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DETERMINATION OF THE OVERALL MOTION CORRELATION TIME FOR SOME GLOBULAR PROTEINS IN DILUTE SOLUTIONS ON THE BASIS OF MAXWELL EFFECT

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The behaviour of globular proteins in a streamline flow of a solution is determined by two effects: (1) the flow induced orientation of their principal axis parallel to the flow direction and (2) the rotational Brownian motion acting against this orientation. As a result, the orientation of the protein principal axes is anisotropic in space and can be described by the distribution function which fulfils a diffusion-type equation. When the external stress field disappears, the anisotropy in the principal axes space distribution vanishes because of the rotational Brownian motion of proteins. The time in which the initial orientation decreases by the factor of e^{-1} is called the overall motion correlation time. For dilute solutions, it can be determined if the intrinsic viscosity, molecular mass and axial ratio of the proteins in solution is known. The intrinsic viscosity has been measured using an Ubbelohde-type capillary microviscometer immersed in a water-bath controlled thermostatically for four globular proteins. The obtained numerical values of the overall motion correlation time are in the following range: from 622 ns (1^0C) up to 111 ns (55^0C) for human IgG immunoglobulin, from 121 ns (1^0C) up to 33.6 ns (45^0C) for human serum albumin, from 65.7 ns (1^0C) up to 14.3 ns (55^0C) for ovalbumin, and from 13.2 ns (1^0C) up to 2.43 ns (55^0C) for hen egg-white lysozyme.

INTRODUCTION

Hydrodynamic properties, such as sedimentation coefficient, intrinsic viscosity, translational and rotational diffusion coefficient, and rotational correlation times are commonly employed sources of information about the overall structure and dynamic behavior of proteins in solution. In particular, the rotational correlation times are important in understanding of many aspects of molecular processes (Wüthrich, 2003). In the case of a globular protein in solution, the rotational correlation times can be determined for the "free" water molecules (bulk water), hydration water molecules (water molecules tightly bound to the protein surface), protein side chains or the rotation of a protein as a whole (Kakalis & Kumosinski, 1992). In the latter case, the rotational correlation time is called the overall motion correlation time.

For a rigid rotator, the components of the Cartesian tensors such as the dipole moment (rank-1 tensor) or the polarizability (rank-2 tensor), are constant in the molecular frame. However, the observations are conducted from the laboratory frame. Here, because of the rotation of a molecule, the components of the Cartesian tensors depend on time. The correlation function describes such a dependence, however not for a single molecule but for the whole ensemble. In practice, it is convenient to express the components of the Cartesian tensors by a linear combination of the spherical tensors and then to study the correlation functions of the latter to unify the results.

Let us assume, that at the time $t = 0$, in the molecular frame connected with a given molecule, the components of the spherical tensor of rank n are $Y_n^k(\Omega_o)$ and let us denote the components of this tensor at time t, determined in the original frame, by $Y_n^k(\Omega)$. The correlation function is then defined as

$$G_n^k(t) = \frac{\left\langle Y_n^{k*}(\Omega) Y_n^k(\Omega_o) \right\rangle}{\left\langle Y_n^{k*}(\Omega_o) Y_n^k(\Omega_o) \right\rangle}, \qquad (1)$$

where (*) marks a complex conjugation and the square brackets denote averages over the all possible changes of orientation of the molecular frame after the time t as well as over all possible initial orientations of the molecular frame with respect to the laboratory one. The correlation function (1) is used to define the rotational correlation times:

$$\tau_n^k = \mathrm{Re} \int_0^\infty G_n^k(t)\, dt. \qquad (2)$$

Re means that only the real part of the integral is taken into account. These correlation times can be obtained from the theoretical models of molecular reorientation. However, the experiments give information only about the effective correlation time τ_n, which is connected with τ_n^k by the relation (Monkos, 1977):

$$\tau_n = \frac{4\pi}{2n+1}\sum_{k=-n}^{n}\left|Y_n^k(\Omega_o)\right|^2 \tau_n^k. \qquad (3)$$

Let us write the above equation for the most interesting cases with $n = 1$ and $n = 2$. In the former one:

$$\tau_1 = \frac{1}{2}\sin^2\vartheta_1\tau_1^{-1} + \cos^2\vartheta_1\tau_1^0 + \frac{1}{2}\sin^2\vartheta_1\tau_1^1 \qquad (4)$$

