PRACA ORYGINALNA

A comparative study on translational diffusion coefficient for some mammalian serum albumins

Porównanie współczynnika dyfuzji translacyjnej dla albumin surowicy kilku ssaków

Karol Monkos

ABSTRACT

BACKGROUND

The aim of the present paper is investigation of the volume fraction dependence of the translational diffusion coefficient for some mammalian serum albumins in aqueous solutions.

MATERIAL AND METHODS

The viscosity of bovine, equine, ovine and rabbit serum albumin aqueous solutions was measured at temperatures ranging from 5°C to 45°C and in a wide range of concentrations. The measurements were performed with an Ubbelohde-type capillary microviscometer.

RESULTS

Translational diffusion coefficient at infinitely dilute solutions $D_o(T)$ can be calculated from generalized Stokes-Einstein equation if the hydrodynamic radius of albumin is known. It gives $D_o(T)$ in the range from 3.5×10^{-11} m^2/s (at 5°C) to 10.210^{-11} m^2/s (at 45°C) for bovine serum albumin, from 3.59×10^{-11} m^2/s (at 5°C) to 10.410^{-11} m^2/s (at 45°C) for equine serum albumin, from 3.42×10^{-11} m^2/s (at 5°C) to 9.92×10^{-11} m^2/s (at 45°C) for ovine serum albumin, and from 3.36×10^{-11} m^2/s (at 5°C) to 9.74×10^{-11} m^2/s (at 45°C) for rabbit serum albumin. Translational diffusion coefficient for higher concentrations $D(T,\Phi)$ can be obtained from the relation: $D(T,\Phi) = D_o(T)\eta_o(T)/\eta(T,\Phi)$, where Φ denotes volume fraction and $\eta_o(T)$ and $\eta(T,\Phi)$ are viscosities of water and solution, respectively, at temperature T.

CONCLUSIONS

The obtained results show that the translational diffusion coefficient decreases linearly with increasing volume fraction, when Φ does not exceed the value of about 0.1. The dependence of the translational diffusion coef-

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ficient on volume fraction in a broader range of Φ, i.e. from dilute to concentrated solutions, is nonlinear and can be described by a stretched exponential function.

KEY WORDS
translational diffusion coefficient, viscosity, Stokes-Einstein equation, serum albumin

STRESZCZENIE

WSTĘP
Celem niniejszej pracy jest zbadanie zależności współczynnika dyfuzji translacyjnej od ułamka objętościowego dla albumin surowicy kilku ssaków w roztworach wodnych.

MATERIAŁ I METODY
Lepkość wodnych roztworów albuminy surowicy wołowej, końskiej, owczej i króliczej zmierzono w zakresie temperatur od 5°C do 45°C i w szerokim zakresie stężeń. Pomiary wykonano przy pomocy kapilarnego mikrowiskozymetru typu Ubbelohde'a.

WYNIKI
Współczynnik dyfuzji translacyjnej dla roztworów rozcieńczonych $D_o(T)$ można obliczyć z uogólnionego równania Stokesa-Einsteina, jeżeli znany jest promień hydrodynamiczny albuminy. Daje to $D_o(T)$ w zakresie od 3.5×10^{-11} m^2/s (w 5°C) do 10.2×10^{-11} m^2/s (w 45°C) dla albuminy surowicy wołowej, od 3.59×10^{-11} m^2/s (w 5°C) do 10.4×10^{-11} m^2/s (w 45°C) dla albuminy surowicy końskiej, od 3.42×10^{-11} m^2/s (w 5°C) do 9.92×10^{-11} m^2/s (w 45°C) dla albuminy surowicy owczej i od 3.36×10^{-11} m^2/s (w 5°C) do 9.74×10^{-11} m^2/s (w 45°C) dla albuminy surowicy króliczej. Współczynnik dyfuzji translacyjnej dla wyższych stężeń $D(T,\Phi)$ można otrzymać z relacji: $D(T,\Phi) = D_o(T)\eta_o(T)/\eta(T,\Phi)$, w której Φ oznacza ułamek objętościowy a $\eta_o(T)$ i $\eta(T,\Phi)$ są, odpowiednio, lepkością wody i roztworu w temperaturze T.

WNIOSKI
Otrzymane wyniki pokazują, że współczynnik dyfuzji translacyjnej maleje liniowo wraz ze wzrostem ułamka objętościowego, jeżeli Φ nie przekracza wartości około 0.1. Zależność współczynnika dyfuzji translacyjnej od ułamka objętościowego w szerszym zakresie Φ, tzn. dla roztworów od rozcieńczonych do stężonych, jest nieliniowa i można ją opisać rozciągniętą funkcją wykładniczą.

