Determination of Some Hydrodynamic Parameters of Ovine Serum Albumin Solutions Using Viscometric Measurements

KAROL MONKOS

Department of Biophysics, Silesian Medical Academy, H. Jordana 19, 41-808 Zabrze 8, Poland

Abstract. The influence of protein concentration and temperature on the viscosity of ovine serum albumin (OSA) solutions was studied. The Mooney equation and a modified Arrhenius formula were used to describe the viscosity-concentration and viscosity-temperature dependence of the solutions, respectively. The effective specific volume, the activation energy and entropy of viscous flow for hydrated OSA were calculated. The axial ratio and the dimensions of the main semi-axes of hydrated OSA were established. At low concentration limit, the temperature dependence of the intrinsic viscosity and Huggins coefficient is presented. Comparison of some hydrodynamic parameters obtained for different proteins has been made.

Key words: viscosity, hydrodynamic parameters, ovine serum albumin

1. Introduction

Albumins are major proteins in the mammalian circulatory system, fulfilling several physiological functions mainly as transporting molecules and as principal contributors to colloid osmotic blood pressure. In blood plasma they account for about 60% of the total proteins corresponding to a concentration of 42 kg/m^3 [1]. They are perhaps the most frequently studied proteins in diverse biophysical and biochemical investigations. Mammalian albumins are moderately large proteins, with molecular mass $M_p = 66.5 \text{ kDa}$ [2]. Determination of amino-acid sequences for several of them showed a common topology and – on the other hand – some differences ([3] and references therein). The differences in amino-acid sequences, in turn, cause some differences in the three-dimensional structure of albumins from various mammals and considerable differences in their physicochemical properties [4, 5]. Unfortunately, detailed investigations on the three-dimensional structure of albumins have been performed only for a few species, including human serum albumin (HSA), bovine serum albumin (BSA) and equine serum albumin (ESA) [3, 6]. For most species, the three-dimensional structure of albumins and – in particular – their conformations in solution is poorly known.

Viscometry still plays an important role in investigations of both conformations and hydrodynamic properties of proteins in solution. The paper presents the results of viscosity measurements for ovine serum albumin (OSA) at temperatures ranging from 5–45 °C and at a wide range of concentrations. On the basis of the modified Arrhenius formula the viscosity-temperature relationship is discussed and the activation energy and entropy of viscous flow for OSA is obtained. The effective specific volume of OSA is also calculated. The modified Arrhenius formula gives, in a natural way, the Mooney equation which describes the viscosity-concentration dependence of a solution. At the same time, it shows the way of calculation of the coefficient S and the self-crowding factor K in the Mooney equation. The dimensions of OSA – approximated as an ellipsoid of revolution with one long axis ($2a$) and two shorter axes ($2b$) – are estimated. At low concentrations, the intrinsic viscosity and the Huggins coefficient – for all measured temperatures – were calculated. Comparison of some hydrodynamic parameters of OSA and previously studied proteins are made.

2. Material and Methods

OSA was purchased from Sigma Chemical Company (A-3264) and was used without further purification. According to the manufacturer's specification this OSA product is not defatted. However, defatting is reported to decrease the stability of albumin and to increase the risk for formation of dimers [7]. From the crystalline form the material was dissolved in distilled water and the solution was filtered by means of filter papers in order to remove possible undissolved fragments. The samples were stored at 4 °C until just prior to viscometry measurements, when they were warmed from 5 °C to 45 °C. The pH values of such prepared solutions changed only insignificantly with the mean value 7.05.

Viscosity measurements in this study were performed with an Ubbelohde glass capillary microviscometer immersed in a water bath controlled thermostatically at $(5-45) \pm 0.1$ °C. The measurements were made in 5 °C intervals. Such a range of temperatures was chosen because slightly above 45 °C flow times (and viscosities) of the OSA solutions increase with increasing temperature and this indicates that thermal denaturation of OSA begins. As has been shown the temperature of denaturation changes with concentration; the lower the protein concentration the higher denaturation temperature. The viscosities of the OSA solutions were measured over a wide range of concentrations, up to 393 kg/m³. However, hydrodynamic parameters for OSA were obtained and discussed on the basis of measurements of viscosity for concentrations only up to about 320 kg/m³. This is because for higher concentrations aggregations of OSA occur and solutions are not then monodisperse. The presence of aggregations has been revealed by the calculation of the axial ratio ($p = a/b$) of OSA using the method described previously [8]. As shown, the mean value and

