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Determination of the glass-transition temperature of proteins from a viscometric approach

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ABSTRACT

All fully hydrated proteins undergo a distinct change in their dynamical properties at glass-transition temperature T_g. To determine indirectly this temperature for dry albumins, the viscosity measurements of aqueous solutions of human, equine, ovine, porcine and rabbit serum albumin have been conducted at a wide range of concentrations and at temperatures ranging from 278 K to 318 K. Viscosity–temperature dependence of the solutions is discussed on the basis of the three parameters equation resulting from Avramov's model. One of the parameter in the Avramov's equation is the glass-transition temperature. For all studied albumins, T_g of a solution monotonically increases with increasing concentration. The glass-transition temperature of a solution depends both on T_g for a dissolved dry protein $T_{g,p}$ and water $T_{g,w}$. To obtain $T_{g,p}$ for each studied albumin the modified Gordon–Taylor equation was applied. This equation describes the dependence of T_g of a solution on concentration, and $T_{g,p}$ and a parameter depending on the strength of the protein–solvent interaction are the fitting parameters. Thus determined the glass-transition temperature for the studied dry albumins is in the range (215.4–245.5) K.

1. Introduction

The temperature at which the properties of material change from liquid-like to solid-like is called glass-transition temperature T_g. In this temperature the viscosity exceeds a value of 10^{13} poise (or 10^{12} Ns/m^2). The glass-transition behavior of synthetic polymers and molecular liquids is well known. Recently, the glass-transition behavior of biopolymers, including proteins and polysaccharides, has received increased attention [1–16]. Most of globular proteins have an ordered three-dimensional flexible structure. This structural, or conformational flexibility is a key component in proteins functions. The functions of proteins like for instance ligand binding, require some flexibility because all interactions lead to at least small rearrangements of atoms and, in consequence, to conformational changes. The energy of the protein is changed with change of its conformation, and like in glasses this can be described by the conformational energy landscape. According to the concept developed by Frauenfelder [17], a protein molecule can assume a very large number of nearly isoenergetic conformational substates, valleys in the protein energy landscape. Protein dynamics involve transitions between its conformational substates. As temperature decreases these transitions become increasingly slower, and at a certain temperature the protein becomes frozen in a specific substate. The freezing of transitions between different conformational substates involves a change in the thermal energy of the protein, and consequently, a change in the heat capacity. As a result, the protein undergoes a glass transition. In the vicinity of glass transition temperature the sharp change in temperature dependence of various physical properties, such as heat capacity, density, elastic modulus, etc. is observed. This allows experimental determination of T_g. The glass transition temperature in hydrated proteins has mainly been estimated by calorimetric and rheological measurements [10,15]. However, it is difficult to measure glass transition temperature experimentally for globular proteins in the dry state. In the present paper T_g for several dry mammalian serum albumins has been obtained from viscosity measurements of aqueous solutions of the albumins, the Avramov's model and the modified Gordon–Taylor equation.

Mammalian serum albumins are moderately large proteins, with nearly identical molecular mass of about 66.5 kDa [18]. Their primary structure is constituted by a single polypeptide chain of about 580 amino-acid residues. Albumins from different mammals exhibit high amino-acid sequence identity with each other [19]. However detail determination of amino-acid sequences for several of them showed some differences [19]. The differences in amino-acid sequences, in turn, cause some differences in the three-dimensional structure of the albumins and considerable differences in their physicochemical properties in solution. It was

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demonstrated by different experimental techniques such as dielectric spectroscopy [20], liquid chromatography [21], electrophoresis [22], viscometry [23–25], calorimetry and fluorescence anisotropy [26,27], circular dichroism [28] or fluorescence spectroscopy and modeling [29].

The study presents the results of viscosity measurements on aqueous solutions of human serum albumin (HSA), equine serum albumin (ESA), ovine serum albumin (OSA), porcine serum albumin (PSA) and rabbit serum albumin (RSA) at temperatures ranging from 278 K to 318 K and over a wide range of concentrations. For each albumin the viscosity-temperature dependence, for a fixed concentration, is analyzed on the basis of equation resulting from the Avramov's model [30]. One of the parameter in this equation is the glass-transition temperature. It appears that the glass-transition temperature of a solution, for each studied albumin, increases with increasing concentration. To establish the glass-transition temperature of the studied dry albumins, in turn, a modified Gordon–Taylor formulae is applied.

2. Materials and methods

The following products of the Sigma (USA) were used in the study: HSA at pH 7.0 (A 1653), ESA (A 9888), OSA (A 3264), PSA (A 2764) and RSA (A 0639). HSA at pH 4.7 was purchased from Polish Chemical Reagents factories. Albumins were used without further purification for all measurements. Aqueous solutions were prepared by dissolving the crystallized albumins in distilled water. To remove possible undissolved fragments the solutions were treated with filter papers. The samples were cooled in a refrigerator (up to 277 K) until just prior to viscometry measurements, when they were warmed from 278 K to 318 K. The pH values of thus prepared solutions were as follows: 7.0 or 4.7 for HSA, 7.4 for ESA, 7.05 for OSA, 6.6 for PSA and 7.0 for RSA. The isoelectric point pI of the studied albumins is: (4.7–4.95) for HSA, (4.65–4.9) for ESA, (4.6–4.9) for OSA, (4.6–4.9) for PSA and (4.6–5.3) for RSA [22]. The pH values of the solutions changed slightly in the whole range of concentrations. The above given values are the average pH.