SŁOWA KLUCZOWE
współczynnik dyfuzji translacyjnej, lepkość, równanie Stokesa-Einsteina, albumina

INTRODUCTION

Albumins are the major protein component of blood plasma, but can also be found in interstitial fluids. They are present in different tissues like gut, liver, muscle or skin. About 30% of the total albumin in the whole animal body is present in the serum [1]. Albumins represent about 60% of total proteins in serum corresponding to a concentration of 42 kg/m^3 [2]. They play an important role in the mammalian circulatory system, mainly as multipurpose transporting molecules and as principal contributors to colloid osmotic blood pressure (provide about 80% of the osmotic pressure of blood). Serum albumins are the principal carriers of fatty acids in the blood which are otherwise insoluble. They also contribute to bodily detoxification by binding poisonous metabolites, such as bilirubin. In clinical situations, their affinity for different drugs such as warfarin, ibuprofen, indomethacin and so on

means that the albumins have a major effect on pharmacokinetics [3]. The binding affinity of any drug to albumin is one of the major factors that determine its availability in various tissues.

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 protoalbumin of about 190 amino acids and molecular mass 22 kDa [4, 5]. 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 [6]. Their secondary structure is formed by 67% of α helix of six turns and 17 disulfide bridges, and is entirely lacking in β-sheet [2]. The albumins from different species exhibit high amino-acid sequence identity with each other. For instance, the equine serum albumin (ESA) molecule exhibit sequence identity in 75.5% with ovine serum albumin (OSA) and in 73.9% with bovine serum albumin (BSA) [5]. These similarities of amino-acid sequence among mammalian albumins lead to the expectations of the common overall shape of them. Detailed investigations on the three-dimensional structure of albumins were performed by X-ray crystallography only for human serum albumin (HSA), BSA and ESA [4, 5, 7]. As appears, HSA in the crystalline state is a heart-shaped molecule and is highly similar to ESA. The tertiary and quaternary structure of HSA and ESA are nearly identical [5].

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). For several mammalian serum albumins those quantities were determined by using viscometric measurements [8–10]. As appears, the values of semi-axes "a" and "b" for different albumins are similar, but not identical. Despite similarities in amino-acid sequences and in the three-dimensional structure of mammalian albumins, their physicochemical properties in solution are quite different. It was showed by different experimental techniques including fluorescence spectroscopy and modelling [2], viscometry [8–10], dielectric spectroscopy [11], liquid chromatography [12], electrophoresis [13], calorimetry and steady-state fluorescence anisotropy [14] and circular dichroism [15].

As mentioned above albumins play an important role as transporting molecules and knowledge about their translational motions is of crucial importance. One of the most important quantity describing the translational motion of molecules is translational diffusion coefficient. This quantity can be experimentally obtained by using different experimental techniques such as fluorescence correlation spectroscopy [16–18], light scattering [19], pulsed-gradient NMR [20–22] and others [23]. Some theoretical methods for prediction of translational diffusion coefficient of proteins are also available. These methods employ simple parameters such as molecular weight [24], radius of gyration [25], both these parameters [26] or sophisticated bead models which use atomic level structure of proteins [27–29]. However, the last methods require high resolution X-ray crystallographic or NMR data that are not always available. In the present study, the translational diffusion coefficient for BSA, ESA, OSA and rabbit serum albumin (RSA) was determined from generalized Stokes-Einstein equation and viscometry measurements. The translational diffusion coefficient for the above albumins was obtained in a wide range of concentrations (from diluted solutions up to concentrated ones) and at temperatures ranging from 5 to 45°C. Concentration dependence of the translational diffusion coefficient was discussed by using a linear approximation (for diluted solutions) and by a stretched exponential function (in the whole range of measured concentrations).

MATERIAL AND METHODS

The following products of the Sigma Chemical Co. were used in this study: BSA (A 4503), ESA (A 9888), OSA (A 3264) and RSA (A 0639). The material was used without further purification for all the measurements. Aqueous solutions were prepared by dissolving the crystallized albumins in distilled water. Such obtained solutions were treated with filter paper in order to remove possible undissolved dust particles. The samples were stored in a refrigerator until just prior to viscometry measurements, when they were warmed from 5 to 45°C in 5°C intervals. The pH values of

such prepared samples were as follows: 5.2 for BSA, 7.4 for ESA, 7.05 for OSA and 7.0 for RSA. These values changed only slightly in the whole range of concentrations. The isoelectric point of the studied albumins is: (4.70 – 5.4) for BSA, (4.65–4.9) for ESA, (4.6–4.9) for OSA and (4.6–5.3) for RSA [13]. So, BSA was studied in its isoelectric point and the other albumins outside of it.