Table I. The numerical values of the OSA solutions viscosity (in centipoises) for all measured concentrations

c [kg/m ³]	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	45 °C
36.5	1.90	1.65	1.42	1.25	1.10	0.990	0.893	0.803	0.739
49.8	2.03	1.75	1.52	1.33	1.18	1.05	0.946	0.856	0.784
72.9	2.35	2.02	1.74	1.53	1.36	1.21	1.09	0.989	0.895
83.2	2.55	2.17	1.88	1.65	1.46	1.30	1.17	1.06	0.966
84.2	2.53	2.17	1.88	1.64	1.46	1.29	1.16	1.06	0.966
101	2.77	2.37	2.05	1.79	1.58	1.41	1.27	1.15	1.04
112	3.02	2.59	2.23	1.96	1.72	1.54	1.38	1.25	1.14
158	4.42	3.75	3.23	2.79	2.44	2.18	1.96	1.76	1.61
174	4.97	4.21	3.61	3.13	2.74	2.44	2.18	1.97	1.81
187	5.59	4.73	4.03	3.49	3.05	2.72	2.42	2.19	2.01
198	6.45	5.42	4.62	4.00	3.48	3.09	2.76	2.49	2.27
243	11.3	9.43	7.96	6.83	5.92	5.22	4.65	4.19	3.85
258	13.4	11.1	9.35	7.99	6.91	6.07	5.39	4.84	4.42
259	13.5	11.2	9.37	8.04	6.94	6.10	5.41	4.86	4.45
270	15.9	13.1	10.9	9.34	8.04	7.05	6.26	5.63	5.14
282	18.9	15.5	12.9	10.9	9.39	8.20	7.24	6.47	5.91
297	24.9	20.2	16.7	14.1	12.1	10.5	9.27	8.30	7.59
317	37.7	30.6	24.8	20.8	17.7	15.3	13.5	12.0	11.0
333	53.0	41.9	33.9	28.1	23.6	20.2	17.6	15.6	14.1
337	60.9	48.4	38.8	32.2	27.0	23.1	19.9	17.7	15.9
348	74.7	58.4	46.7	38.3	32.0	27.2	23.5	20.7	18.8
349	74.8	58.5	46.9	38.7	32.4	27.6	24.0	21.2	19.1
361	107	81.8	65.2	52.9	43.8	37.1	31.8	27.8	25.1
368	134	103	81.0	65.6	53.9	45.4	38.8	33.8	30.3
378	177	135	106	83.9	69.3	58.0	49.3	42.6	37.4
385	254	191	146	116	93.1	77.4	65.4	55.9	49.8
393	314	225	175	137	111	91.4	76.4	65.1	57.5

standard deviation of p – calculated for concentrations up to 320 kg/m³ – are as follows: $p = 3.06 \pm 0.11$. At higher concentrations the axial ratio for OSA increases and this indicates that proteins create aggregates of two monomer molecules. This will be also discussed later. Solution densities and protein concentrations were measured by weighing, as described earlier [8]. The experimental data of solution viscosities for all measured concentrations and temperatures are presented in Table I.

3. Results and Discussion

Viscosity-temperature dependence for biological macromolecules solutions may be quantitatively described both by the Vogel-Tammann-Fulcher equation and by the modified Arrhenius formula [9]. In each case different parameters for a studied system can be obtained. In the present paper, the discussion of the viscosity-temperature relationship of OSA solutions is limited only to the modified Arrhenius formula. It was quite recently successfully applied to several proteins [10–14], and has the form:

$$\eta = \exp \left(-\frac{\Delta S_s}{R} + D_s T + \frac{\Delta E_s}{RT} \right) \quad (1)$$

where D_s is a parameter, and ΔS_s and ΔE_s are the entropy and activation energy of viscous flow of solution and R , T are the gas constant and the absolute temperature, respectively. The activation energy of viscous flow can be interpreted as the minimum energy required for a molecule of the solution to jump from one equilibrium position in the liquid to the next. To fit the above formula to the experimental values of η and T , the numerical values of ΔS_s , D_s and ΔE_s are needed. For all concentrations, they were calculated by using the least squares method, as described previously [10]. In particular, for water the parameters are as follows: $\Delta S_w = 242 \text{ J/mol K}$, $D_w = 2.55 \times 10^{-2} \text{ K}^{-1}$ and $\Delta E_w = 35.8 \text{ kJ/mol}$. Figure 1 shows the results of such calculations for all measured concentrations. As seen, ΔE_s

Figure 1. Plot of the solution activation energy ΔE_s versus concentration. Experimental points (•) were obtained by using the least squares method; the curve shows the fit according to relation (2) with the parameters given in the text.

is a monotonically increasing function of concentration. This may be explained by assuming that the activation energy ΔE_s is a superposition of the activation energy of water ΔE_w and protein ΔE_p molecules. The detailed analysis has shown that this leads to the relation [10]:

$$\Delta E_s = \frac{c}{\alpha - \beta c}(\Delta E_p - \Delta E_w) + \Delta E_w \quad (2)$$

where $\alpha = \rho_w M_p / M_w$ and $\beta = \alpha \xi - 1$. The quantities c , ρ_w , ξ , M_p and M_w denote the solute concentration and water density in kg/m^3 , the effective specific volume of a protein and the molecular masses of the dissolved proteins and water, respectively. The effective specific volume is the constant of proportionality between the effective molar volume and the molar mass of a macrosolute.

