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A comparison of solution conformation and hydrodynamic properties of equine, porcine and rabbit serum albumin using viscometric measurements

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Abstract

This paper presents the results of viscosity determinations on aqueous solutions of equine, porcine and rabbit serum albumin over a wide range of concentrations and at temperatures ranging from 5 °C to (42–45) °C. The results are compared with human and bovine serum albumin previously studied. Viscosity–temperature dependence is discussed on the basis of the modified Arrhenius formula. The effective specific volume, the activation energy and entropy of viscous flow for all investigated albumins are compared. Viscosity–concentration dependence, in turn, is discussed on the basis of Mooney equation. Based on the assumption that theoretical and experimental values of Simha factor – at high temperature limit – are equal to each other, the hydrodynamic volume of the studied albumins has been calculated. The numerical values of a self-crowding factor were also obtained. At low concentration limit, the numerical values of the intrinsic viscosity and of Huggins coefficient were compared.

Keywords: Albumin; Activation energy; Effective specific volume; Hydrodynamic volume; Intrinsic viscosity; Huggins coefficient

1. Introduction

For understanding of many physiological phenomena, a knowledge about hydrodynamic properties and conformation of native proteins, in particular serum albumin, is of fundamental importance. Albumin is present in different tissues like gut, liver, muscle or skin, and about 30% of the total albumin in the whole animal body is present in the serum [1]. As the major protein in the circulatory system in vertebrates (it represents more than 50% of total protein in serum) it plays an important role, mainly as a multipurpose transport molecule and as a principal contributor to colloid osmotic blood pressure. The amino-acid sequences determined for a number of mammalian albumins show that they have internal sequence homology suggesting the proteins evolved from a common proto-albumin of about 190 amino acids and molecular mass 22 kDa (Refs.[2,3] and references therein). Mammalian serum albumins are moderately large proteins, with primary structure constituted by a single polypeptide chain of about 580 amino-acid residues and molecular mass M_p=66.5 kDa [4]. The albumins from different species exhibit high amino-acid sequence identity with each other. For instance, the equine serum albumin (ESA) molecule exhibits the highest sequence identity with human serum albumin (HSA) (76.1%) followed by porcine serum albumin (PSA) (76%), ovine serum albumin (OSA) (75.5%) and bovine serum albumin (BSA) (73.9%) [3]. The HSA, in turn, has 76% of sequence identity with BSA [5]. These similarities of amino-acid sequence among mammalian albumins lead to the expectation of the common (but not identical) overall shape or topology of them. Unfortunately, detailed investigations on the three-dimensional structure of albumins have been performed by X-ray crystallography only for a few species, including HSA, BSA and ESA [2,3,5,6]. For instance, HSA in the crystalline state is a heart-shaped molecule [2] and is highly similar to ESA; despite differences in amino-acid

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sequence, both the tertiary and quaternary structures of HSA and ESA are nearly identical [3].

In solution the environment of albumins is different than in the crystalline state and their conformation may change. Usually, a conformation of the experimentally studied albumins is then approximated by an ellipsoid of revolution with one long semi-axis (a) and two shorter semi-axes (b) [7–17]. However, it is worth to emphasize that for most species the conformation of albumin in solution is poorly known. The determination of the length of main axes for ESA, PSA and RSA in aqueous solution is one of the goals of this paper. Despite similarities in amino-acid sequences and in the three-dimensional structure of albumins from various mammals, their physicochemical properties in solution are quite different. It was proved by different experimental techniques including dielectric spectroscopy [18], liquid chromatography [19], electrophoresis [20], calorimetry and steady-state fluorescence anisotropy [21], circular dichroism and viscosity [22], fluorescence spectroscopy and modelling [23]. However, as far as I know, there is no paper in which the comparison of hydrodynamic properties of albumins from different species would be made on the basis of viscometric measurements. Only for BSA and HSA could the comprehensive data be found in the literature [13,17].

