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Effect of Heavy-Metal Ions on Dynamic Characteristics of Collagen Molecules in Solutions

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Abstract—Effect of heavy (Pb^{2+}, Cs^{+}) and light (Na^{+}) metal ions on the molecular-dynamic characteristics of type-I collagen in aqueous solution was studied using the method of dynamic light scattering. It was found that the dependence of the translational diffusion coefficient D_t from pH solutions has a nonlinear form with a pronounced extremum close to the isoelectric point of the protein (pI 6.0). For pure aqueous solution of protein there is a maximum of D_t in isoelectric point. For collagen solutions with the addition of heavy-metal salts the minimum of D_t was observed near the isoelectric point. This fenomenon is connected with the formation of protein nanoclusters in solution. With concentration of heavy metal ions increasing translational diffusion coefficient Dt decreases, which shows on increasing of aggregation effect. The addition of sodium ions in aqueous solution of collagen containing heavy metal ions sharp decreasing of the translational diffusion of molecules is observed. That can be connected with the rise of scattering particles masses.

Keywords: type-I collagen, heavy-metal ions, lead, cesium, method of dynamic light scattering, isoelectric point, translational diffusion coefficient

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INTRODUCTION

The study of physical processes that occur in protein solutions that contain heavy-metal ions, such as lead and cesium, is necessary to understand the influence of pathogenic factors on biological objects. Automobile exhausts and industrial emissions contain heavy metals at high concentrations, which exert not only an external adverse effect on the human habitat, but can also enter into the body with inhaled air and deposit on human skin.

Collagen is a fibrillar protein that forms the basis of body connective tissue, such as tendon, bone, cartilage, skin, and provides its strength and elasticity [1]. Currently, 28 various types of collagen have been described. They differ from each other in their amino-acid sequences and also by the modification degree, i.e., the hydroxylation and glycosylation intensities. The most abundant of them is type-I collagen, which is the base of the skin of all living beings. In the present work we studied type-I collagen obtained from calf skin.

Proteins in aqueous solutions are prone to aggregate depending on their physico-chemical state. This process is often irreversible and can be a sign of some serious disease in humans. Pathological changes in the structure of collagen fibrils are the cause of several diseases of the connective tissue and are characterized by lesions of organs, namely, joints, heart, blood vessels, muscles, and skin tissue.

Aggregation and other processes that occur with protein macromolecules in solutions depend on the nature of proteins, their concentration, type of solvent, salts, metal ions, acids, bases, pH, temperature and other factors.

It has been experimentally shown that the dynamic parameters of globular (albumin and gamma-globulin) and fibrillar (collagen) proteins do not change when light metal (for example, sodium) ions are added to their solutions [2]. When heavy-metal ions (for example, lead, potassium, and cadmium) were added, a decrease in the coefficient of translation diffusion of molecules was observed, which is likely to be due to the increasing masses of the diffusing particles. From this we can conclude that the presence of heavy metals "launches" the process of protein clustering in solution.

The properties of monomolecular layers of collagen and a product of its denaturation, namely, gelatin were investigated, and the effects of trivalent metal ions (Fe^{3+}, Al^{3+}, and Cr^{3+}) on the behavior of the protein molecules in the monolayers was studied [3]. It was shown that the layers of gelatin and collagen are least expanded on substrates at pH values close to the isoelectric point of the protein (pH 6.0 for collagen and pH 4.8 for gelatin). It was found that introduction

of Fe^{3+} ions in the aqueous substrate has a strong condensing effect on collagen and gelatin monolayers. The authors attributed this effect to the formation of two-dimensional complexes of macromolecules with Fe^{3+} ions at the water–air interface. It was also found that the Cr^{3+} ions have a lower condensing effect on monolayers of collagen and gelatin and that Al^{3+} ions affect only the gelatin properties.

The purpose of the present work was to study the molecular-dynamic processes that occur in aqueous solutions of type-I collagen at various changing parameters of the medium (pH, protein concentration, and ionic strength), including the study of the harmful effects of toxic metal ions (Pb^{2+} and Cs^{+}) using the method of dynamic light scattering.