Proteins in solution are surrounded by water, whose structure differs from that of bulk water, and which stabilizes structure of proteins and stimulates the activity of the active site. This hydration shell must be taken into account when the mass of the hydrodynamic particle is calculated. The molecular mass of hydrated protein is $M_h = M_p(1 + \delta)$, where δ denotes the amount of grams of water associated with the protein per gram of protein. For globular proteins a value of (0.3–0.4) has been obtained from different methods [15] and, in particular, for albumin $\delta = 0.35$ [16]. It gives the hydrodynamic mass of OSA $M_h = 89.775 \text{ kDa}$. So, in Eq. (2) we should replace M_p by M_h and then $\alpha = 4.988 \times 10^6 \text{ kg/m}^3$. The only unknown quantities in the Eq. (2) are ΔE_p and ξ , and these were taken as two adjustable parameters. By using the least squares method the following values were obtained: $\Delta E_p = 2.13 \times 10^5 \text{ kJ/mol}$ and $\xi = 2.06 \times 10^{-3} \text{ m}^3/\text{kg}$. As seen in Figure 1, Eq. (2) then gives a good approximation to the experimental values, but only up to a concentration of about 320 kg/m^3 .

The values of two other parameters are presented in Figure 2. As seen, both ΔS_s and D_s monotonically increase with increasing concentration and they can be described by the identical equations as ΔE_s , but ΔE_p and ΔE_w should be replaced by $(\Delta S_p, \Delta S_w)$ and (D_p, D_w) , respectively [10]. In these relations $(\Delta S_p, \xi)$ and (D_p, ξ) are adjustable parameters. The best fit to the experimental values, for $\Delta S_p = 1.13 \times 10^6 \text{ J/mol K}$, $\xi = 2.12 \times 10^{-3} \text{ m}^3/\text{kg}$ and $D_p = 245 \text{ K}^{-1}$, $\xi = 2.07 \times 10^{-3} \text{ m}^3/\text{kg}$, was obtained and the results are presented in Figure 2. As for ΔE_s , the above mentioned equations give good approximations to the experimental values of ΔS_s and D_s only for concentrations up to about 320 kg/m^3 (the results for higher concentrations are not shown in Figure 2). Because relations connecting the parameters ΔE_s , ΔS_s and D_s with concentration have been obtained for monodisperse systems, the above results suggest that for concentrations higher than 320 kg/m^3 , the aggregation of OSA cannot be neglected in the interpretation of the viscosity data. It is worth to note that the problem of aggregation at highly concentrated solutions of HSA has been investigated by using small-angle neutron scattering and Monte Carlo simulation [17]. The authors proved that up to

Figure 2. Plots of the coefficients ΔS_s (x) and D_s (●) versus concentration. Experimental points were obtained by using the least squares method; the curves show fits according to relations analogous to relation (2).

the concentration of at least 260 kg/m^3 the whole data set could be explained by a model of monomeric HSA. The analysis of viscosity data, in turn, suggests that HSA solutions are monodisperse up to at least 369 kg/m^3 [14].

The relative viscosity η_r is defined as the ratio of viscosity of the solution and the solvent. For proteins in aqueous solutions, its dependence on concentration is often described by the equation introduced by Mooney [18]:

$$\eta_r = \exp \left[\frac{S\Phi}{1 - K\Phi} \right] \quad (3)$$

where Φ denotes the volume fraction of the dissolved proteins and S and K are the Simha and the self-crowding factors, respectively. The volume fraction $\Phi = N_A V_h c / M_h$ and N_A is Avogadro's number. It is interesting to note that Mooney's equation can be obtained in a natural way from the modified Arrhenius formula, if we insert into it the concentration dependence of ΔE_s (Eq. 2), ΔS_s and D_s . This leads to an equation identical with Mooney's formula, with the Simha factor [10]:

$$S = \frac{M_w}{\rho_w N_A V_h} \left[- \left(\frac{\Delta S_p - \Delta S_w}{R} \right) + (D_p - D_w)T + \frac{\Delta E_p - \Delta E_w}{RT} \right] \quad (4)$$

and the self-crowding factor

$$K = \left(\xi - \frac{M_w}{\rho_w M_h} \right) \frac{M_h}{N_A V_h}. \quad (5)$$

As seen, the numerical values of S can be obtained only when the hydrodynamic volume V_h of the dissolved proteins is known. Unfortunately, V_h for OSA is unknown. However, for several proteins such calculations were made [10, 11, 13, 14] and it was shown that the Simha factor decreases with increasing temperature. At low temperatures, the rotational Brownian motion of proteins – produced as a reaction to the random heat motions of the solvent – is negligible, and all particles should be aligned almost parallel to the direction of flow. Because globular proteins have an unusually large dipole moment [19], the dipole-dipole interactions between the proteins then create additional attractive forces between the flowing planes of the liquid, and the viscosity (as well as S) is high. As the temperature increases, the average angle between the neighbouring dipoles increases. As a result, the dipole-dipole interaction forces decrease and the viscosity also decreases.