This effective correlation time can be obtained, for globular proteins, from dielectric spectroscopy (Moser *et al*, 1966; Miura *et al*, 1994; Kabir *et al*, 2003). For the symmetric top molecules $\tau_n^k = \tau_n^{-k}$ and relation (4) can be reduced to the form:

$$\tau_1 = \sin^2\vartheta_1\tau_1^1 + \cos^2\vartheta_1\tau_1^0. \qquad (5)$$

The symbol ϑ_1 denotes an angle between the dipole moment of the molecule and its axis of symmetry. For instance, for bovine serum albumin $\vartheta_1 = 50^0$ (Moser *et al*, 1966). When the dipole moment and the axis of symmetry of the molecule overlap, the effective correlation time τ_1 is equivalent to τ_1^0; when the dipole moment is perpendicular to the axis of symmetry of the molecule τ_1 is determined by $\tau_1^{\pm 1}$. For $n = 2$, in the case of the symmetric top molecules, the relation (3) gives:

$$\tau_2 = \frac{3}{4}\sin^4\vartheta_2\tau_2^2 + 3\sin^2\vartheta_2\cos^2\vartheta_2\tau_2^1 + \frac{1}{4}\left(3\cos^2\vartheta_2 - 1\right)^2\tau_2^0. \qquad (6)$$

This time can be experimentally determined, for globular proteins, with the help of the fluorescence depolarization method (Vos *et al*, 1987; Gryczynski *et al*, 1988; Bucci & Steiner, 1988; Takeda & Yamamoto, 1990; Takeda *et al*, 1991), EPR spectroscopy (Ebert *et al*, 1981; Steinhoff 1990), perturbed angular correlations of the γ-ray method (Danielsen *et al*, 1991) or NMR spectroscopy (Endre & Kuchel, 1986; Kakalis & Kumosinski, 1992; Belton, 1994). In the latter case, the symbol ϑ_2 means the angle between the electric field gradient inside a molecule and its axis of symmetry. It is well known that the rotational correlation times are connected with the rotational diffusion coefficient D_R by a simple relation: $\tau_n = [n(n + 1) D_R]^{-1}$. So, in theory, $\tau_1 = 3\tau_2$.

In the present study, the overall motion correlation time τ_R, equivalent to τ_2, was determined on the basis of Maxwell effect. The intrinsic viscosity, which is needed for the calculation of τ_R, was measured using an Ubbelohde microviscometer for human serum albumin (HSA), human IgG immunoglobulin (HIgG), ovalbumin and hen egg-white lysozyme (HEWL). The numerical values of τ_R were obtained over a wide range of temperatures: from the vicinity of the freezing point up to the temperature of thermal denaturation of the globular proteins investigated. All values of τ_R were obtained for proteins in dilute solutions.

MATERIALS

Highly purified HSA, hen ovalbumin and HEWL were purchased from Sigma Chemical Company and HIgG from Polish Chemical Reagents factories, and were used without further purification for all the measurements. From the crystalline form the material was dissolved in distilled water and then the solutions were filtered through filter papers in order to remove possible undissolved fragments. The samples were stored at 4^0C until viscometry measurements, when they were warmed from 5^0C to 45^0C for HSA and from 5^0C to 55^0C for the others proteins, with a step of 5^0C. The pH values of such prepared samples were as follows: 7.0 for HSA, 5.6 for HIgG, 6.4 for ovalbumin and 7.0 for HEWL. These values fluctuated slightly with different protein concentrations.

VISCOMETRY

Viscosity was measured with an Ubbelohde-type capillary microviscometer with a flow time for water of 28.5 s at 25^0C. The microviscometer was calibrated using cooled boiled distilled water. It was placed in a water-bath controlled thermostatically and the immersion heater was capable of maintaining the water temperature to $\pm 0.1^0$C. The same microviscometer was used for all measurements and was mounted so that it always occupied the same position in the bath. Measurements started after a several minut delay to ensure that the system had reached equilibrium. The solution to be studied was passed through the microviscometer before any measurements were made. Flow times were recorded to within 0.1 s.

The viscosity measurements were made from 5^0C to 45^0C (or 55^0C) in 5^0C intervals. Such a range of temperatures was chosen because above 45^0C for HSA, and above 55^0C for the other studied proteins the

thermal denaturation occurs. Beyond the temperature of denaturation the secondary structure of albumin becomes partially irreversible (Takeda *et al*, 1991) and the proteins show a highly pronounced tendency to aggregate (Katakam & Banga, 1995; Yoshioka *et al*, 1997). This heat-induced aggregation reverses upon cooling.

