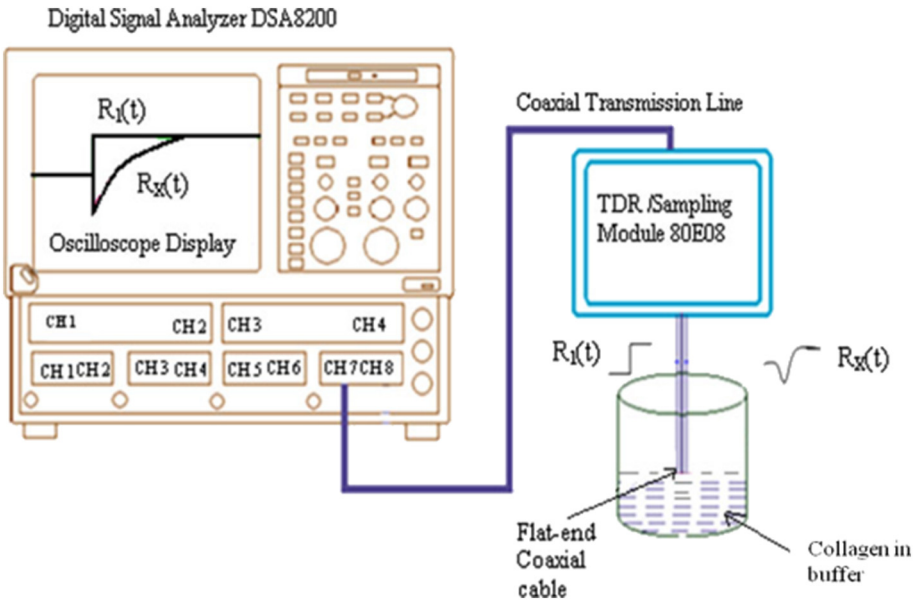

Fig. 1. Experimental setup of time domain reflectometry.

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