Simha [20] showed that at temperatures when Brownian motion prevails and the orientation of dissolved proteins is completely random, the factor S (which will be denoted as S_{Sim}) depends on their shape. The exact formula for S_{Sim} – obtained by Simha for tri-axial ellipsoids – contains elliptic integrals which can be soluble numerically. However, for prolate ellipsoids of revolution with the axial ratio p , the relation between S_{Sim} and p can be describe with great precision by the asymptotic formula [15]:

$$p = -10.71584 + 2.79158 S_{\text{Sim}} + 1.622009 S_{\text{Sim}}^2 + 0.01556169 S_{\text{Sim}}^3 - 0.192997 S_{\text{Sim}}^4 + 0.02060718 S_{\text{Sim}}^5 \quad (6)$$

for $1.1 \leq p < 2$, and

$$p = -3.80413 + 2.8712 S_{\text{Sim}} - 0.3908319 S_{\text{Sim}}^2 + 0.03612282 S_{\text{Sim}}^3 - 0.001733981 S_{\text{Sim}}^4 + 0.0000332711 S_{\text{Sim}}^5 \quad (7)$$

for $2 \leq p < 10$.

For the mean value of p obtained for OSA ($p = 3.06$) the Eq. (7) gives $S_{\text{Sim}} = 3.76$. The experimental values of S have been previously obtained for hydrated ovalbumin [13] and HSA [14], and for unhydrated BSA [10], lysozyme [11] and human IgG immunoglobulin [21]. If the hydration shell around the proteins is taken into account, small corrections in calculations are needed. The values of S for ovalbumin and HSA, and corrected values for BSA, lysozyme and human IgG immunoglobulin obtained from relation (4) in the high temperature limit (i.e. in the vicinity of denaturation temperature), and denoted as S_{exp} are presented in

Table II. Values of the axial ratio p , the theoretical values of Simha factor obtained from relations (6) and (7) S_{Sim} , the experimental values of Simha factor obtained in the high temperature limit S_{exp} , and pH of the measured solutions with respect to their isoelectric point pI , for previously studied proteins

Protein	p	S_{Sim}	S_{exp}	pH	pI
HSA	3.95 [22]	4.60	4.52	7.0	4.70–4.95 [4]
Ovalbumin	2.74 [22]	3.48	3.44	6.4	4.59–4.71 [22]
Lysozyme	1.5 [23]	2.64	2.58	7.0	11.0–11.2 [22]
Human IgG	5.34 [22]	6.22	7.51	5.6	5.80–6.60 [22]
BSA	3.5 [24]	4.16	4.94	5.2	4.70–5.40 [4,25]

Table II. As seen, the values of S_{exp} and S_{Sim} are very similar for HSA, ovalbumin and lysozyme i.e. for proteins for which the viscosity measurements were made for solutions at pH values far from the isoelectric point. For human IgG immunoglobulin and BSA, in turn, the viscosity measurements were made for solutions at pH in the vicinity of the isoelectric point and, as seen in Table II, S_{exp} is significantly higher than S_{Sim} . So, we can state that $S_{\text{exp}} \geq S_{\text{Sim}}$. Taking this into account, one can assess – from Eq. (4) – the value or the upper limit of the hydrodynamic volume of dissolved proteins:

$$V_h \leq \frac{M_w}{\rho_w N_A S_{\text{Sim}}} \left[- \left(\frac{\Delta S_p - \Delta S_w}{R} \right) + (D_p - D_w) T_h + \frac{\Delta E_p - \Delta E_w}{R T_h} \right] \quad (8)$$

where T_h is a temperature in the high temperature limit. In the above relation the equality is obtained for solutions far from the isoelectric point and the inequality is for solutions at (or in the vicinity of) the isoelectric point. The isoelectric point for OSA is in the range of (4.6–4.9) [4]. So, for the OSA solutions studied here, pH is far from the isoelectric point. Using $T_h = 318.16$ K and the numerical values of the parameters obtained earlier, the above relation gives for OSA: $V_h = 182 \text{ nm}^3$. Because – for the prolate ellipsoid of revolution – $V_h = (4/3)\pi ab^2$ and $p = a/b$, then $V_h = (4/3)\pi pb^3$. It gives the values of the main semi-axes of the hydrated OSA molecule: $a = 7.4 \text{ nm}$ and $b = 2.4 \text{ nm}$. It is worthwhile to note that quite recently the effective semi-axes of hydrated HSA: $a = 8.2 \text{ nm}$ and $b = 2.1 \text{ nm}$ [14] have been obtained. These similarities in the overall shape of mammalian albumins is a reflection of their high amino-acid sequence identity. For example, the ESA molecule exhibits the highest sequence identity with HSA (76.1%) followed by porcine serum albumin (76%), OSA (75.5%) and BSA (73.9%) [3].

The hydrodynamic volume, in turn, enables calculation of the self-crowding factor K from Eq. (5). Taking into account the values of the effective specific volume and the molecular mass of hydrated OSA obtained earlier, we get $K = 1.7$. A comparison of the self-crowding factor for some earlier studied proteins is given below.

