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Broadband acoustic spectra of aqueous solutions of human serum albumin

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Abstract

Between 0.2 and 2000 MHz, sonic absorption spectra of solutions of albumin in water and in aqueous phosphate buffer systems have been measured at different temperatures (15–35 °C), pH values (1.8–12.3), and protein concentrations (0.2–4% w/w). Broad absorption spectra, indicating the presence of various relaxation processes, have been found. Depending on the albumin concentration, the spectra can be analytically represented by a sum of the asymptotic high-frequency contribution and up to four Debye-type relaxation terms. Other relaxation models, including one based on a continuous distribution of relaxation times, also appropriately describe the measured spectra. Low-frequency relaxation processes with relaxation times between about 30 and 300 ns appear to be due to cooperative conformational changes of the polypeptide chain. High-frequency processes with relaxation times between 0.3 and 3 ns seem to reflect elementary reactions of the albumin molecule that are associated with changes in the hydration properties.

Proteins are the most abundant biomolecules in living cells. They are fundamental in all aspects of cell structure and function and thus hold a key position in the molecular logic of life. Therefore, the kinetics and molecular dynamics of protein systems are among the most challenging topics in the life sciences. Additional demands for a better understanding of the behaviour of proteins as a function of external parameters arise from biomolecule-based technologies in food production, pharmaceutical industries, and various further applications. Despite the interest in protein systems, our knowledge of their microdynamics is rather empirical. This is particularly true for human serum albumin (HSA) which, due to its conformational flexibility, is able to reversibly bind many ligands and to serve as a major transport protein in blood. We thus found it interesting to perform a study of the broadband ultrasonic relaxation of human serum albumin solutions as a function of protein concentration, pH, and temperature.

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Ultrasonic spectrometry is a powerful method for studying elementary processes in liquids [1]. It has already been applied in investigations into the properties of HSA as well as bovine serum albumin solutions [2–5]. In view of the multiple molecular mechanisms contributing to the acoustic absorption spectra of protein systems, most of the former measurements were conducted over too small a frequency band. Choi *et al* [4] have performed broadband measurements, revealing relaxations associated with conformational transitions of the protein and also with proton-transfer reactions. These researchers proposed a novel relaxation time distribution function to some extent analogous to functions describing precritical and critical demixing in liquids. Interestingly, critical phenomena have in fact been observed in protein–water solutions [6, 7].

Here we applied resonator methods at frequencies $f < 15$ MHz, utilizing a plano-concave and a biplanar cell, and a pulse-modulated wave transmission method at $f > 3$ MHz, employing three different variable-path-length cells to cover the frequency range from 200 kHz to 2 GHz [8,9]. The experimental error in the attenuation coefficient (α) data was $\Delta\alpha/\alpha = 0.07$ at $f < 3$ MHz and $\Delta\alpha/\alpha = 0.02$ at $f > 3$ MHz. Albumin from SIGMA (Deisenhofen, FRG), claimed to be 96% pure, was used as delivered. Analytical grade chemicals (FLUKA, Deisenhofen; MERCK, Darmstadt, FRG) were used for the buffer solutions. In figure 1, as an example, two ultrasonic attenuation spectra are displayed in the format α/f^2 versus f for albumin solutions at different pH values. For both solutions the α/f^2 data monotonically decrease with f to asymptotically reach a high-frequency value B'. Despite the rather small difference in pH, the spectra significantly deviate from one another at low frequencies. Also shown in figure 1 in the excess attenuation $\times$ wavelength spectrum for the albumin–water solutions at almost neutral pH (=6.8). The excess attenuation $\times$ wavelength is defined by $(\alpha\lambda)_{exc} = \alpha\lambda - c_s B' f$, where c_s denotes the sound velocity and $\lambda = c_s/f$. For purposes of comparison, the $(\alpha\lambda)_{exc}$ data for the buffer solution used in the measurement at pH = 7.4 are presented additionally in figure 1. The phosphate buffer displays Debye-type relaxation with discrete relaxation time in the upper part of the frequency range. Hence contributions from the buffer cannot cause the difference in the low-frequency part of the albumin solution spectra.

The excess attenuation spectra of the albumin solutions extend over a much broader frequency range than the Debye relaxation term of the buffer. This broadness of the $(\alpha\lambda)_{exc}$ spectra is characteristic of all solutions studied so far. Similarly broad shapes of albumin solution spectra have been reported previously [4]. It is illustrated in figure 1 that the excess attenuation spectra of HSA solutions, within the frequency range of the measurements, may be analytically represented by a sum of (up to) four Debye terms. Applying such a relaxation model, the attenuation $\times$ wavelength is given by

$$\alpha\lambda = \sum_{i=1}^{4} \frac{A_i \omega\tau_i}{1 + (\omega\tau_i)^2} + Bf \tag{1}$$

where the A_i and τ_i, $i = 1, \ldots, 4$ are relaxation amplitudes and relaxation times, respectively, $\omega = 2\pi f$, and $B = B' c_s$. However, various other relaxation spectral functions are likewise suitable for describing the measured spectra within the limits of experimental error. Among these models is a superposition of two semi-empirical Cole–Cole spectral terms [10], each of which is based on a symmetrical continuous relaxation time distribution:

$$\alpha\lambda = \frac{A_a \phi_a \cos\varphi_a}{1 + 2\phi_a \sin\varphi_a + \phi_a^2} + \frac{A_b \phi_b \cos\varphi_b}{1 + 2\phi_b \sin\varphi_b + \phi_b^2} + Bf. \tag{2}$$