1. THEORETICAL BASES OF THE METHOD OF DYNAMIC LIGHT SCATTERING

The method of dynamic light scattering is used to study the changes in the dynamic parameters of molecules (for example, translational diffusion coefficient or hydrodynamic radius of the particles). The method studies the correlation function of the fluctuations of the scattered light intensity caused by the Brownian motion of the particles. For solutions of macromolecules, the correlation function $c(t)$, which describes molecular motion, can be associated with translational diffusion coefficient D_t:

$$c(t) = a\langle E^*(0)E(t)\rangle = c_0\exp(-D_t k^2 t). \quad (1)$$

The appropriate method for determining $c(t)$ is called the method of photon correlation. It is known that there is a relationship between the function of the spectral density of molecular motion $S(\omega)$ and $c(t)$ in the form:

$$S(\omega) = \frac{1}{2\pi}\int_{-\infty}^{\infty} c(t)e^{iat}dt. \quad (2)$$

The function $S(\omega)$ can be determined by the method of light mixture [4]. During dynamic light scattering, the radiation is detected at a constant angle (usually, 90°). The diffuse motion of the particles in solution increases the fluctuations in the intensity of scattered light at the micro-second time interval.

In the simplest case of an ideal translational diffusion of small, compared with the 1/q value, non-interacting identical spherical particles in solution, it can be shown that the power spectrum of the photocurrent is a Lorentz curve with the half-width $\Gamma = q^2 D_t$, and the correlation function is an exponent with the relaxation time $\tau_{rel} = 1/\Gamma$. Thus, the translation diffusion coefficient D_t can be easily determined by the spectrum.

The characteristic relaxation time of the fluctuations is proportional to the size of the particles. In the case of a polydispersed solution, i.e., if it contains particles of different sizes, the photocurrent spectrum is a continuous set (integral) of the Lorentz curves with different half-widths:

$$g_1(\tau) = \int G(\Gamma)e^{-\Gamma t}d\Gamma. \quad (3)$$

Therefore, to find the particle-size distribution (diffusion coefficient) it is necessary to solve the inverse spectral sum in the form of an integral equation with the Lorentz kernel.

For polydisperse particles or particles with characteristic dimensions comparable to the wavelength of the original beam, the auto-correlation function is the sum of the contributions of intensities of particles of different sizes and different diffusion modes. In this case, the interpretation of D_t is a rather difficult task.

The relationship between the static and dynamic parameters of light scattering in solutions of macromolecules was considered in [5]. The concentration dependence of translational diffusion coefficient D_t can be represented by a virial decomposition in low concentrations. According to this, the relationship between coefficient D_t, molecular mass M, coefficient of intermolecular interaction B, and intrinsic viscosity of the protein solution $[\eta]$ is determined by the equation:

$$D_t = D_0\{1 + (2BM - [\eta])c\}. \quad (4)$$

Intrinsic viscosity $[\eta]$ is the limiting viscosity value at $c \longrightarrow 0$. D_0 is the translational diffusion coefficient defined by the formula (3).

2. THE OBJECT OF STUDY

We used aqueous solutions of type-I collagen obtained from calf skin (Sigma Aldrich, Germany) with the addition of lead acetate $Pb(CH_3COO)_2$, cesium chloride CsCl, and sodium chloride NaCl.

Collagen is the major structural protein of the intercellular matrix. It comprises 25–33% of the total protein in the human body and 6% of the body mass. The name "collagen" unites a family of closely related fibrillar proteins, which are the major protein components of skin, bones, tendons, cartilage, blood vessels, and teeth. In different tissues, different types of collagen predominate, and this, in turn, is determined by the role that collagen plays in a particular organ or tissue. A total of 95% of the total collagen in the human body is collagen of the I, II, and III types, which form very strong fibrils. Type-I collagen type is part of the skin, tendons, bones, cornea, placenta, arteries, liver, and human dentin.

The collagen molecule is a right-handed helix made up of three α-strands. Such a formation is known as tropocollagen. One coil of α-helix contains three amino-acid residues. Collagen molecules are not connected "end to end," between them a gap of 35–40 nm occurs. Electron microscopy shows that the collagen fibrils are composed of molecules that are

