

ALTERATION OF THE OPTICAL PARAMETERS OF BLOOD SERUM PROTEINS DURING CEREBRAL CIRCULATION DISORDERS

A. V. Boiko, V. B. Koshelev, G. P. Petrova, Yu. M. Petrusevich, O. E. Fadyukova,
and D. I. Ten

E-mail: petrova@phys.msu.su

Static and dynamic light scattering techniques are used to investigate the parameters of aqueous serum solutions in rats suffering from cerebral circulation disorders. Comparison between the results obtained and control data shows a reduction of the effective masses of the scattering particles and their hydrodynamic radii in the case of vascular pathologies. This gives reason to believe that the sensitivity of the light scattering method is adequate for the analysis and monitoring of vascular diseases.

INTRODUCTION

The development of pathological processes in the organism is accompanied by changes in a number of molecular parameters of blood serum proteins.

In this work, we have studied the alteration of the molecular parameters of proteins in aqueous solutions of animal (rat) serum, caused by an artificially induced pathology, with a view to developing novel physical methods for diagnosing cerebral circulation disturbances. The investigations were conducted by static and dynamic light scattering methods. Artificially induced ischemic and hemorrhagic strokes were used as a pathology in the animals under study.

Ischemic strokes develop as a result of reduction of cerebral blood flow and limited inflow of oxygen and glucose into neural tissues. While accounting for a mere 2% of the total mass of human body, the brain consumes 20–25% of the oxygen coming into the organism and up to 70% of free glucose. Various experimental models have been worked out to study the mechanisms responsible for the development of cerebral ischemia in experiments with animals. In the present work, we used the model of hypoxia resulting from carotid ligation. Hemorrhagic stroke in a special strain of rat (Krushinskii–Molodkina's) was induced by exposure to strong noise.

INVESTIGATION METHODS

The scattering of light in blood serum solutions depends on the physical parameters of the dissolved molecules, albumin and globulin in the first place.

The static and dynamic light scattering techniques prove very effective in studying the intermolecular interaction, mobility, and polarization properties of macromolecules, including aqueous protein solutions.

According to the Debye theory [1], the following relation holds for dilute solutions of macromolecules:

$$\frac{cH}{R_{90}} = \frac{1}{M} + 2Bc + \dots, \quad (1)$$

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which allows the experimentally measured quantity R_{90} —the Rayleigh coefficient or turbidity—to be represented in the form of a virial expansion in small concentrations. The constant H is determined by the parameters of the experimental setup and the refractive index increment of the solution under study. The method makes it possible to directly determine the molecular mass M by measuring R_{90} for several concentrations and extrapolating the relationship obtained to the concentration $c = 0$. The second virial coefficient, B , characterizes the extent of deviation of the behavior of the solution from the ideal one and serves as a measure of the intermolecular interaction in the solution.

In the case of electrically charged macromolecules, such as proteins, the effect of the surface charge of a molecule on its behavior in solution, its intermolecular interaction parameter in particular, proves very important.

The dynamic parameters of macromolecules can be studied by the correlation spectroscopy method. With this method, subject to study is the correlation function of the scattered light intensity fluctuations due to the Brownian motion of the particles of the solution of interest. This can help determine the translational diffusion coefficients of the particles and their hydrodynamic radii.

The authors of [2] have demonstrated that the concentration dependences of the translational diffusion coefficient and scattering parameter are governed by one and the same virial coefficient. To illustrate, the relation between the coefficient D_t , the mass M of the molecule, and the characteristic viscosity η of the protein solution is defined by the equation

$$D_t = D_0\{1 + (2BM - [\eta])c\}, \quad D_0 = \frac{kT}{6\pi\eta_0 R}, \quad (2)$$

where R is the radius of the particle.

In classical formulas (1) and (2), one cannot find any explicit dependence on the charge state of the protein molecule [1]. However, one can suppose that both the coefficient B and the characteristic viscosity η are functions of the electrostatic parameters of the macromolecule. This was experimentally verified in [2], where the coefficient D_t was shown to depend nonlinearly on the charge on the surface of the protein molecule (the pH value) and to have a minimum at the isoelectric point, like the parameter B .