Solution densities were measured by weighing. Protein concentrations were determined by a dry weight method in which the samples were dried at high temperature for several hours.

RESULTS AND DISCUSSION

A stimulated birefringence in liquids or solutions can be induced by an external electric field (Kerr effect) or magnetic field (Cotton-Mouton effect) as well as by the mechanical force like shear stress in a streamline flow (Maxwell effect). The latter appears when the molecules of a flowing liquid or particles dissolved in a solution are non-spherical and have anisotropic polarizability (Cwietkow *et al*, 1968). In a non-flowing liquid, because of Brownian motions, the distribution of the principal axis of molecules in space is isotropic, and - despite of anisotropy of the molecules - the liquid is optically isotropic. When a liquid flows, the hydrodynamic forces cause the additional translational and rotational motions of molecules and the distribution of the principal axis of molecules in space becomes anisotropic.

At low temperatures, when the Brownian motion is negligible, the most probable direction of the principal axis of a molecule is that parallel to the direction of the flow. At higher temperatures, the stimulated orientation of molecules induced by the flow of a liquid is weakened because of the rotational Brownian motion of molecules. As a result, the privileged direction of the molecules orientation makes some angle with the direction of the liquid flow, which depends on the velocity gradient and on the shape of the molecules.

In the steady-state, the orientation of the molecules principal axis is anisotropic in space and can be described by the distribution function of all possible directions, which fulfils a diffusion-type equation (Cwietkow *et al*, 1968). If the external forces stimulating the liquid flow disappear, the anisotropy of the distribution of the principal axis in the space vanishes because of the rotational Brownian motion of molecules. The time in which the initial orientation decreases by the factor of e^{-1} is called the overall motion correlation time τ_R and it is connected with the rotational diffusion coefficient by the relation $\tau_R = (6D_R)^{-1}$ (Cwietkow *et al*, 1968). So, τ_R is equivalent to the rotational correlation time τ_2.

For dilute solutions, τ_R can be obtained from the following relation (Cwietkow *et al*, 1968; Acuña *et al*, 1987; Ferrer *et al*, 2001):

$$\tau_R = \frac{M[\eta]\eta_o}{RTF(p)}, \tag{7}$$

where M and R denote the molecular mass of the dissolved protein and the gas constant, respectively; $[\eta]$ and η_o mean the intrinsic viscosity and the solvent viscosity at temperature T; $F(p)$ is a function of the axial ratio $p = a/b$ of dissolved protein modeled as a prolate ellipsoid of revolution with one long semi-axis (a) and two shorter semi-axes (b). The above function can be presented in the form $F(p) = \nu/6\Pi$, where ν is a Simha factor (Simha, 1940) and Π is the Perrin function (Perrin, 1934).

The exact formula for ν obtained by Simha for tri-axial ellipsoids includes elliptic integrals which can be soluble numerically. However, for prolate ellipsoids of revolution the relation between ν and p can be described by the asymptotic formulae (Harding, 1997):

$$p = -10.71584 + 2.79158\nu + 1.622009\nu^2 + 0.01556169\nu^3 - 0.192997\nu^4 + +0.02060718\nu^5 \tag{8}$$

for $(1.1 \le p < 2)$, and

$$p = -3.80413 + 2.8712\nu - 0.3908319\nu^2 + 0.03612282\nu^3 - 0.001733981\nu^4 + 0.0000332711\nu^5 \tag{9}$$

for $(2 \le p < 10)$. The Perrin function, in turn, has the following form:

$$\Pi \equiv \left[\frac{0.4}{2g_1^{-1} + 4g_2^{-1}} + \frac{0.4}{5g_1^{-1} + g_2^{-1}} + \frac{0.2}{6g_1^{-1}}\right], \tag{10}$$

where

$$g_1 = \frac{2(p^2-1)}{3p(p-S)}, \qquad g_2 = \frac{2(p^4-1)}{3p(2p^2-1)S - p} \tag{11}$$

and

$$S = \frac{\ln\left(p + \sqrt{p^2-1}\right)}{\sqrt{p^2-1}}. \tag{12}$$

The relation (7) allows a calculation, in a simple and convenient way, of the overall motion correlation time for different type of molecules in dilute solutions. It has been applied, among others, to human fibrinogen (Acuña *et al*, 1987), food biopolymers (Kokini, 1994),

hetero-polysaccharide pullulan (Dais & Viachou, 2001), scleroglucan (Vlachou *et al*, 2001) and bovine serum albumin (Ferrer *et al*, 2001).