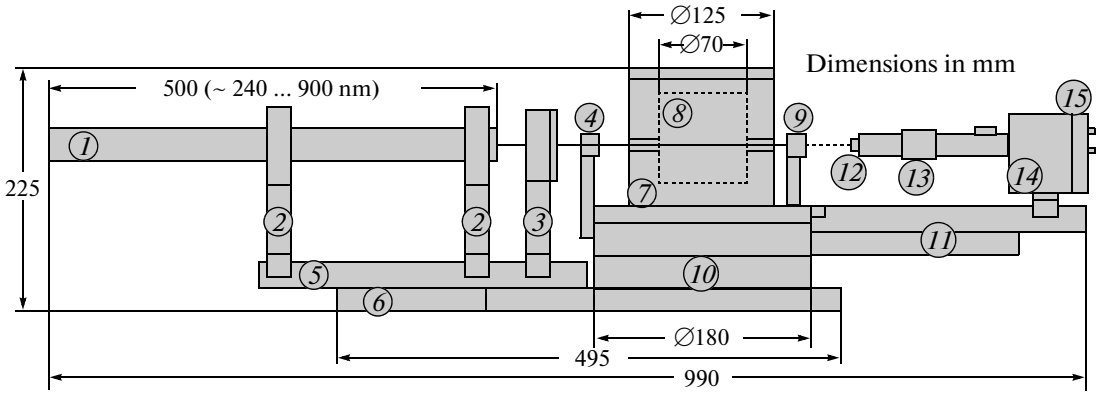

Fig. 1. Scheme of the experimental apparatus. An optical bench (*5*) and precision turntable (*10*) are mounted on a rigid base plate (*6*). A Diode laser (*1*) and the focusing optics (*3*) are fixed on the bench. The thermostat (*7*) and the sample-cell holder (*8*) are coaxial with the turntable axis. The photon-counting system (*14*) is mounted on the rotating arm of turntable (*11*). The photon-counting system consists of receiving optics (*13*) with interchangeable pinholes for selection of the aperture (*12*), a low-noise photomultiplier that operates in a photon-counting mode, and a rapid amplifier-discriminator (*15*) with a through direct-current tract and a special high-voltage power supply for PMT with no spurious correlations.

displaced with respect to each other by about 67 nm (a unit that is designated by the letter D and varies depending on the state of substance hydratation). It is considered that such a structure maximizes the resistance of the aggregate to tensile loads [6].

The choice of the investigated metals was determined by their abundance in nature. Lead in large amounts can be found in the environment and is ingested with inhaled air or deposited on the skin. Lead and its compounds are extremely toxic. When entering in the body, lead accumulates in the bones and causes their destruction. The maximum permissible concentration of lead compounds in air is 3.8 mg/m^3, and that of lead acetate, which was used in this study, 0.01 mg/m^3 [7].

Cesium is related to the group of heavy metals. Cs^{137} is a beta-gamma-emitting radioisotope, one of the main components of the radioactive contamination of the biosphere. It is contained in radioactive wastes and discharges of the plants that recycle wastes from nuclear-power plants.

Sodium is an integral part of all human biological fluids, such as blood, sweat, and lymph. In this regard, it is useful to study the mechanism of interaction of the collagen molecules with ions of heavy (lead and cesium) and light (sodium) metals.

The investigations were carried out at different ionic strengths of the solutions, i.e., at different concentrations of metal ions. The ionic strength of the solution μ was calculated using the formula (5):

$$\mu = \frac{1}{2}\sum z_i^2 c_i, \qquad (5)$$

where c_i is the molar concentration of the ion particle and z_i is the number of the elementary charges.

3. EXPERIMENTAL APPARATUS

We used a Photocor-FC optical unit (Fig. 1) with a diode laser (wavelength 647 nm and power 25 mV) [5, 3]. The measurements were carried out at 90°; the signal from the photomultiplier (Perkin Elmer) was analyzed using a software package [8]. Room temperature (20°C) was kept in the cell with the test solution using a thermostat integrated in the instrument.

4. RESULTS AND DISCUSSION

The nonlinear dependences of the translational-diffusion coefficient D_t on pH with a maximum near the protein's isoelectric point (pH 6.0), which corresponds to the minimum value of the surface charge of the protein molecule, were obtained in the study of the dynamic parameters of the collagen molecules in pure solution and in solutions with additives of salts, such as NaCl and $CaSO_4$ [2]. In accordance with (5), the translational diffusion coefficient D_t should decrease if the protein's intrinsic viscosity exceeds the product of the molecule mass by the coefficient of intermolecular interaction. Indeed, according to the literature [9], the intrinsic viscosity of collagen (1150 cm^3/g) is higher by more than two orders of magnitude than the viscosity of globular proteins, such as albumin (3.7 cm^3/g).

In the present study, we obtained the pH-dependences of the translational diffusion coefficient D_t for collagen molecules in a pure aqueous solution (concentration of protein $c = 0.05$ mg/ml) and in solutions with additives of lead acetate $Pb(CH_3COO)_2$, cesium chloride CsCl, and sodium chloride NaCl (Figs. 2–5).