In the present study, the results of viscosity determination on aqueous solutions of ESA, PSA and RSA at a wide range of concentrations and temperatures are presented. Based on these results the viscosity–temperature and viscosity–concentration relationships are discussed. From a modified Arrhenius formula, such rheological quantities as activation energy and entropy of viscous flow, and the effective specific volume for all investigated albumins are calculated. On the basis of Mooney equation, in turn, viscosity–concentration dependence is discussed. From a comparison of experimental and theoretical values of the Simha factor, at the high temperature limit, the volume of hydrated molecule for each investigated albumin is obtained. A comparison of the self-crowding factor K of studied albumins is also made. At low concentrations, the temperature dependence of the intrinsic viscosity and of Huggins coefficient is presented.

2. Materials and methods

The following products of the Sigma Chemical Co. were used in this study: ESA (A 9888), PSA (A 2764) and RSA (A 0639). The material was used without further purification for all the measurements. From the crystalline form the material was dissolved in distilled water and then filtered by means of filter papers in order to remove possible undissolved fragments. The samples were stored in a refrigerator until just prior to viscometry measurements, when they were warmed from 5 to 45 °C for ESA and RSA, and from 5 to (42–45) °C for PSA, mainly by steps of 5 °C. The pH values of such prepared samples were outside of their isoelectric point and were as follows: 7.4 for ESA, 6.6 for PSA and 7.0 for RSA. These values changed only slightly in the whole range of concentrations. The isoelectric point of the studied albumins is: (4.65–4.9) for ESA, (4.6–4.9) for PSA and (4.6–5.3) for RSA [20]. It is worth noting that serum albumins in solution, in the vicinity of neutral pH, have stable conformation [3].

The viscosity measurements were performed using an Ubbelohde-type capillary microviscometer placed in a water-bath controlled thermostatically with a precision of ±0.1 °C. The details of the method are described elsewhere [17]. The upper limit of temperature for which the viscosity measurements were made has been established by the temperature of denaturation. Above the temperature of denaturation the albumins show a highly pronounced tendency to aggregate, but this heat-induced aggregation reverses upon cooling. For the temperatures above the temperature of denaturation viscosity of the albumins solutions increases with increasing temperature. However, as appears the temperature of denaturation changes with concentration of the solution, and the lower albumin concentration the higher denaturation temperature. For ESA and RSA the temperature of denaturation is only slightly higher than 45 °C. This is also the case for PSA up to the concentration of about 227 kg/m^3, and for the higher concentrations up to 386 kg/m^3 the temperature of denaturation gradually decreases up to 42 °C. The highest temperature at which the denaturation does not yet occur we will call the high temperature limit.

The viscosity was measured up to the high temperature limit over a wide range of concentrations: from 12.9 up to 391 kg/m^3 for ESA, from 34.3 up to 386 kg/m^3 for PSA and from 13.9 up to 370 kg/m^3 for RSA. However, hydrodynamic parameters for all studied albumins were obtained and discussed only in a narrower range of concentrations: from low concentrations up to 367 kg/m^3 for ESA, up to 195 kg/m^3 for PSA and up to 300 kg/m^3 for RSA. This is because for higher concentrations the aggregations of albumins occur and solutions are not then mono-disperse. The presence of aggregates has been revealed by the calculation of the axial ratio ($p=a/b$) of studied albumins. As has been shown many years ago by Polson [24], the relative viscosity – for a given concentration – depends on the axial ratio of dissolved proteins. His results, limited only to very low concentrations, were later extended up to the high concentrations [25]. Based on this method, the following axial ratios have been obtained (in the narrower range of concentrations given above, i.e. in the mono-disperse range): 3.15 for ESA, 3.11 for PSA and 3.25 for RSA. For the higher concentrations, the axial ratio of studied albumins increases and this indicates that the albumins create ensembles formed by “end to end” aggregations of two monomer molecules. The additional argument for aggregation of albumins in this range of concentrations is given below. It is worth to note that the problem of aggregation at highly concentrated solutions of HSA has been previously investigated by using the small-angle neutron scattering and Monte Carlo simulation [12].