At low concentrations, i.e. in the region of dilute solutions, the properties of solutions are determined by the behaviour of an individual protein. The relation between the solution viscosity and the concentration may be then expressed by the following polynomial [26]:

$$\frac{\eta_{sp}}{c} = [\eta]\{1 + k_1[\eta]c + k_2[\eta]^2c^2 + \dots\} \quad (9)$$

where $[\eta] = \lim_{c \rightarrow 0} \eta_{sp}/c$ is the intrinsic viscosity and the specific viscosity $\eta_{sp} = \eta_r - 1$. The dimensionless parameters k_1, k_2 are the coefficients of expansion, and k_1 is called the Huggins coefficient. In our case, the formula (3) can be expanded in the power series of the concentration. An expansion identical to that in Eq. (9) can be obtained from:

$$[\eta] = \frac{1}{\alpha} \left[- \left(\frac{\Delta S_p - \Delta S_w}{R} \right) + (D_p - D_w)T + \frac{\Delta E_p - \Delta E_w}{RT} \right] \quad (10)$$

$$k_1 = \frac{1}{2} \left\{ \frac{2\beta}{-(\Delta S_p - \Delta S_w)/R + (D_p - D_w)T + (\Delta E_p - \Delta E_w)/RT} + 1 \right\}. \quad (11)$$

The above parameters are usually obtained from the plot of η_{sp}/c versus concentration, by linear extrapolation of the experimental results to the zero concentration; the intercept is then equal to $[\eta]$ and k_1 is obtained from the corresponding slope. However, even for small concentrations the dependence of η_{sp}/c versus concentration is not perfectly linear, so the linear extrapolation can give a serious error in $[\eta]$ and k_1 [26]. The alternative method presented above was earlier successfully applied to several proteins [10–14]. It is worth noting that the numerical values of the intrinsic viscosity calculated from relation (10) at $t = 25^\circ\text{C}$ ($[\eta] = 2.66 \times 10^{-3} \text{ m}^3/\text{kg}$ for lysozyme [11] and $[\eta] = 3.86 \times 10^{-3} \text{ m}^3/\text{kg}$ for ovalbumin [13]) agree very well with the values obtained in the traditional way at the same temperature ($[\eta] = 2.7 \times 10^{-3} \text{ m}^3/\text{kg}$ for lysozyme and $[\eta] = 3.9 \times 10^{-3} \text{ m}^3/\text{kg}$ for ovalbumin [27]). Moreover, the values of $[\eta]$ obtained for some mammalian IgG immunoglobulins [12] lie within the range of $[\eta] = (6\text{--}14) \times 10^{-3} \text{ m}^3/\text{kg}$ reported in the literature [22]. The numerical values of $[\eta]$ and k_1 for OSA are presented in Table III. As seen, $[\eta]$ slowly decreases and k_1 increases with increasing temperature. Because the intrinsic viscosity should be independent of temperature only for perfectly rigid macromolecules, our results indicate that this is not the case for OSA.

The Huggins coefficient represents the quantitative measure of the intermolecular interactions. Because the hydrodynamic interactions for different liquids and solutions are different, the numerical values of k_1 published in the literature [10–14, 26] vary widely. As is seen in Table III, for OSA k_1 increases monotonically with increasing temperature. This is the case for all globular proteins studied earlier [10–14]. The higher coefficient of expansion k_2, k_3 and so on in Eq. (9), are connected with the Huggins coefficient [11] and are omitted here. The experimental values of

Table III. The numerical values of the intrinsic viscosity $[\eta]$ and the Huggins coefficient k_1 for OSA calculated from Eqs (10) and (11), respectively

$t[^\circ\text{C}]$	$[\eta] \times 10^3 [\text{m}^3/\text{kg}]$	k_1
5	4.95 ± 0.12	0.921
10	4.87 ± 0.12	0.928
15	4.80 ± 0.12	0.934
20	4.74 ± 0.12	0.939
25	4.69 ± 0.12	0.944
30	4.65 ± 0.11	0.948
35	4.62 ± 0.11	0.951
40	4.60 ± 0.11	0.953
45	4.59 ± 0.11	0.954

these coefficients are highly desirable because they provide a test for any theoretical treatment of dilute solution viscosity.

4. Comparison of Some Hydrodynamic Parameters for Different proteins

It is interesting to compare the hydrodynamic parameters calculated for OSA with those obtained earlier for human IgG immunoglobulin [12, 21], HSA [14], BSA [10], ovalbumin [13] and lysozyme [11]. Such parameters for hydrated proteins are presented in Table IV.

The activation energy of viscous flow is given per 1 mol (in kJ/mol) and per 1 molecule (in J). It is worthwhile to compare the activation energy with the mean energy $\langle E \rangle$ of translational heat motion of proteins. This energy is independent of molecular mass and at $t = 25^\circ\text{C}$ (the mean temperature of viscosity measurements for albumins) is equal to $\langle E \rangle = 6.17 \times 10^{-21} \text{ J}$. The activation energy for water molecules is $\Delta E_w = 5.94 \times 10^{-20} \text{ J}$, i.e. only one order of magnitude higher than $\langle E \rangle$. Meanwhile, the activation energy of presented proteins is higher than $\langle E \rangle$ by 4 (for lysozyme) and even by 6 orders of magnitude (for human IgG). The higher activation energy in comparison with $\langle E \rangle$, the lesser probability of gathering this energy by the molecule. So, the activation energy (and viscosity of a solution) strongly depends on the molecular mass of dissolved proteins. However, the values of ΔE_p obtained for OSA, HSA and BSA (which have nearly identical molecular masses) show that this factor is not the only one which influences ΔE_p . The activation energies for OSA and HSA were obtained at neutral pH and have similar values. Measurements for BSA, in turn, were made at the isoelectric point (Table II) and the activation energy is here significantly higher than for OSA and HSA.