To calculate the overall motion correlation time from equation (7), the molecular mass of the proteins is needed. However, for hydrated and unhydrated globular proteins this quantity is not the same. In physiological conditions, proteins are in water and the protein-water interactions are one of the dominant factors which influence their structure, function and dynamics. Water molecules in protein solutions may be classified into three categories: (1) buried internal, (2) ordered on the protein surface and (3) disordered (Wade *et al*, 1991). Internal water molecules are of particular significance because they fill the cavities inside proteins and play an important role in the process of folding of the protein polypeptide chain and in maintaining its stability. They are considered as integral parts of a protein. The ordered water molecules are linked through hydrogen bonds to the oxygen and nitrogen atoms or to the polar groups on the protein surface. The disordered water molecules belong to the bulk water. The above was clearly revealed by dielectric spectroscopy (Miura *et al*, 1994; Miura *et al*, 1995; Yokoyama *et al*, 2001; Kabir *et al*, 2003).

In the dielectric spectrum of a protein solution several ranges of dispersion can be distinguished. The first one, called β-dispersion, is observed in the range of 0.1 – 1 MHz and is connected with the orientational relaxation of dipoles of the whole proteins. The second one, called δ-dispersion, is due to the orientational relaxation of water molecules in the hydration shell or side chains of protein. Water molecules surrounding apolar moieties have relaxation frequencies about 0.1 GHz; those in the vicinity of polar moieties have greater mobility and relaxation frequency in the range (4 – 8) GHz (Kabir *et al*, 2003). The last range, called γ-dispersion, is in the microwave frequency range (about 17 GHz at 20^0C) and corresponds to the orientational relaxation of bulk water dipoles.

The most direct method to elucidate the details of the protein hydration shell in the crystal state is high-resolution x-ray and neutron scattering (Pérez *et al*, 1999; Zanotti *et al*, 1999; Perkins, 2001; Chatake *et al*, 2003). Especially, the neutron scattering method with a resolution of 1.5 – 1.6 Å gives information about both localization of water molecules on the protein surface and about their spatial orientation. However, the best method of high-resolution investigation of protein hydration in solution is NMR (Denisov & Halle, 1995a, 1995b; Wüthrich, 2003).

The above mentioned methods have shown that proteins in solution are surrounded by one or two shells of water molecules having quite different physical properties than those of bulk water molecules (Gregory *et al*, 1993; Denisov & Halle, 1995a, 1995b; Svergun *et al*, 1998 Pouliquen & Gallois, 2001). As appears, the first hydration shell has an average density about 10% larger than that of the bulk water; "bound" water molecules have rotational correlation times 3 to 6 orders of magnitude longer than those in bulk water and different heat absorption. Protein hydration is usually defined as a process of successive adding of water to a dry protein, until reaching the level of hydration beyond which further addition of water produces no change and only dilutes the protein (Steinbach & Brooks, 1993). The level of protein hydration is usually marked by δ and denotes the amount of grams of water associated with the protein per a gram of the protein. This quantity does not depend on temperature (Takeda *et al*, 1991; Miura *et al*, 1994; Ferrer *et al*, 2001) and concentration (Menon & Allen, 1990) of a solution. The "bound" water molecules migrate with the protein and therefore contribute to its hydrodynamic mass and volume. In particular, the molecular mass of the hydrated protein $M = M_p(1 + \delta)$, and M_p is the molecular mass of the unhydrated protein. Moreover, these water molecules should contribute to the hydrodynamic friction – in the translational and rotational movements of the protein – in the same way as the protein atoms. So, the amount of water from the hydration shell should be taken into account in the calculation of any hydrodynamic parameter depending on the mass or volume of the protein studied.

HEWL is a small globular protein of the molecular mass M_p = 14.32 kDa (Squire & Himmel, 1979). It can be treated as prolate ellipsoid of revolution with the main axes a = 4.5 nm and b = 3 nm. It gives the axial ratio p = 1.5. The effect of HEWL hydration has been exhaustively studied by several authors (Gregory *et al*, 1993; Steinbach & Brooks, 1993; Pérez *et al*, 1999; Yokoyama *et al*, 2001). As appears, HEWL remains inactive at the level of hydration lower than 0.2 g water/g protein. The onset of activity occurs around 0.2 g water/g protein but the full hydration of HEWL is achieved at δ = 0.38. It gives the molecular mass of hydrated HEWL M = 19,762 kDa. A comparison of the solution and the crystal infrared spectra has shown a very close similarity of the structure of HEWL in solution and in crystals (Hadden *et al*, 1995). Moreover, an analysis of the viscosity of HEWL aqueous solutions showed that its axial ratio remains almost unchanged in solution (Monkos, 1997).