The viscosity measurements of albumins solutions were conducted by using an Ubbelohde-type capillary microviscometer with a flow time for water of 28.5 s at 298 K. It was placed in a water-bath controlled thermostatically with a precision of ±0.1 K and was mounted so that it always occupied the same position in the bath. Flow times were recorded to within 0.1 s. The microviscometer was calibrated using cooled boiled distilled water and the same microviscometer was used for all measurements. Measurements started after a few minutes delay to ensure that the system reached equilibrium. For each concentration, the solution was passed once through the microviscometer before any measurements were made. For most concentrations the viscosity measurements were taken from 278 K to 318 K mainly by steps of 5 K. At temperatures slightly higher than 318 K the thermal denaturation of the studied albumins occurs and the lower the protein concentration the higher the denaturation temperature.

The viscosity of the studied albumins is discussed here in the mono-disperse range, i.e. from 8.2 kg/m^3 up to 369 kg/m^3 for HSA at pH 7.0, from 9.5 kg/m^3 up to 328 kg/m^3 for HSA at pH 4.7, from 13 kg/m^3 up to 367 kg/m^3 for ESA, from 36 kg/m^3 up to 317 kg/m^3 for OSA, from 34 kg/m^3 up to 195 kg/m^3 for PSA and from 14 kg/m^3 up to 300 kg/m^3 for RSA. For higher concentrations the aggregations of albumins occur and solutions become poly-disperse. The problem is discussed in detail elsewhere [23,24]. The above concentration ranges may be expressed by a suitable range of weight fractions as follows: from 0.0082 up to 0.34 for HSA at pH 7.0, from 0.0096 up to 0.306 for HSA at pH 4.7, from 0.0128 up to 0.333 for ESA, from 0.0359 up to 0.293 for OSA, from 0.0341 up to 0.185 for PSA and from 0.0138 up to 0.277 for RSA. Solution densities were measured by weighing. Albumin concentrations were determined using a dry weight method in which the samples were dried at high temperature for several hours.

3. Results and discussion

One of the models of viscous flow for glass-forming systems is the Avramov's model [30]. According to the model molecules in a flowing liquid jump from the holes formed by the nearest neighbors to one of the adjoining holes. During the jumps the molecule has to overcome some energy barrier which, in general, is different for different molecules. The frequency of the jumps decreases exponentially with increasing the energy barrier. The assumption that the jumps frequency follows a Poisson distribution allows calculation of the average jump frequency. It depends on a dispersity and a maximum value of the energy barrier. Moreover, in the model one assumes that viscosity of the liquid is inversely proportional to the average frequency of these jumps. As a final result the temperature dependence of liquid viscosity is obtained. For solutions, when viscosity depends both on temperature and concentration, this dependence can be written in the following way:

$$\eta(c,T) = \eta_\infty(c)\ \exp\left[\frac{\Theta(c)}{T}\right]^{\alpha(c)} \quad (1)$$

where $\eta_\infty(c)$, $\Theta(c)$ and $\alpha(c)$ are concentration dependent parameters.

To fit the viscosity from the Avramov's relation to the experimental values of viscosity, the numerical values of the parameters $\eta_\infty(c)$, $\Theta(c)$ and $\alpha(c)$ are needed. The calculations of these parameters were conducted by applying a non-linear regression procedure in the computational statistical program. Fig. 1 shows the results of viscosity measurements for HSA at pH 4.7, ESA and OSA aqueous solutions for high concentrations. The curve shows the fit to the experimental points according to relation (1), with the parameters obtained by the mentioned above method. As seen this function gives very good fit over the whole range of measured temperatures. For the smaller concentrations the situation is the same. This is also the case for the other albumins discussed here. In Avramov's relation (1) the parameter $\Theta(c) = T_g(c)\varepsilon^{1/\alpha(c)}$. The parameter $T_g(c)$ denotes the glass transition temperature for a solution and the quantity ε means the ratio of the activation energy corresponding to its value at the maximum of the probability distribution function to a dispersity of the activation energy.

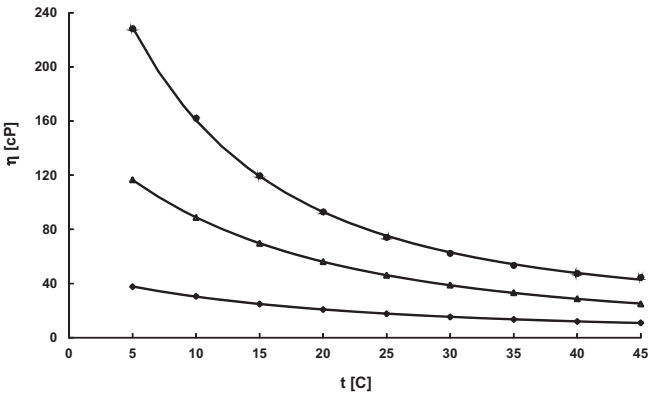

Fig. 1. Temperature dependence of the viscosity of HSA at pH 4.7 (•), ESA (△) and OSA (♦) aqueous solutions for concentrations: c = 328, 367 and 317 kg/m^3, respectively. The curves show the fit obtained by using Eq. (1) with the parameters: $\eta_\infty(c)$ = 2135 cP, $\Theta(c)$ = 3058 K and $\alpha(c)$ = 9118 for HSA; $\eta_\infty(c)$ = 5761 cP, $\Theta(c)$ = 3424 K and $\alpha(c)$ = 5302 for ESA; $\eta_\infty(c)$ = 318 cP, $\Theta(c)$ = 3308 K and $\alpha(c)$ = 5233 for OSA.