Table IV. Activation energy of viscous flow ΔE_p , effective specific volume ξ , specific volume ν , self-crowding factor K , Simha factor S , intrinsic viscosity $[\eta]$ and Huggins coefficient k_1 for some previously studied proteins

	Human IgG	HSA	BSA	Ovalbumin	Lysozyme
ΔE_p [kJ/mol]	1.19×10^6	1.86×10^5	5.96×10^5	8.49×10^4	5.34×10^4
ΔE_p [J]	1.98×10^{-15}	3.09×10^{-16}	9.90×10^{-16}	1.41×10^{-16}	8.87×10^{-17}
ξ [m ³ /kg]	2.25×10^{-3}	2.15×10^{-3}	1.62×10^{-3}	1.95×10^{-3}	2.60×10^{-3}
ν [m ³ /kg]	0.95×10^{-3}	1.02×10^{-3}	1.05×10^{-3}	1.08×10^{-3}	0.92×10^{-3}
K	2.37	2.10	1.55	1.81	2.82
S	$t = 5^\circ\text{C}$	10.0	4.81	5.73	3.78
	$t = 20^\circ\text{C}$	9.07	4.64	5.21	3.62
	$t = 40^\circ\text{C}$	8.07	4.53	4.95	3.49
	$t = 5^\circ\text{C}$	9.59×10^{-3}	4.90×10^{-3}	6.49×10^{-3}	4.07×10^{-3}
$[\eta]$ [m ³ /kg]	$t = 20^\circ\text{C}$	8.67×10^{-3}	4.73×10^{-3}	5.90×10^{-3}	3.90×10^{-3}
	$t = 40^\circ\text{C}$	7.75×10^{-3}	4.62×10^{-3}	5.60×10^{-3}	3.75×10^{-3}
	$t = 5^\circ\text{C}$	0.715	0.938	0.718	0.979
	$t = 20^\circ\text{C}$	0.738	0.954	0.740	1.00
k_1	$t = 40^\circ\text{C}$	0.766	0.964	0.753	1.02
					1.54

The total electrostatic interactions between two proteins can be qualitatively described as a combination of repulsive interactions between net charges of the proteins and attractive dipole-dipole interaction between their dipole moments. In the case of proteins discussed here the dipole moments are as follows: 121 D for lysozyme [28], 250 D for ovalbumin [29], 700 D for HSA [30], 384 D for BSA [30] and 1200 D for immunoglobulin [31]. At pH values outside of the isoelectric point, the Coulomb repulsion partially balances the dipole-dipole interaction. At pH values in the vicinity of (or at) the isoelectric point, in turn, the net charges on the proteins are small (or equal to zero) and the attractive dipole-dipole interaction dominates. This is the case for BSA, and this shows that for proteins of similar molecular masses the activation energy is mainly influenced by the strength of protein-protein interaction. The differences in the activation energy for OSA and HSA suggest that some protein-solvent interactions and differences in the overall structure or proteins may be also important. The above results suggest that – for a given protein – the activation energy reaches a maximum value at the isoelectric point.

As is seen from Eq. (5), the self-crowding factor K does not depend on temperature. However, it is worthwhile to remark that for all proteins discussed here the following inequality occurs: $\xi \gg M_w/\rho_w M_h$. It means that – in a good approximation – the self-crowding factor may be presented in the form $K = \xi/\nu$, where $\nu = N_A V_h/M_h$ is the specific volume of hydrated protein. As is seen in Table IV, ν is very similar for all proteins discussed. Differences among the values of the self-crowding factor is caused mainly by the differences between the specific

volumes of the effective specific volume of the proteins (Table IV). The results obtained for the studied albumins suggest that – for a given protein – ξ reaches a minimum value at the isoelectric point. Some theoretical support of this, on the basis of the analysis of virial expansion for the hard sphere model of BSA, has been previously given [32]. In this case the authors obtained the effective specific volume in the range from $0.8 \times 10^{-3} \text{ m}^3/\text{kg}$ (at pH 5.1) up to $1.7 \times 10^{-3} \text{ m}^3/\text{kg}$ (at pH 7.6). However, comparison of these values with those obtained experimentally for albumins discussed here shows that the hard sphere model for serum albumin molecules is not a good one. Mooney showed, on the basis of purely geometric arguments, that for rigid spherical particles, values of K should lie between 1.35 and 1.91. For the proteins discussed here, only for OSA, BSA and ovalbumin do the values of K lie within this range. There is no estimations of K for aspherical particles, but the presented experimental values (Table IV) show that in this case the values of K should lie in a wider range than for spherical particles.