Ovalbumin is the major globular protein of chicken egg white. The crystal structure of the protein, as revealed by X-ray crystallography, indicates that the ovalbumin molecule is approximately a tri-axial ellipsoid with overall dimensions $7 \times 4.5 \times 5$ nm and a molecular mass M_p = 45 kDa (Stein *et al*, 1991). The level of hydration for ovalbumin δ = 0.36 (Young, 1963) and it gives the molecular mass of hydrated ovalbumin M = 61.2 kDa. Analysis of viscosity of ovalbumin

solutions has shown (Monkos, 2000) that the hydration shell around the ovalbumin molecule is not uniform; water molecules cover some part of the protein surface leaving the other part uncovered. As a result, the hydrated ovalbumin is more elongated than unhydrated one, and can be approximated by an ellipsoid of revolution with the axial ratio p = 2.74 (Young, 1963).

HSA is the major protein component of blood plasma and plays an important role mainly in the transport of a wide variety of substances. In the crystal state it is a heart-shaped molecule with a molecular mass M_p = 66.479 kDa (He & Carter, 1992; Dockal *et al*, 1999). However, in solution, the HSA conformation is commonly approximated by an ellipsoid of revolution, but to interpret the experimental data quite different values of main axes have been used by different investigators (Monkos, 2004 and references therein). From the hydrodynamic point of view the hydrated HSA can be treated as a prolate ellipsoid of revolution with the main axes 16.4 nm and 4.2 nm (Monkos, 2004).
Careful measurements of the level of hydration gave δ = 0.379 (Baranowska & Olszewski, 1996) and it yields to the molecular mass of hydrated HSA M = 91.675 kDa.

Immunoglobulins IgG, IgA, IgM, IgD and IgE constitute about 20% of the total plasma proteins. Of the above five classes, the IgG immunoglobulins make approximately 75% of the total serum immunoglobulins (Goodman, 1991). The basic unit of IgG is a four polypeptide chains structure, with a molecular mass M_p = 156 kDa, comprising two identical light chains and two identical heavy chains which are folded in three well-defined globular structures, two F_{ab} fragments and one F_c fragment (Al-Lazikani *et al*, 1997). The level of hydration is different for F_{ab} and F_c fragments: δ = 0.5 and 0.7, respectively (Harding, 2001). It gives – for the whole IgG molecules - the average value of hydration δ = 0.567 and the molecular mass of hydrated IgG immunoglobulins M = 244.4 kDa. HIgG molecules in solution behave as prolate ellipsoids of revolution with the axial ratio p = 5.34 (Young, 1963; Monkos & Turczynski, 1999). The above given axial ratios for the proteins investigated have been used in calculations of the values of the function $F(p)$ from equation (7). The values obtained are presented in Table 1.

Table 1. Values of the unhydrated molecular mass M_p, the level of hydration δ, the hydrated molecular mass M, the axial ratio p, the Simha factor ν from equation (8) and (9), the Perrin function Π from equations (10) – (12) and $F(p) = \nu/6\Pi$ for the proteins studied

Protein	Human IgG immunoglobulin	Human serum albumin	Ovalbumin	Hen egg-white lysozyme
M_p [kDa]	156	66.479	45	14.32
$\delta\left[\frac{g\ H_2O}{g\ protein}\right]$	0.567	0.379	0.36	0.38
M [kDa]	244.4	91.675	61.2	19.762
p	5.34	3.95	2.74	1.5
ν	6.22	4.61	3.48	2.64
Π	0.352	0.270	0.199	0.122
$F(p)$	2.95	2.85	2.92	3.60

The intrinsic viscosity $[\eta] = \lim_{c \to 0} \eta_{sp} / c$, where the specific viscosity $\eta_{sp} = \eta_r - 1$ and c is the solute concentration in kg/m^3. The relative viscosity $\eta_r = \eta/\eta_o$ and η denotes the viscosity of a solution. The usual procedure for treating viscosity data consists of plotting the η_{sp}/c against concentration and extrapolating it to the intercept which is equal to $[\eta]$. In the present paper another method, proposed earlier (Monkos, 1996), is used. It was successfully applied for lysozyme (Monkos, 1997; Monkos, 2001), some mammalian IgG immunoglo-bulins (Monkos & Turczynski, 1999), ovalbumin (Monkos, 2000) and HSA (Monkos, 2004). The numerical values of $[\eta]$, obtained by using this method, at temperatures from 1^0C up to 55^0C (up to 45^0C for HSA) are gathered in Table 2.