VISCOMETRY

Viscosity measurements were conducted by using an Ubbelohde-type glass capillary microviscometer immersed in a water bath controlled thermostatically at (5 – 45) ± 0.1°C. A solution passed once through the viscometer before any measurements were made. Measurements started after several minutes delay to ensure the system reached equilibrium and were made in 5°C intervals. 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. For the temperatures above the temperature of denaturation viscosity of the albumins solutions increases with increasing temperature. The viscosity was measured over a wide range of concentrations: from 17.6 kg/m^3 up to 363 kg/m^3 for BSA, from 12.9 kg/m^3 up to 367 kg/m^3 for ESA, from 36.5 kg/m^3 up to 320 kg/m^3 for OSA and from 13.9 kg/m^3 up to 300 kg/m^3 for RSA, i.e. in the mono-disperse range. In this range of concentrations the viscosity data could be interpreted by assuming a model of monomeric albumins molecules. The details are presented in previous papers [9–10]. Solution densities were measured by weighing and albumin concentrations were determined by a dry weight method in which samples were dried at high temperatures for several hours.

RESULTS AND DISCUSSION

Biological processes often depend on diffusive transport of macromolecules within cells and tissues. In particular, diffusion of proteins plays an important role in such diverse biological processes as enzymatic catalysis and molecular transport. For example, intracellular protein diffusion appears to be a highly effective mechanism of oxygen and carbon dioxide transport. To accomplish most of their physiological functions proteins have to meet and recognize each other. The random translational and rotational Brownian motion of proteins is necessary to encounter the reacting partners and to adopt the adequate mutual orientation required for subsequent docking and reaction. Then, knowledge about conditions under which protein diffusion occurs within cells is of crucial importance. A first step towards achievement of this knowledge is the study of protein diffusivity in solutions with protein concentrations as high as those found inside cells. One of the most important hydrodynamic parameter describing dynamic behavior of proteins in solution is translational diffusion coefficient D.

Translational and rotational movements of macromolecules (in particular, proteins) occur simultaneously as a consequence of the spatial imbalance of random collisions with solvent molecules within a short time interval. The problem of large particles diffusing in solution was studied by Einstein at infinite dilution [30]. In this limit, interactions between large particles can be neglected and the interaction between the large particles and the solvent particles can be replaced by a randomly fluctuating force. The problem can be then solved by using the usual Fick's diffusion equation, which defines the translational diffusion coefficient.

The size of water molecules is negligible in comparison with the size of proteins molecules. It means that proteins in aqueous solutions can be treated as Brownian particles immersed in an ideal, homogeneous, isotropic and continuous solvent. Translational diffusion of such Brownian particles is driven by thermal energy and is hindered by friction experienced by the particles. The translational diffusion coefficient of spherical particles at infinite dilution can be then obtained from a fluctuation-dissipation theory developed by Einstein [30]. This theory along with the results of macroscopic continuum hydrodynamics [31] yields the following Stokes-Einstein equation:

$$D_o(T) = \frac{kT}{6\pi\eta_o(T)R_h} \qquad (1)$$

where k is Bolzmann's constant, T is the absolute temperature, $\eta_o(T)$ is the solvent viscosity

and R_h is the hydrodynamic radius of the immersed particles. In the case of spherical particles, the hydrodynamic radius R_h is equal to their radius.

Majority of proteins in aqueous solutions is aspherical. However, in the first approximation they can be modeled by an ellipsoid of revolution with one long semi-axis (a) and two shorter semi-axes (b). The diffusion of ellipsoidal molecules was studied by Perrin [32]. According to this study the hydrodynamic radius of molecules with a shape of prolate ellipsoid of revolution can be expressed in the following way:

$$R_h = \frac{\sqrt{a^2 - b^2}}{\ln\left(\frac{a + \sqrt{a^2 - b^2}}{b}\right)} \qquad (2)$$

The measure of asphericity of a molecule is the axial ratio p = a/b. In general, the greater value of the axial ratio, the greater value of the hydrodynamic radius of a molecule. For native HSA and BSA it has been experimentally proved that the hydrodynamic radius does not depend on solution pH and temperature [33]. Equation (1) for non-spherical molecules is called generalized Stokes-Einstein equation.