The numerical values of the Simha factor decrease with increasing temperature (Table IV). However, for each studied protein those values – for a given temperature – are quite different. In particular, they are different in the high temperature limit (Table II). Accordance between the values of the Simha factor experimentally established in the high temperature limit (S_{exp}) and calculated on the basis of the Simha model (S_{sim}), for solutions of proteins at pH not at the isoelectric point, shows that the repulsive Coulomb forces sufficiently compensate the attractive forces from the dipole-dipole interaction, the resultant of these forces is so weak that the rotational heat motion of proteins destroys the effect of the orientation of the principal axes of the proteins to the flow direction, and the conditions of applicability of the Simha model are fulfilled. Contrary to this, when the pH of a solution is at (or in the vicinity of) the isoelectric point, the net charges of proteins are equal (or nearly equal) to zero, and the dipole-dipole interaction dominates. In this case, even in the high temperature limit, the rotational Brownian motion of proteins only partially destroys the ordering of the principal axes of proteins and, as for human IgG and BSA (Table II), $S_{\text{exp}} > S_{\text{sim}}$.

The intrinsic viscosity measures the contribution of a protein to the viscosity of a solution in which it is dissolved. This quantity is strongly dependent on the molecular mass of the protein (Table IV). Moreover, the intrinsic viscosity depends on the pH of the solution [22]. The obtained values of $[\eta]$ – for a given temperature – for OSA (Table III) and HSA (Table IV) are nearly identical, but they are substantially lesser than those for BSA (Table IV). These results suggest that – for a given protein – the intrinsic viscosity reaches a maximum value at the isoelectric point.

Contrary to $[\eta]$, there is no correlation between the Huggins coefficient and molecular mass of dissolved proteins. Despite differences in molecular masses, quite similar values of k_1 – for a given temperature – for human IgG and BSA (both studied at the isoelectric point), and for HSA (or OSA) and ovalbumin have been obtained. The Huggins coefficients for OSA and HSA – for a given temperature – are nearly identical and they are substantially greater than k_1 for BSA. The

obtained results suggest that – for a given protein – the Huggins coefficient reaches a minimum value at the isoelectric point.

5. Conclusions

The viscosity of OSA aqueous solutions at temperatures ranging from 5 to 45 °C – at the vicinity of neutral pH – may be quantitatively described by a modified Arrhenius formula (1). Analysis of the dependence of the three parameters in this formula on concentration allows, among others things, calculation of the activation energy ($\Delta E_p = 2.13 \times 10^5$ kJ/mol) and entropy ($\Delta S_p = 1.13 \times 10^6$ J/mol K) of viscous flow for hydrated OSA. The effective specific volume of OSA ($\langle \xi \rangle = 2.08 \times 10^{-3}$ m³/kg) has also been obtained. The axial ratio of OSA – modeled as a prolate ellipsoid of revolution – ($p = 3.06 \pm 0.11$) was calculated and the main semi-axes ($a = 7.4$ nm and $b = 2.4$ nm) were estimated. The asymptotic form of Mooney's equation for small concentrations enables calculation of the intrinsic viscosity (Eq. (10)) and the Huggins coefficient (Eq. (11)). Both quantities depend on temperature: $[\eta]$ decreases and k_1 increases with increasing temperature. The above conclusions are the results of analysis of viscosity data for OSA solutions up to the concentration of about 320 kg/m³. For the higher concentrations: (i) the calculated axial ratio of OSA molecules increases with increasing concentration, and (ii) the values of ΔE_s , ΔS_s and D_s obtained from relation (2) and analogous relations for ΔS_s and D_s do not fit to the experimental points, suggesting that oligomers of OSA molecules in solution arise at higher concentrations.

References

1. Gelamo, E.L., Silva, C.H.T.P., Imasato, H. and Tabak, M.: Interaction of Bovine (BSA) and Human (HSA) Serum Albumin With Ionic Surfactants: Spectroscopy and Modeling, *Biochem. Biophys. Acta* **1594** (2002), 84–99.
2. Dockal, M., Carter, D.C. and Rüker, F.: The Three Recombinant Domains of Human Serum Albumin, *J. Biol. Chem.* **274** (1999), 29303–29310.
3. Ho, J.X., Holowachuk, E.W., Norton, E.J., Twigg, P.D. and Carter, D.C.: X-ray and Primary Structure of Horse Serum Albumin (*Equus caballus*) at 0.27-nm Resolution, *Eur. J. Biochem.* **215** (1993), 205–212.
4. Miller, I. and Gemeiner, M.: An Electrophoretic Study on Interactions of Albumins of Different Species With Immobilized Cibacron Blue F3G A, *Electrophoresis* **19** (1998), 2506–2514.
5. Dimitrova, M.N., Matsumura, H., Dimitrova, A. and Neitchev, V.Z.: Interaction of Albumins From Different Species With Phospholipids Liposomes, *Int. J. Biol. Macromol.* **27** (2000), 187–194.
6. He, X.M. and Carter, D.C.: Atomic Structure and Chemistry of Human Serum Albumin, *Nature* **358** (1992), 209–215.
7. Peters, T.: *Advances in Protein Chemistry*, Vol. 37, Academic Press, New York, 1985, pp. 161–245.
8. Monkos, K. and Turczynski, B.: Determination of the Axial Ratio of Globular Proteins in Aqueous Solution Using Viscometric Measurements, *Int. J. Biol. Macromol.* **13** (1991), 341–344.
9. Vinogradov, G.V. and Malkin, A.Ya.: *Rheology of Polymers*, Mir publishers, Moscow, 1980.