The molecular mass and the axial ratio of hydrated proteins as well as the experimentally obtained intrinsic viscosity allow calculation of the overall motion correlation time from equation (7). The numerical values of τ_R for all investigated proteins are presented in Table 2. As seen, the temperature greatly influences the global rotational motion of proteins. For instance, HIgG rotational motion at $t = 55^0$C is about 5.6 times faster than at $t = 1^0$C. For a given temperature, τ_R depends on the molecular mass of the protein in a direct way as in equation (7) and indirectly through the intrinsic viscosity. As seen from Table 2, the higher the molecular mass of the protein the higher the intrinsic viscosity.

Table 2. Numerical values of the intrinsic viscosity $[\eta]$ obtained from experiment and the overall motion correlation time τ_R calculated from equation (7) for the proteins studied.

	Human IgG immunoglobulin		Human serum albumin		Ovalbumin		Hen egg-white lysozyme	
t [C]	$[\eta]\times10^3$ [m^3/kg]	τ_R [ns]	$[\eta]\times10^3$ [m^3/kg]	τ_R [ns]	$[\eta]\times10^3$ [m^3/kg]	τ_R [ns]	$[\eta]\times10^3$ [m^3/kg]	τ_R [ns]
1	9.88	622	4.96	121	4.13	65.7	3.16	13.2
2	9.80	595	4.95	116	4.11	63.1	3.13	12.6
3	9.73	569	4.93	112	4.10	60.6	3.11	12.0
4	9.66	545	4.91	108	4.08	58.3	3.08	11.5
5	9.59	522	4.90	104	4.07	56.1	3.05	11.0
10	9.26	426	4.83	86.3	4.01	46.7	2.94	8.95
15	8.95	353	4.78	73.1	3.95	39.4	2.83	7.40
20	8.67	296	4.73	62.7	3.90	33.7	2.74	6.21
25	8.41	251	4.69	54.4	3.86	29.2	2.66	5.27
30	8.17	215	4.66	47.6	3.82	25.4	2.59	4.52
35	7.95	186	4.63	42.1	3.78	22.4	2.54	3.93
40	7.75	162	4.62	37.4	3.75	19.8	2.49	3.44
45	7.57	142	4.61	33.6	3.73	17.7	2.45	3.05
50	7.41	126	-	-	3.71	15.9	2.42	2.72
55	7.27	111	-	-	3.70	14.3	2.41	2.43

Unfortunately, there are not many experimental reports on the overall motion correlation time of globular proteins, in literature. For HEWL at 20^0C the values: 7.2 ns (Vos *et al*, 1987), 7.4 ns (Belton, 1994) and 8 ns (Halle & Davidivic, 2003) have been reported and at 21^0C the value 4.7 ns (Kakalis & Kumosinski, 1992) has been obtained. The value 6.21 ns (at 20^0C) obtained in the present paper lies in the middle of this range. For bovine serum albumin (a sister molecule to HSA) the rotational correlation time τ_2 values: 40 ns (Ferrer *et al*, 2001) or 41.7 ns (Bucci & Steiner, 1988) at 20^0C, and 76 ns (Moser *et al*, 1966) at 25^0C have been obtained. They are of the same order of magnitude as for HSA.

Proteins are dynamic systems. Besides the global rotational motion, in each individual protein molecule different sorts of motions appear: local vibrations of atoms and groups, changes of relative positions of whole domains, rotations of side chains and so on. For most experimental techniques a separation of these motions is not a trivial problem. Usually, the obtained correlation times represent the overlay of different motions: a global reorientation and one or more localized motions (Danielsen *et al*, 1991; Vlachou *et al*, 2001; Dais & Vlachou, 2001). The Maxwell effect partially allows such a separation (because the results depend only on one sort of molecular motion) and gives an opportunity to study – in a simple and convenient way – a global rotational motion of a protein.

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