As can be seen from Fig. 2, in a pure aqueous solution of collagen the pH-dependence of translational diffusion coefficient has a nonlinear form that is close to parabolic with a maximum at the isoelectric point of the protein pI 6.0 (Fig. 2, curve *1*). When lead ions are

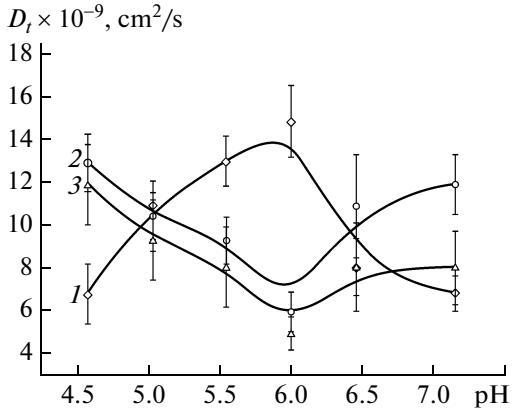

Fig. 2. The pH-dependence of the translation diffusion coefficient D_t for collagen in (*1*) pure aqueous solution and with the $Pb(CH_3COO)_2$ additives at (*2*) $\mu = 10^{-4}$ mol/l and (*3*) $\mu = 10^{-3}$ mol/l.

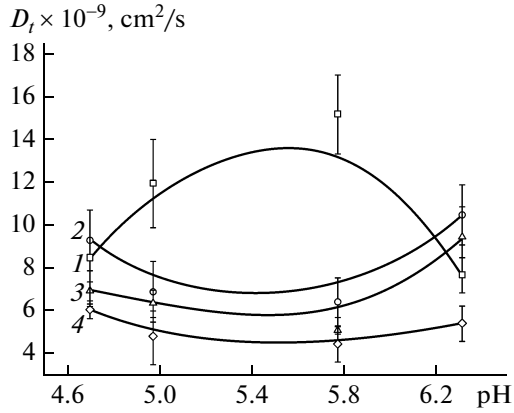

Fig. 3. The pH-dependence of the translation diffusion coefficient D_t for collagen in (*1*) pure aqueous solution and with the CsCl additives at (*2*) $\mu = 10^{-5}$ mol/l, (*3*) $\mu = 10^{-4}$ mol/l, and (*4*) $\mu = 10^{-3}$ mol/l.

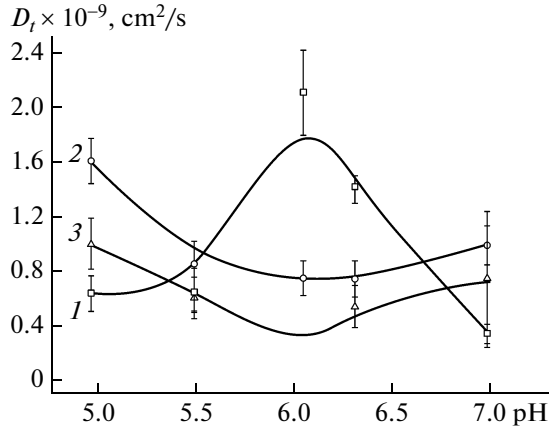

Fig. 4. The pH-dependence of the translation diffusion coefficient D_t for collagen in (*1*) pure aqueous solution and with additives of (*2*) $Pb(CH_3COO)_2$ $\mu_{Pb} = 10^{-3}$ mol/l, (*3*) $Pb(CH_3COO)_2$ $\mu_{Pb} = 10^{-3}$ mol/l and NaCl $\mu_{Na} = 10^{-3}$ mol/l.

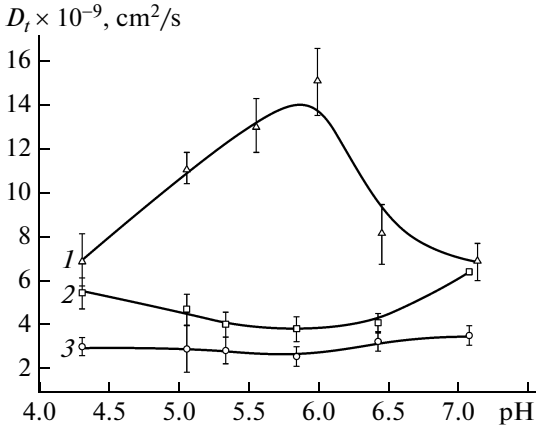

Fig. 5. The pH-dependence of the translation diffusion coefficient D_t for collagen in (*1*) pure aqueous solution and with additives of (*2*) CsCl $\mu_{Cs} = 10^{-3}$ mol/l, (*3*) CsCl $\mu_{Cs} = 10^{-3}$ mol/l and NaCl $\mu_{Na} = 10^{-3}$ mol/l.

