ISSN 0027-1349, Moscow University Physics Bulletin, 2011, Vol. 66, No. 2, pp. 190–195. © Allerton Press, Inc., 2011.
Original Russian Text © T.N. Tikhonova, G.P. Petrova, Yu.M. Petrusevich, K.V. Fedorova, V.V. Kashin, 2011, published in Vestnik Moskovskogo Universiteta. Fizika, 2011, No. 2, pp. 82–87.

Formation of Dipole Nanoclusters in Blood Serum Protein Solutions Containing Europium and Potassium Ions

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Received October 25, 2010; in final form, November 27, 2010

Abstract—Aqueous solutions of major serum proteins (albumin and g-globulin) with small concentrations of potassium and europium ions were investigated with the use of photon-correlation spectroscopy and atomic-force microscopy. The coefficients of translation diffusion, as well as the effective radiuses of the scattering particles in the solutions as a function of pH and salt concentration, were obtained. It was found that protein dipole nanoclusters form in these solutions, which was confirmed by AFM methods as well.

Keywords: serum albumin, g-globulin, dynamic light scattering, photon-correlation spectroscopy, atomic-force microscopy, translation diffusion coefficient, isoelectric point

DOI: 10.3103/S0027134911020172

INTRODUCTION

In recent years, protein aggregation processes have been widely discussed because of their importance in many areas of biotechnological, pharmaceutical, and medical research. Despite this, the global understanding of the phenomena and its main mechanisms remains far from complete. Protein aggregation is characterized by multiple interactions, in particular, by conformational changes and intermolecular interactions, which affect each other strongly. The hierarchy of these mechanisms depends on the medium properties and on some physical and chemical parameters, such as temperature, pH, ionic force, etc [1].

A new physical phenomena has been discovered in work conducted at the Physics Department of MSU, viz., the formation of dipole clusters in solutions of various proteins containing ions of heavy metals [2–4].

We investigated the formation of protein nanostructures in solutions of albumin and gamma-globulin containing ions of potassium and europium with the use of photon-correlation spectroscopy and atomic-force microscopy.

PHYSICAL MODEL

It was found earlier that cluster formation depends on metal ion radius [2–4]. Interaction of these ions with a protein surface involves, as a rule, their hydrated shells. In cases where protein solutions contain small ions like Na^+ (the ion radius equals 0.87 Å), dipole clusters are not formed, because sodium ions are located near the protein surface surrounded by water molecules and cannot bind directly with the negative charges on the protein.

The energy of the ion and the water dipole molecule binding, determined by equation

$$E_{pq} = \frac{q^2 p_w^2}{12\pi\varepsilon r_0^4 kT},$$

is inversely proportional to the fourth power of the ionic radius. In this case, it may be of the same order or less than the heat energy kT. In these cases, the water cannot stay on ion surfaces. This is observed for ions with large radii, such as Cs^+, Rb^+, Cd^+, Ce^+, Pb^{2+}, and Eu^{3+}, as well as K^+. (Here, q is the ion charge, p_w is the water molecule dipole moment, ε is water permittivity ~80, and r_0 is the metal ion radius.)

In interacting with the protein surface directly, a metal ion with a large radius is bound more strongly to negatively charged groups on the protein and can form a Coulomb complex on a protein macromolecule with a common hydrated shell. In this case, the metal ions compensate completely for the local surface charge of the protein molecule [5].

The effective decrease of the protein surface charge that takes place as a result of strong binding of metal ions with a large radius and the macromolecule can lead to a situation where the main type of interaction between the protein molecules is a dipole–dipole attraction instead of Coulomb repulsion, because the

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proteins have abnormally high dipole moments (several hundred Debyes).

Changes in the intermolecular interaction pattern lead to the emergence of molecular nanostructures in protein solutions, viz., dipole protein clusters.