10. Monkos, K.: Viscosity of Bovine Serum Albumin Aqueous Solutions as a Function of Temperature and Concentration, *Int. J. Biol. Macromol.* **18** (1996), 61–68.
11. Monkos, K.: Concentration and Temperature Dependence of Viscosity in Lysozyme Aqueous Solutions, *Biochim. Biophys. Acta* **1339** (1997), 304–310.
12. Monkos, K. and Turczynski, B.: A Comparative Study on Viscosity of Human, Bovine and Pig IgG Immunoglobulins in Aqueous Solutions, *Int. J. Biol. Macromol.* **26** (1999), 155–159.
13. Monkos, K.: Viscosity Analysis of the Temperature Dependence of the Solution Conformation of Ovalbumin, *Biophys. Chem.* **85** (2000), 7–16.
14. Monkos, K.: On the Hydrodynamics and Temperature Dependence of the Solution Conformation of Human Serum Albumin From Viscometry Approach, *Biochim. Biophys. Acta* **1700** (2004), 27–34.
15. Harding, S.E.: The Intrinsic Viscosity of Biological Macromolecules. Progres in Measurements, Interpretation and Application to Structure in Dilute Solution, *Prog. Biophys. Mol. Biol.* **68** (1997), 207–262.
16. Squire, P.G. and Himmel, M.E.: Hydrodynamics and Protein Hydration, *Arch. Biochem. Biophys.* **196** (1979), 165–177.
17. Sjöberg, B. and Mortensen, K.: Interparticle Interactions and Structure in Nonideal Solutions of Human Serum Albumin Studied by Small-Angle Neutron Scattering and Monte Carlo Simulation, *Biophys. Chem.* **52** (1994), 131–138.
18. Mooney, M.J.: The Viscosity of a Concentrated Suspension of Spherical Particles, *J. Colloid. Sci.* **6** (1951), 162–170.
19. Antosiewicz, J.: Computation of the Dipole Moments of Proteins, *Biophys. J.* **69** (1995), 1344–1354.
20. Simha, R.: The influence of Brownian Movement on the Viscosity of Solutions, *J. Phys. Chem.* **44** (1940), 25–34.
21. Monkos, K. and Turczynski, B.: Viscosity-Temperature Dependence for Human Gamma-Globulin Aqueous Solutions at a Wide Range of Concentrations, *Curr. Top. Biophys.* **21** (1997), 113–116.
22. Young, E.G.: Occurrence, Classification, Preparation and Analysis of Proteins, in M. Florkin and E.H. Stotz (eds.) *Comprehensive Biochemistry*, Elsevier, Amsterdam, 1963, pp.1–53.
23. Squire, P.G. and Himmel, M.E.: Hydrodynamics and Protein Hydration, *Arch. Biochem. Biophys.* **196** (1979), 165–177.
24. Nesmelova, I.V., Skirda, V.D. and Fedotov, V.D.: Generalized Concentration Dependence of Globular Protein Self-Diffusion Coefficients in Aqueous Solutions, *Biopolymers* **63** (2002), 132–140.
25. Kanal, K.M., Fullerton, G.D. and Cameron, I.L.: A Study of the Molecular Sources of Nonideal Osmotic Pressure of Bovine Serum Albumin Solutions as a Function of pH, *Biophys. J.* **66** (1994), 153–160.
26. Bohdanecky, M. and Kovar, J.: *Viscosity of Polymer Solutions*, Elsevier, Amsterdam, 1982.
27. Lefebvre, J.: Viscosity of Concentrated Protein Solutions, *Rheol. Acta* **21** (1982), 620–625.
28. Takashima, S.: The Structure and Dipole Moment of Globular Proteins in Solution and Crystalline States: Use of NMR and X-ray Databases for the Numerical Calculation of Dipole Moment, *Biopolymers* **58** (2001), 398–409.
29. Tanford, C.: *Physical Chemistry of Macromolecules*, Wiley, New York, 1961.
30. Moser, P., Squire, P.G. and O’Konski, C.T.: Electric Polarization in Proteins – Dielectric Dispersion and Kerr Effect. Studies of Isoionic Bovine Serum Albumin, *J. Phys. Chem.* **70** (1966), 744–756.
31. Oncley, J.L.: Electric Moments and Relaxation Times of Protein Molecules, *J. Phys. Chem.* **44** (1940), 1103–1113.
32. Zimmerman, S.B. and Minton, A.P.: Macromolecular Crowding: Biochemical, Biophysical, and Physiological Consequences, *Annu. Rev. Biophys. Biomol. Struct.* **22** (1993), 27–65.