Proteins in solution are surrounded by water molecules, whose structure differs from that of bulk water. As has been showen by dielectric spectroscopy, such molecules of water may be classified into three categories: (i) buried internal, (ii) ordered on a protein surface and (iii) disordered [34, 35]. Internal water molecules fill cavities in proteins interior and participate in maintaining of the stability of a protein polypeptide chain. Ordered water molecules on protein surface, in turn, are attached to the oxygen, nitrogen, and polar groups through hydrogen bonding. Water molecules from these two groups are an integral part of a protein and contribute to its hydrodynamic mass and volume. Moreover, water molecules ordered on a protein surface contribute to the hydrodynamic friction in the same way as protein atoms. It means that they influence the translational diffusion of proteins. The measure of protein hydration is the hydration level δ, defined in terms of the time-averaged mass of water associated with a protein per unit anhydrous mass of protein. For albumins δ = 0.35 [36]. The molecular mass of hydrated protein is $M_h = M_p(1 + \delta)$ and it gives the hydrodynamic mass of albumins M_h = 89.775 kDa. Semi-axes of hydrated ESA, OSA and RSA were obtained from the analysis of temperature dependence of viscosity of their solutions [9, 10]. These quantities and hydrodynamic radii calculated from equation (2) are gathered in table I.

Tab. I. The numerical values of the long semi-axis (a), the shorter semi-axes (b) and the hydrodynamic radius Rh for the studied albumins.

Tab. I. Wartości liczbowe długiej półosi (a), krótszych półosi (b) oraz promień hydrodynamiczny Rh badanych albumin.

Albumin	a [nm]	b [nm]	Rh [nm]
BSA [20,36]	7.70	2.20	3.83
ESA [9]	7.16	2.27	3.74
OSA [10]	7.41	2.42	3.92
RSA [9]	7.75	2.39	4.0

As seen from generalized Stokes-Einstein relation to calculate the translational diffusion coefficient of discussed here albumins in the limit of infinite dilution the values of hydrodynamic radius and water viscosity η_o are needed. The numerical values of η_o can be found in the standard physicochemical tables. The values of $D_o(T)$ obtained from relation (1) for the studied albumins are gathered in table II. Unfortunately there are very few experimental results of $D_o(T)$ for these al-

Tab. II. The numerical values of the translational diffusion coefficient Do(T) (in 10–11 m^2/s) for investigated albumins in aqueous solutions calculated on the basis of equation (1) and (2).

Tab. II. Wartości liczbowe współczynnika dyfuzji translacyjnej Do(T) (w 10–11 m^2/s) dla badanych albumin w roztworach wodnych, obliczone na podstawie równań (1) i (2).

	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C
BSA	3.50	4.14	4.83	5.58	6.38	7.24	8.15	9.13	10.2
ESA	3.59	4.24	4.95	5.71	6.53	7.42	8.35	9.35	10.4
OSA	3.42	4.04	4.72	5.44	6.23	7.07	7.96	8.91	9.92
RSA	3.36	3.97	4.63	5.35	6.12	6.94	7.82	8.75	9.74

bumins in the literature. The appropriate values one can find only for BSA. In this case, at t=20°C values of $D_o(T)$ are 5.42×10^{-11} m²/s [37] and 5.93×10^{-11} m²/s [26,37]. The value of $D_o(T)=5.58\times10^{-11}$ m²/s (table II) obtained from the Stokes-Einstein relation at this temperature is within this range. The experimental value of $D_o(T)=6.75\times10^{-11}$ m²/s obtained at t=27°C [38], in turn, agrees very well with the value $D_o(T)=6.72\times10^{-11}$ m²/s calculated from equation (1) at the same temperature. Quite recently, the Stokes-Einstein relation was applied to calculation of $D_o(T)$ for hen egg-white lysozyme [39]. The value of $D_o(T)=11.0\times10^{-11}$ m²/s obtained in this case at 20°C agrees very well with the experimental values which are in the range $(10.6 - 11.2)\times10^{-11}$ m²/s ([39] and references therein). The above results strongly suggest that the generalized Stokes-Einstein relation with the hydrodynamic radius obtained from Perrin formula can be applied to calculation of the translational diffusion coefficient in the limit of infinite dilution for hydrated proteins for which spatial dimensions are precisely known.