TEST SUBJECTS. ORDER OF SOLUTION PREPARATION. EXPERIMENTAL UNITS

Two soluble proteins were used in this work: human albumin (HSA) and g-globulin (IgG) (Sigma). The test solutions were prepared in laboratory conditions immediately before beginning the experiments. The protein was weighed on an Adventured electron analytical balance, allowing measurements with a precision of up to 0.1 mg. The solutions were prepared in Clinicon cuvettes with a volume of ~5 ml. Medical-grade water in shot ampoules was used as the solvent for the protein solutions preparations.

For the sake of convenience and in order to avoid damage to local protein structures from concentrated reagents, preparation of the systems under investigation must be conducted in several steps:

(1) preparation of a protein primary solution at a concentration of 50 mg/ml (the total solution quantity was calculated with the estimated number of experiments taken into account);

(2) preparation of salt solutions with the appropriate ionic force value, according to the following equation:

$$\mu = \frac{1}{2}\sum (z_i^+ n^c + z_i^- n^a),$$

where z and n are the charges and the partial concentration of the cations and anions in the solution (the total amount is calculated with the estimated number of experiments taken into account);

(3) changing the concentration of free protons in the solvent (water or salt solution) to reach a given pH value through the addition of a small amount of weak HCl or KOH solutions (the chemical vessel is a glass ampoule with a volume of 5 ml);

(4) preparation of the final system through the addition of 20 μl of solution (1) into solution (3). As a result, we obtained a protein solution with the final concentration of 0.2 mg/ml. After this, we measured the pH again;

(5) the protein solution concentrations were varied within limits from 0.2 to 1.4 mg/ml.

Samples for AFM were prepared as follows. Freshly cleaved graphite was used as the substrate. It was coated with glutaric aldehyde, which was used to hold biological tissue for investigation by electron microscopy. Then, the aldehyde was washed away with water and the substrate was coated with the protein solution. When using the AFM, the albumin concentration in the solution did not change and was always equal to 0.8 mg/ml. The protein solution remained on the graphite for some time and then it was pumped out with the use of a measuring dropper. This was done so that separate protein molecules would remain on the graphite substrate.

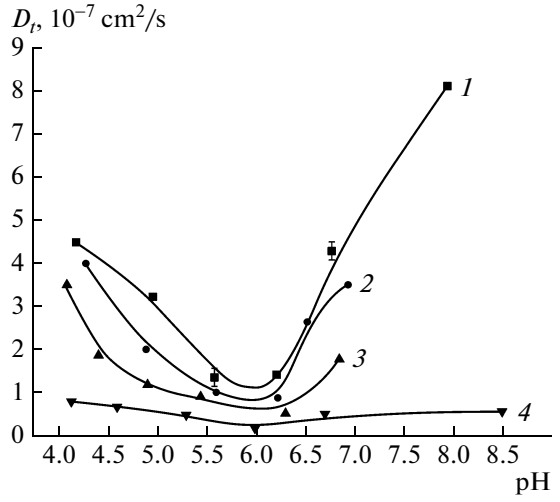

Fig. 1. Dependence of the translation diffusion coefficient D_t of g-globulin on pH value for aqueous solutions with addition of KCI salt. *1*, Aqueous solution of g-globulin; *2*, solution of γ-globulin with KCl (I = 0.001 mol/l); *3*, solution of γ-globulin with KCl (I = 0.005 mol/l); *4*, solution of γ-globulin with KCl (I = 0.01 mol/l).

In the present work, albumin and g-globulin aqueous solutions were investigated using two methods: dynamic light scattering and AFM. The experiments on dynamic light scattering were conducted with the use of a Photocor Complex photon-correlation spectrograph [6]. The AFM experiments were conducted with the use of an AFM Solver P47 unit [7].

RESULTS AND DISCUSSION

In this work the translation diffusion coefficients of albumin and g-globulin in solutions containing KCl with variation of the pH and the ionic force were obtained. The results are shown in Figs. 1 and 2. As one can see from the dependences presented, the Debye values decreased in the region of the protein's dielectric point (at pH 5 and 6, respectively) with the growth in the K^+ ion concentration.