added to the solution, the character of dependence changes; a minimum now occurs near the isoelectric point (Fig. 2, curves *2* and *3*) in contrast to the pure solution. This is likely to be due to the change of the nature of the interactions of collagen molecules in aqueous solution (from Coulomb to dipole–dipole interactions) and to the formation of protein nanoclusters [5].

Figure 2 shows that the minimum at the protein's isoelectric point becomes deeper with increasing ionic strength. This indicates that the mass of the scattering particles also increases with increasing concentration of Pb^{2+} ions in solution, which is associated with an increased clustering effect.

Similar relationships were obtained for aqueous solutions of collagen with cesium salt (Fig. 3). Figure 3 shows that the pH-dependence of the translational diffusion coefficient D_t of the collagen molecules in aqueous solution containing Cs^+ ions also has a nonlinear form with a minimum at the protein isoelectric point and decreases with increasing concentration of heavy-metal ions (Fig. 3, curves *2–4*).

It was also found that the addition of sodium ions in aqueous solutions of collagen containing heavy-metal (Pb^{2+}) ions results in a decrease in translational

diffusion coefficient at the protein isoelectric point (Fig. 4, curves *2* and *3*). From this it can be concluded that sodium ions enhance the effect of clustering of molecules in an aqueous protein solution that contains ions of the heavy metal Pb^{2+}.

A similar effect was observed when NaCl was added to an aqueous protein solution containing Cs^+ ions (Fig. 5, curves *2* and *3*).

CONCLUSIONS

It was found that the addition of $Pb(CH_3COO)_2$ and CsCl to aqueous solutions of collagen results in a decrease of the translation diffusion coefficient at the protein's isoelectric point (pI 6.0), which is associated with the growth of the mass of scattering particles during the formation of protein nanoclusters.

It was revealed that the minimum in the pH-dependence of translational diffusion coefficient becomes deeper with increasing concentrations of Cs^+ and Pb^{2+} ions in aqueous solutions of collagen, which is associated with an increase in the mass of scattering particles.

It was found that the addition of Na^+ ions to aqueous solutions of collagen containing Cs^+ and Pb^{2+} ions leads to a decrease of the translational diffusion coefficient and to an enhancement of the clustering effect.

Thus, the effect produced by the heavy metals results in a change in the molecular-dynamic parameters of the collagen molecules in aqueous solutions, i.e., a decrease in the translational diffusion coefficient and an increase of the linear dimensions of particles. The content of heavy metals in the environment is rapidly increasing as a result of human activities; therefore, the study of the effects of those harmful factors on the human body, including the mucous membranes and skin, is of great practical importance for medicine, ecology, and biophysics. Data, obtained with the model protein solutions, makes possible the determination of potential patological process in human body, which occure because of adverse ecological enviroment conditions.

REFERENCES

1. R. P. Boot-Handford and D. S. Tuckwell, BioEssays **2** (25), 142 (2003).
2. I. A. Sergeeva, N. V. Sokol, M. S. Ivanova, et al., Moscow Univ. Phys. Bull. (Engl. Transl.) **64** (4), 446 (2009).
3. A. D. Lebedev, Yu. N. Levchuk, A. V. Lomakin, et al., *Laser Correlation Spectroscopy in Biology* (Kiev, 1987) [in Russian].
4. H. Z. Cummins and E. R. Pike, *Photon Correlation and Light Beating Spectroscopy* (Plenum, New York, 1974; Moscow, 1978).
5. Yu. M. Petrusevich and G. P. Petrova, Biomed. Radioelektron., No. 3, 41 (2000).
6. *Biochemistry: Textbook for Universities*, Ed. by E. S. Severin (2003) [in Russian].
7. R. K. Rajaram and J. K. Mohana Rao, Z. Kristallogr. - Cryst. Mater. **160** (3–4), 225 (1982).
8. http://www.photocor.ru/dynals.ht.
9. E. Marshell, *Biophysical Chemistry* (Moscow, 1981), Vol. 1 (Moscow, 1981) [in Russian].