In many cases, in particular, in biological processes in living cells and tissues knowledge about the translational diffusion coefficient in the limit of infinite dilution is not sufficient. To obtain the translational diffusion coefficient D(c,T) for diluted, semi-diluted or concentrated solutions one can use the method proposed by Wang et al [40]. According to this method, to obtain the translational diffusion coefficient of proteins in solutions for concentrations higher than zero, the solvent viscosity η_o in the Stokes-Einstein relation should be replaced by the macroscopic solution viscosity $\eta(c,T)$. This assumption leads to the following relation:

$$\mathbf{D(c,T) = D_o(T)\frac{\eta_o(T)}{\eta(c,T)}} \qquad (3)$$

Correctness of the above equation was successfully verified experimentally for different proteins such as *Lumbricus* hemoglobin, tetrameric hemoglobin and ovalbumin by Gros [23]. However, it is worth to note that the above equation is correct only in the case when the size of solvent molecules is negligible in comparison to the size of dissolved particles. In the case when the local viscosity is influenced by the presence of macromolecular co-solutes – such as proteins, RNA's etc. the relation (3) has to be corrected to a somewhat modified form $D=D_o(\eta_o/\eta)^q$ [17]. As has been experimentally showed the exponent q in this relation is less or equal to 1 and it depends on the co-solvent's dimension and mass. The deviations from relation (3) appear when the molecular mass of cosolvent is higher than 10^3 and become more distinct as the molecular mass of the co-solvent increases.

The translational diffusion coefficient of a protein at high concentrations was experimentally obtained for the first time by Wang et al [40] for ovalbumin. One can also find, among other, the results of the investigations of proteins diffusion in concentrated solutions for ribonuclease, myoglobin, aldolase [41], lysozyme [20, 39] and barstar [20]. In the present paper the translational diffusion coefficient of discussed here albumins in the whole range of measured concentrations and temperatures has been calculated on the basis of relation (3).

Protein's translational diffusion coefficient is often used as a test of the aggregation state of proteins in low concentrated solutions. Therefore, it is important to know the concentration dependence of this coefficient in dilute solutions. In the case of dilute solutions in which only monomers of protein are present, the translational diffusion coefficient of the protein depends linearly on concentration. This dependence one can describe in the following way [42–44]:

$$\mathbf{D(c,T) = D_o(T)(1 - K_D\Phi)} \qquad (4)$$

The linear regression coefficient K_D is a measure of interparticle interaction and denotes the volume fraction of the particles. The volume fraction $\Phi=N_AVc/M_h$ where N_A and V denote Avogadro's number and the hydrodynamic volume of one dissolved protein, respectively, and c is concentration in kg/m³. Depending on the kind of interactions between the proteins the coefficient K_D can be negative or positive. When the resultant of forces between the proteins is attractive the coefficient K_D is positive and this means that the translational diffusion coefficient decreases with increasing concentration. When the resultant of protein's interactions change from attraction to repulsion the coefficient K_D becomes negative and D(c,T) increases with increasing concentration.

As mentioned above, protein molecules in water solution are surrounded by a hydration shell of water molecules. The hydrodynamic volume of one dissolved protein modeled as prolate ellipsoid of revolution with one long semi-axis (a) and two shorter semi-axes (b) is $V = 4/3\ \varpi ab^2$. Taking the numerical values of semi-axes for hydrated albumins from table I one can obtain the following values of the hydrodynamic volume: 156.1 nm^3 for BSA, 154.5 nm^3 for ESA, 181.8 nm^3 for OSA and 185.4 nm^3 for RSA. Because the hydrodynamic mass of albumins M_h=89.775 kDa, therefore their volume fraction one can calculate in the following way: $\Phi=1.047\times10^{-3}\ m^3/kg \times c$ for BSA, $\Phi=1.036\times10^{-3}\ m^3/kg \times c$ for ESA, $\Phi=1.219\times10^{-3}\ m^3/kg \times c$ for OSA and $\Phi=1.244\times10^{-3}\ m^3/kg \times c$ for RSA. The translational diffusion coefficient for the studied albumins, calculated from relation (3), depends linearly on concentration only up to the concentration of about 100 kg/m^3 (it corresponds to Φ of about 0.1). In this range of concentrations, and for temperatures ranging from 5°C to 45°C, the dependence is linear with the correlation coefficient $r \cong -0.998$. Concentration dependence of the translational diffusion coefficient for the studied albumins in dilute solutions is presented in figure 1. The numerical values of the linear regression coefficient K_D, in turn, in the whole range of measured temperatures are gathered in table III.