EXPERIMENTAL RESULTS

Serum is the liquid part of blood, separated from the blood corpuscles and fibrinogen. Normal serum contains 90% of water, 9% of proteins, and 0.87% of dissolved salts [3, 4]. Table 1 lists the main protein fractions of blood serum and their concentrations.

Experiments in our work were conducted with animals of two groups: the 1st group—controls—and the 2nd group—experimental animals—rats operated on for the purpose of carotid ligation or exposed to noise.

The scattering properties of aqueous blood serum solutions were studied with an experimental setup built around a He-Ne laser ($\lambda = 632$ nm) and a scattered light photoelectron detector [5].

Table 1
Blood Serum Proteins [4]

Main protein fractions	Concentration	
	g/l	%
Albumins	35–45	56.5–66.8
Globulins	23–35	33.2–43.5
α_1 -globulins	3–6	3.0–5.6
α_2 -globulins	4–9	6.9–10.5
β -globulins	6.0–11.0	7.3–12.5
γ -globulins	7.0–15.0	12.9–19.0
Fibrinogen	2.0–4.0	0.2–0.4

The main parameters investigated in our experiments included the integral light scattering intensity (measured at an angle of 90° to the incident light beam), the mass of the scattering particles, and the intermolecular interaction coefficient.

Table 2 lists the results of examination by the static scattering method of aqueous solutions of blood serum samples taken from animals suffering from a cerebral circulation disorder caused by carotid ligation. The samples were taken both prior to and after the operation. These samples correspond to the series *Ischemic stroke (experiment)*. The results obtained for these samples are compared with the results of examination of blood serum samples taken from animals subjected to the so-called false operation (without carotid ligation). These results are presented in Table 2 as *Ischemic stroke (control)*. It can be seen from the table that in the case of ischemic stroke the effective mass of the scattering particles decreases.

Table 2

Experimental Values of the Molecular Mass M ,
Second Virial Coefficient B , and Mean Scattering
Particle Radius for Rat Blood Serum Samples

Sample no.	M_{before} , g/mol	M_{after} , g/mol	R_{before} , nm	R_{after} , nm
<i>Ischemic stroke (experiment)</i>				
1	115 740	85 000	78.7	44.1
2	28 000	20 200	65.4	32.1
3	21 000	16 600	57.5	42.6
4	78 700	55 000	66.7	50.4
5	83 000	76 000	44.2	31.7
<i>Ischemic stroke (control)</i>				
1	74 100	76 000	32.3	40.2
2	29 948	23 347	60.1	56.3
3	70 500	69 100	51.1	47.5
4	35 900	31 500	48.8	37.6
5	20 500	20 000	56.5	55.6
<i>Hemorrhagic stroke (experiment)</i>				
1	38 000	41 600	38.7	62.5
2	42 600	39 200	31.7	14.2
3	36 000	15 200	50.5	23.6
4	29 154	24 509	59.3	50.1
5	37 300	32 258	33.4	14.9
<i>Hemorrhagic stroke (control)</i>				
1	31 900	28 500	23.9	23.6
2	76 400	77 000	30.2	30.5
3	81 200	76 000	67.8	64.5
4	24 700	23 100	38.7	20.9
5	36 088	39 717	32.6	31.6
6	16 700	17 900	11.8	18.5

Presented in the second half of Table 2 are the molecular parameters of aqueous solutions of blood serum samples taken from rats suffering from noise-induced hemorrhagic stroke (indicated as *Hemorrhagic stroke (experiment)*). In this case, as in the case of ischemic stroke, the effective mass of the scattering particles is observed to decrease somewhat. For comparison, we also took blood serum samples from rats of the same strain (Krushinskii-Molodkina's). The results for these samples are indicated in Table 2 as *Hemorrhagic stroke (control)*. One can see from the table that the effective scattering particle masses for the control samples have closer values.