According to the Stokes–Einstein formula, a decrease of the translation diffusion coefficient by approximately two times means that the radius of the scattering particles doubles. In this case, the particle (clusters) mass increases approximately by a factor of ten compared to the protein's molecular mass. Thus, the γ-globulin and albumin molecules aggregate near their isoelectric points due to the effects of the potassium ions.

Potassium plays an important role in an organism's vital functions. The electrical properties (rest potential and action potential) of most cells are determined by

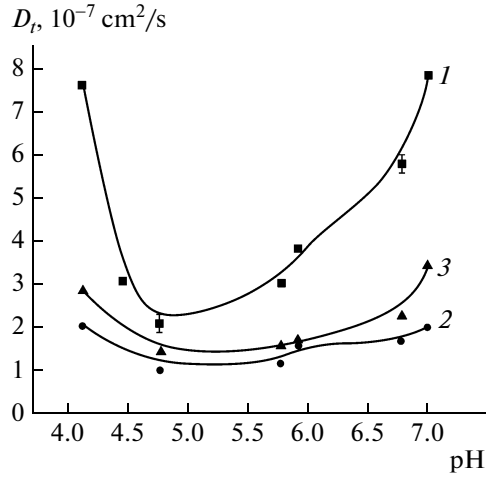

Fig. 2. Dependence of the translation diffusion coefficient D_t of albumin on pH value for aqueous solutions with addition of KCl salt. *1*, Aqueous solution of albumin; *2*, solution of albumin with KCl (I = 0.01 mol/l); *3*, solution of albumin with KCl (I = 0.005 mol/l).

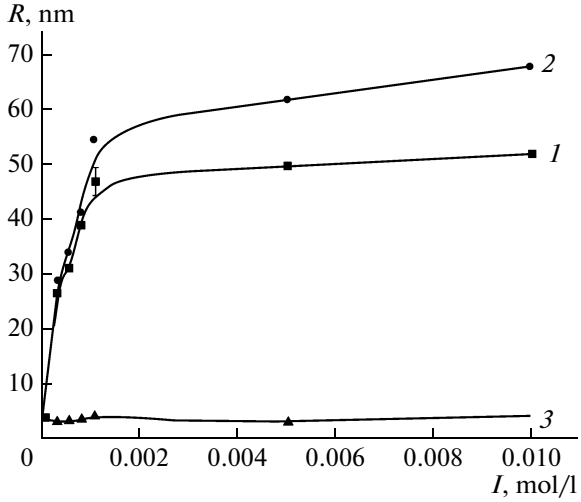

Fig. 3. Graph of albumin nanocluster radius dependence on the solution's ionic force. *1*, Aqueous solution of albumin with KCl; *2*, albumin solution with $Eu(NO_3)_3$; *3*, albumin solution with KCl upon ultrasonic treatment.

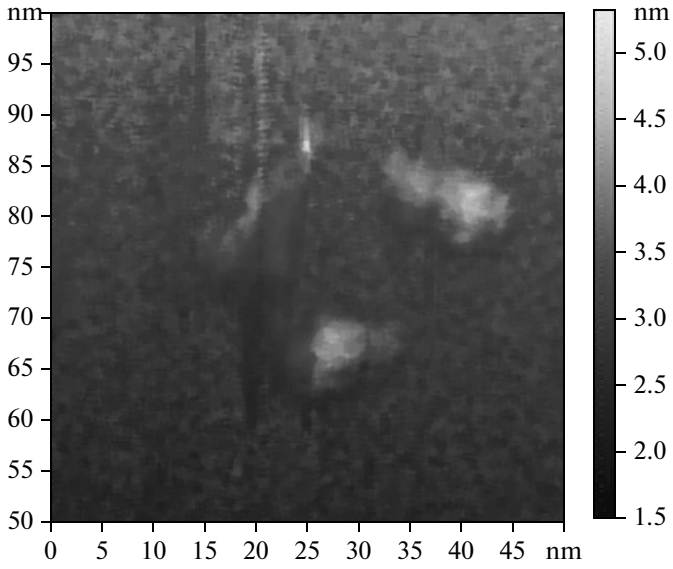

Fig. 4. The Albumin molecule structure obtained with the use of a tunneling microscope.