As seen from table III the linear regression coefficient K_D, for a given temperature, is different for different albumins. However, for each albumin it has tendency to decreasing with increasing temperature. For spherical particles the coefficient K_D should be equal to 1.5 [42]. For the studied albumins this quantity changes from 3.47 (for OSA at 45°C) to 4.55 (for BSA at 5°C). It shows that in the range of dilute solutions the rate of decreasing of translational diffusion coefficient with increasing of the volume fraction is higher for aspherical particles than for spherical ones. Moreover, all values of the linear regression coefficient K_D are positive, i.e. the translational diffusion coefficient deceases with increasing concentration. It means, that for dilute albumins solutions both in and outside of their isoelectric point the resultant of forces between the albumins is attractive.

Knowledge of the translational diffusion coefficient of proteins in dilute solutions is often insufficient because the diffusion of proteins inside cells, in extracellular environment and even in some physiological fluids is hindered by the very high concentrations of proteins. For example, the concentration of protein and

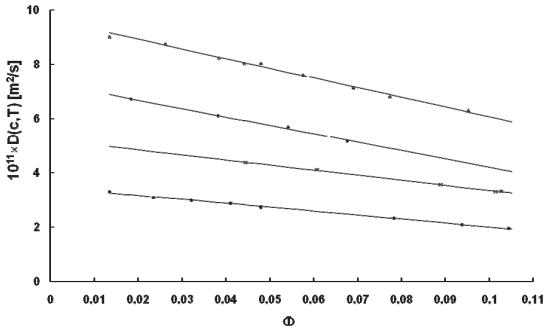

Fig. 1. Plot of the translational diffusion coefficient vs. volume fraction for RSA at 45°C (Δ), BSA at 30°C (♦), OSA at 20°C (×) and ESA at 5°C (•) in aqueous solutions. Experimental points were obtained on the basis of equation (3); the straight lines show the fit according to equation (4) with the parameters: Do(T) = 9.622×10-11 m^2/s, KD = 3.696 for RSA; Do(T) = 7.286×10-11 m^2/s, KD = 4.229 for BSA; Do(T) = 5.214×10-11 m^2/s, KD = 3.553 for OSA; Do(T) = 3.465×10-11 m^2/s, KD = 4.201 for ESA.

Ryc. 1. Wykres zależności współczynnika dyfuzji translacyjnej od ułamka objętościowego dla RSA w 45°C (Δ), BSA w 30°C (♦), OSA w 20°C (×) i ESA w 5°C (•) w roztworach wodnych. Punkty doświadczalne otrzymano na podstawie równania (3); linie proste pokazują dopasowanie do równania (4) z parametrami: Do(T) = 9.622×10-11 m^2/s, KD = 3.696 dla RSA; Do(T) = 7.286×10-11 m^2/s, KD = 4.229 dla BSA; Do(T) = 5.214×10-11 m^2/s, KD = 3.553 dla OSA; Do(T) = 3.465×10-11 m^2/s, KD = 4.201 dla ESA

Tab. III. The numerical values of the linear regression coefficient KD for investigated albumins in aqueous solutions calculated on the basis of equation (4).

Tab. III. Wartości liczbowe współczynnika regresji liniowej KD dla badanych albumin w roztworach wodnych obliczone na podstawie równania (4).

	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C
BSA	4.55	4.51	4.39	4.33	4.25	4.23	4.27	4.29	4.29
ESA	4.20	4.15	4.13	4.09	4.08	4.07	4.05	4.03	3.99
OSA	3.66	3.54	3.56	3.55	3.55	3.52	3.49	3.55	3.47
RSA	3.77	3.74	3.70	3.87	3.66	3.71	3.66	3.70	3.70

RNA molecules in the cytoplasm of *Escherichia coli* is 300-400 g/l and they occupy 20-30% of the total cellular volume [45]; muscle cells contain about 23% protein by weight, red blood cells have about 35% protein by weight, eye lens contains crystalline proteins at concentrations of (20–50)% by weight [19] and so on. An understanding of the translational diffusion phenomenon of proteins in concentrated solutions, i.e. in solutions containing high concentrations of a single solute and in crowded solutions, i.e. in solutions where a solute is present in a concentrated solution of another solute is necessary to correctly model passive intracellular transport. This process regulates such cellular functions as signal transduction [46], self-assembly of supramolecular structures [47], kinetics of reaction [48], embryogenesis [49], gene transcription