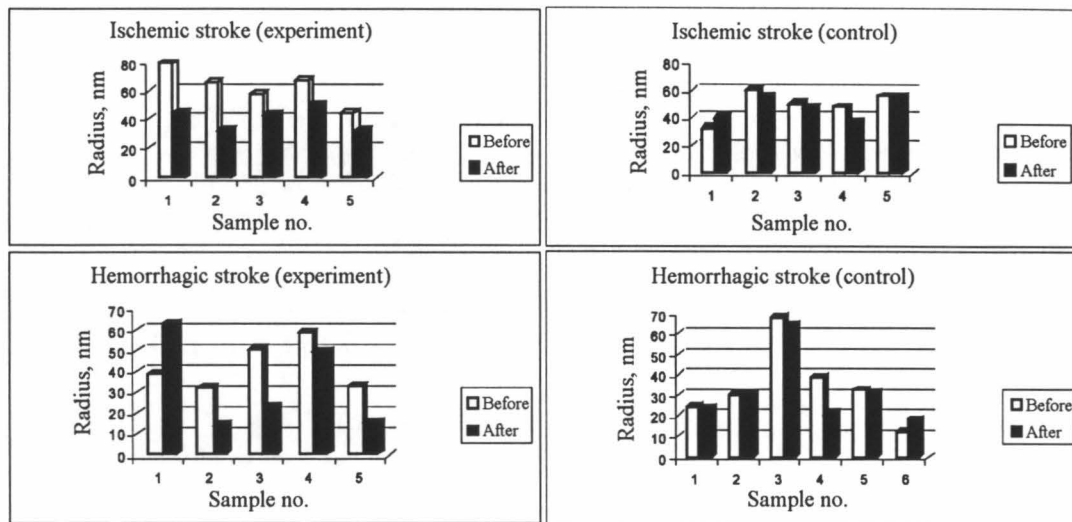


Fig. 1

Diagrams for the effective radius values.

The dynamic parameters—the diffusion coefficient and the mean effective radius of the scattering particles — were measured with a laser correlation spectrometer [6]. The wavelength of light scattered by particles in the Brownian motion differs from that of the exciting source. The photomultiplier detecting light beams differing in wavelength operates in this case as quadrature detector. The output photocurrent is a pulse train governed by the resultant light intensity fluctuations. The scattered light intensity correlation function thus obtained is used to determine the diffusion coefficient and hydrodynamic radii of the particles.

An optical photon correlator was used to examine by the dynamic light scattering method ten blood serum samples taken from male rats (prior to and after operation for carotid ligation) and also 11 blood serum samples from female rats (prior to and after hemorrhagic stroke). We determined the mean effective radii of the particles from their translational diffusion coefficient in double blind experiments.

The values of the effective radii of particles measured for the experimental and control blood serum samples are also presented in Table 2 and Fig. 1. The results obtained show a substantial difference in this parameter between healthy animals (before operation) and the animals having the operation.

Analysis of Table 2 shows that the calculated mass M of the scattering particles and the intermolecular interaction parameter (coefficient B) depend on the effective radius R of the particles that governs the dynamic diffusion coefficient. All the above quantities alter perceptibly in the case of pathology being considered.

The cause of the reduction of the effective radii and masses of the scattering particles in the case of vascular pathology is as yet not perfectly understood. It is probably due to the inadequacy of the classical formulas used to calculate the static and dynamic parameters of the scattering particles in the case of strong electrostatic interactions. To draw more detailed conclusions requires additional experiments, all the more so since the blood serum parameters strongly depend on the individual characteristics of the animals.

CONCLUSIONS

We used the static and dynamic light scattering methods to measure the molecular parameters of the blood serum of rats suffering from various vascular pathologies. Comparison between the results obtained and control data revealed a reduction of the effective masses of the scattering particles and their radii in the case of ischemic and hemorrhagic strokes.

We believe that light scattering techniques may prove sufficiently sensitive for analysis and monitoring of vascular diseases.

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Department of Molecular Physics
Department of Low-Temperature Physics