Herein A_x, $x = a, b$, denote the relaxation amplitudes, $\phi_x = (\omega\tau_x)^{1-h_x}$, and $\varphi_x = 0.5\pi h_x$. The τ_x are the principal relaxation times and the h_x are parameters that control the widths of the underlying relaxation time distribution functions.

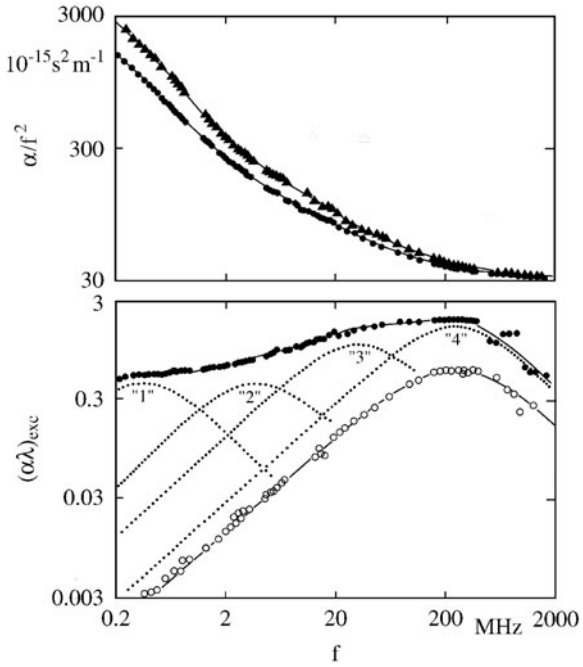

Figure 1. Ultrasonic absorption spectra in the frequency-normalized format for albumin (4% w/w) in water (●; pH = 6.8) and in a phosphate buffer solution (▲; pH = 7.4) at 15.6 °C. Also shown as a function of frequency f is the excess absorption × wavelength (bottom) for the albumin/water system (●) and for the phosphate buffer (○; 0.01 mol l^{-1} NaH_2PO_4 + 0.09 mol l^{-1} Na_2HPO_4, 15.6 °C). The latter spectrum displays a Debye-type relaxation term. Dotted curves indicate the decomposition of the albumin solution spectrum into four Debye terms.

Relaxation functions which are based on models of precritical [11] and critical [12] order parameter fluctuations have also been considered. Though some of these functions fit nicely to the shape of the spectra, unreasonable parameter values have been obtained from the fitting procedures. Further ultrasonic relaxation spectra and additional information from other specific experimental methods are required to enable definite conclusions to be drawn regarding the particular characteristics of the relaxation model of HSA solutions. Nevertheless, some interesting aspects of different relaxation regions can already be extracted from the measured spectra. For simplicity, let us consider a four-Debye-term model (figure 1).

The relaxation times for terms '1' and '2' are of the order of 0.4 μs and 40 ns and are almost independent of temperature T. The independence of T is usually attributed to cooperative segmental motions of the protein chains [13]. Our spectra at different pH values between 1.8 and 12.3 reveal these conformational changes of the HSA molecule to be controlled by the hydronium-ion concentration of the protein solution.

The relaxation time for term '3' is of the order of some nanoseconds. It exhibits normal Arrhenius behaviour but is almost independent of protein concentration c and of the pH of the solution as well. The corresponding relaxation amplitude A_3 varies monotonically with c and the pH. These relaxation properties suggest that term '3' also reflects an intramolecular mechanism, probably a side-group rotation, coupling to the sonic field via hydration-water-density changes. The relaxation time τ_4 of the high-frequency relaxation term is of the order of 600 ps for solutions of 4% (w/w) HSA and 200–300 ps for albumin systems of distinctly lower albumin content (0.2% w/w). This unusual concentration dependence of τ_4 suggests a

relaxation due to small segments in albumin subdomains which might be hindered at a high protein concentration.

Our first spectroscopic measurements of albumin systems show the high potential of ultrasonic spectrometry for revealing different modes of conformational changes and rotational isomerizations of proteins in solution. Additional measurements are in progress that should enable us to more clearly relate the relaxation regimes in the ultrasonic spectra to underlying molecular mechanisms.

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