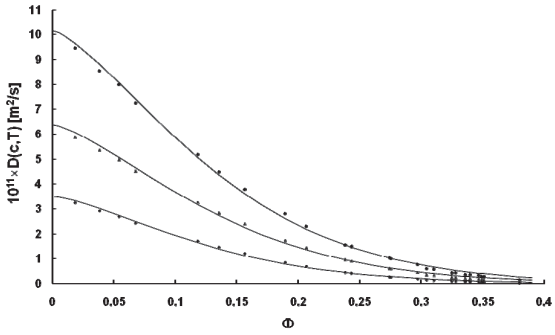

Fig. 2. Plot of the translational diffusion coefficient vs. volume fraction of BSA in aqueous solutions at t = 45°C (•), t = 25°C (▲) and t = 5°C (♦). Experimental points were obtained on the basis of equation (3); the curves show the fit according to equation (5) with the parameters: Do = 10.16×10-11 m^2/s, β = 14.32 and ν = 1.417 at t = 45°C; Do = 6.38×10-11 m^2/s, β = 14.73 and ν = 1.428 at t = 25°C; Do = 3.5×10-11 m^2/s, β = 16.29 and ν = 1.437 at t = 5°C.

Ryc. 2. Wykres zależności współczynnika dyfuzji translacyjnej od ułamka objętościowego BSA w roztworach wodnych dla t = 45°C (•), t = 25°C (▲) i t = 5°C (•). Punkty doświadczalne otrzymano na podstawie równania (3); krzywe pokazują dopasowanie zgodnie z równaniem (5) z parametrami: Do = 10.16×10-11 m^2/s, β = 14.32 i ν = 1.417 dla t = 45°C; Do = 6.38×10-11 m^2/s, β = 14.73 i ν = 1.428 dla t = 25°C; Do = 3.5×10-11 m^2/s, β = 16.29 i ν = 1.437 dla t = 5°C.

[50] or transport of small molecules and ions [51].

Proteins diffusion, both in concentrated solutions and crowded solutions, is influenced by strong intermolecular interactions. It causes that the dependence of the translational diffusion coefficient of proteins on concentration becomes non-linear. It can be described be a stretched exponential function [16, 18, 52]:

$$D(c,T) = D_0(T)\exp\left(-\beta\,\Phi^{\nu}\right) \qquad (5)$$

where β and ν are scaling parameters. Figure 2 shows a plot of the translational diffusion coefficient vs. volume fraction for BSA for three temperatures in the whole range of measured concentrations. Figure 3, in turn, shows this plot for ESA at t = 5°C, OSA at t = 25°C and RSA at t = 45°C. In the last case, different temperatures were chosen to avoid partial overlapping of the results. The curves show the fit to the experimental points obtained by using the above relation with β and ν treated as adjustable parameters. The numerical values of those parameters obtained in such a way for the studied albumins are presented in Table IV and V. As seen the scaling parameter β decreases with increasing temperature for all studied here albumins. For each fixed temperature, it reaches the highest value for BSA. This is the only albumin which was studied in solutions at isoelectric point. This suggests that, for a given protein, the rate of decreasing of the translational diffusion coefficient with increasing concentration is the highest in solutions at isoelectric point. The second parameter ν, in turn, is – for a given albumin - constant in the range of estimated errors, in the whole range of measured temperatures. However, as for parameter β, it also reaches the highest value for BSA for each fixed temperature. Because of lack of any theoretical treatment of the problem of concentration dependence of the translational diffusion coefficient, the experimental values of the scaling parameters β and ν are very valuable. Their values for a different sort of proteins can give indications for building such a theory, and in the same time can be a test for the theory.

CONCLUSIONS

Stokes-Einstein equation with the hydrodynamic radius calculated on the basis of Perrin formula allows the calculation of the translational diffusion coefficient for serum albumins in aqueous solution in the limit of zero concentration. Such way obtained translational diffusion coefficient decreases

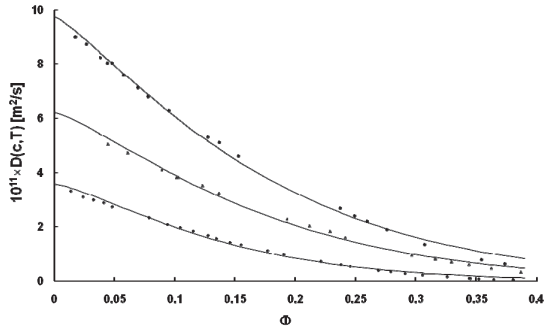

Fig. 3. Plot of the translational diffusion coefficient vs. volume fraction of RSA at t = 45°C (●), OSA at t = 25°C (▲) and ESA at t = 5°C (♦) in aqueous solutions. Experimental points were obtained on the basis of equation (3); the curves show the fit according to equation (5) with the parameters: Do = 9.739×10-11 m^2/s, β = 7.756 and ν = 1.214 for RSA; Do = 6.226×10-11 m^2/s, β = 8.403 and ν = 1.255 for OSA; Do = 3.587×10-11 m^2/s, β = 11.64 and ν = 1.294 for ESA.