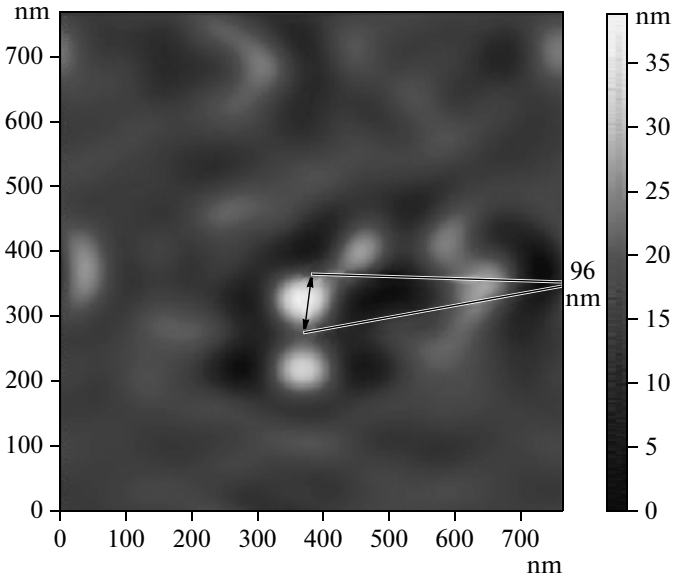

Fig. 5. A 2D image of the particles formed in the albumin solution upon addition of KCl. The image was obtained with the use of AFM.

this ion, as well as by the calcium and sodium ions. That is why these ions are called potential generating ions. It is known that sodium is present in blood and in plasmatic liquid of the intercellular space, while calcium in humans is located mainly inside cells. Thus, almost 50 times more potassium occurs in myocytes than in the intercellular space. Much energy is consumed to maintain such a concentration imbalance. With the use of molecular machines, viz., ATPases, sodium is pumped out of cells and potassium is pumped in. When a person is in a pathological state, their cell membranes may deteriorate (cell lysis). In this case, the potassium leaving the cells disturbs the protein synthesis process and leads to macromolecular clustering.

As an example of the influence of a metal with a large ionic radius on albumin, results can be presented from measuring the radii of clusters formed in protein solutions containing small concentrations of europium salt (Fig. 3). This figure also shows the influence of the potassium ion on albumin for comparison.

The same graph also shows the dependence of the particle radius on the solution's ionic force upon treating the solution with ultrasound (3). A solution con-

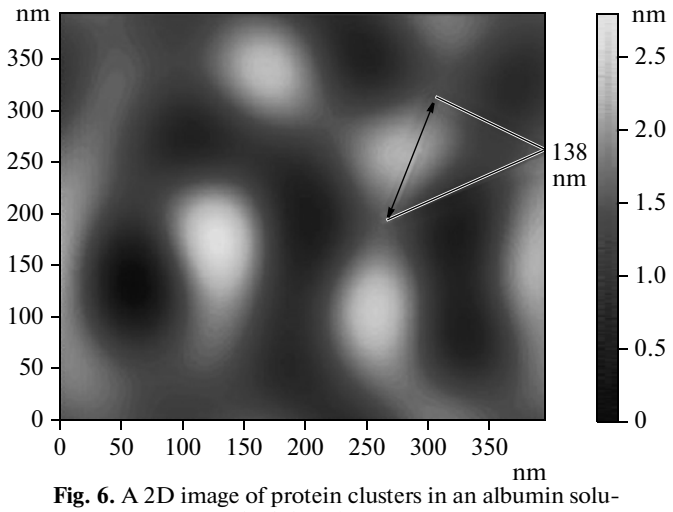

Fig. 6. A 2D image of protein clusters in an albumin solution containing $Eu(NO_3)_3$ salt.

taining formed particles was placed in an ultrasonic bath with a power of 50 W for 7–10 min. Then, the solution was removed from the ultrasonic bath and investigated with the use of photon-correlation spectroscopy. It turned out that ultrasound destroys nanoclusters, and the particle size decreased to about 5 nm, which, within the accuracy of the measurement, is the size of the albumin molecule.