Ryc. 3. Wykres zależności współczynnika dyfuzji translacyjnej od ułamka objętościowego RSA dla t = 45°C (●), OSA dla t = 25°C (▲) i ESA dla t = 5°C (♦) w roztworach wodnych. Punkty doświadczalne otrzymano na podstawie równania (3); krzywe pokazują dopasowanie zgodnie z równaniem (5) z parametrami: Do = 9.739×10-11 m^2/s, β = 7.756 i ν = 1.214 dla RSA; Do = 6.226×10-11 m^2/s, β = 8.403 i ν = 1.255 dla OSA; Do = 3.587×10-11 m^2/s, β = 11.64 i ν = 1.294 dla ESA.

with increasing temperature. In the range of dilute solutions, i.e. when the volume fraction of albumins does not exceed the value of approximately 0.1, the translational diffusion coefficient decreases linearly with increasing concentration. Linear regression coefficient for a given albumin slightly decreases with increasing temperature (within the range of the experimental errors). For each fixed temperature, it reaches the highest value for BSA, which was studied in solutions at isoelectric point. Concentration dependence of the translational diffusion coefficient from dilute to concentrated solutions is non-linear and can be described by a stretched exponential function. Two scaling parameters β and ν in this function depend on temperature in a different manner. The parameter decreases with increasing temperature, and the second parameter ν, in the range of estimated errors, is constant in the whole range of measured temperatures for all studied albumins. However, for each fixed temperature, both parameters reach the highest value for BSA, i.e. for albumin studied in solutions at isoelectric point.

Tab. IV. The numerical values of the scaling parameter β from equation (5) for investigated albumins in aqueous solutions.
Tab. IV. Wartości liczbowe parametru skalowania β z równania (5) dla badanych albumin w roztworach wodnych.

	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C
BSA	16.29 ±0.91	15.78 ±0.88	15.30 ±0.88	14.97 ±0.84	14.73 ±0.80	14.59 ±0.77	14.53 ±0.71	14.39 ±0.68	14.32 ±0.62
ESA	11.64 ±0.78	11.36 ±0.76	11.29 ±0.73	11.20 ±0.71	11.11 ±0.68	11.09 ±0.65	11.05 ±0.63	10.94 ±0.62	10.89 ±0.62
OSA	8.91 ±0.54	8.68 ±0.53	8.60 ±0.50	8.48 ±0.49	8.40 ±0.48	8.37 ±0.47	8.30 ±0.46	8.30 ±0.44	8.31 ±0.46
RSA	8.52 ±0.55	8.26 ±0.54	8.18 ±0.51	8.16 ±0.45	8.00 ±0.46	7.90 ±0.43	7.86 ±0.43	7.76 ±0.41	7.75 ±0.39

Tab. V. The numerical values of the scaling parameter ν from equation (5) for investigated albumins in aqueous solutions.
Tab. V. Wartości liczbowe parametru skalowania ν z równania (5) dla badanych albumin w roztworach wodnych.

	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C
BSA	1.437 ±0.030	1.435 ±0.030	1.430 ±0.032	1.428 ±0.031	1.428 ±0.031	1.430 ±0.030	1.432 ±0.028	1.426 ±0.027	1.417 ±0.024
ESA	1.294 ±0.035	1.288 ±0.035	1.293 ±0.034	1.296 ±0.034	1.300 ±0.033	1.304 ±0.032	1.308 ±0.031	1.307 ±0.031	1.310 ±0.031
OSA	1.257 ±0.034	1.249 ±0.035	1.253 ±0.033	1.252 ±0.033	1.255 ±0.033	1.258 ±0.032	1.258 ±0.032	1.261 ±0.031	1.261 ±0.032
RSA	1.216 ±0.033	1.208 ±0.033	1.212 ±0.032	1.219 ±0.029	1.216 ±0.030	1.212 ±0.028	1.215 ±0.028	1.210 ±0.027	1.214 ±0.026

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