With the use of the atomic-force microscope (AFM), images of the main serum proteins albumin and γ-globulin were obtained for the first time, as well as images of protein clusters that formed in the solutions upon addition of metal ions with large ionic radii. Graphite with a high level of surface orientation (HOG), which is often used in work with biomaterials, was used as the substrate [8, 9].

As an example, the photos shown in Figs. 4–6 show the images of albumin molecules and protein aggregates in albumin solutions with potassium and europium ions.

The photo shown in Fig. 4 was obtained with the use of a tunneling microscope; it gives an idea of the size of an albumin molecule (~8 nm).

The photo shown in Fig. 5 was obtained with the use of AFM. Larger formations can be seen in it that emerged in the albumin solution upon addition of KCI. As can be seen, the particle size increased almost by a factor of ten.

The photo shown in Fig. 6, which was obtained with the use of AFM, shows an image of the particles that formed in the albumin solution upon the addition of the salt $Eu(NO_3)_3$.

The interaction of albumin with potassium and europium salts was investigated using the method of photon-correlation spectroscopy as well. The sizes of the protein nanoclusters, which were obtained with

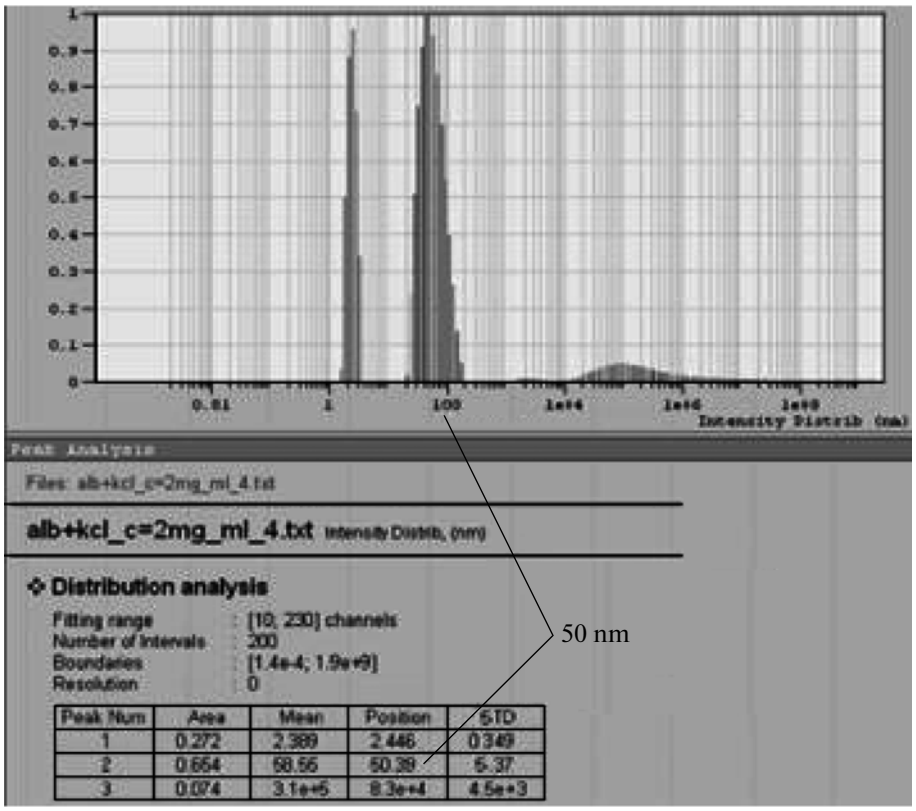

Fig. 7. The radius of nanoclusters in an albumin solution upon addition of KCl. The radius was detected by a correlation spectrograph.

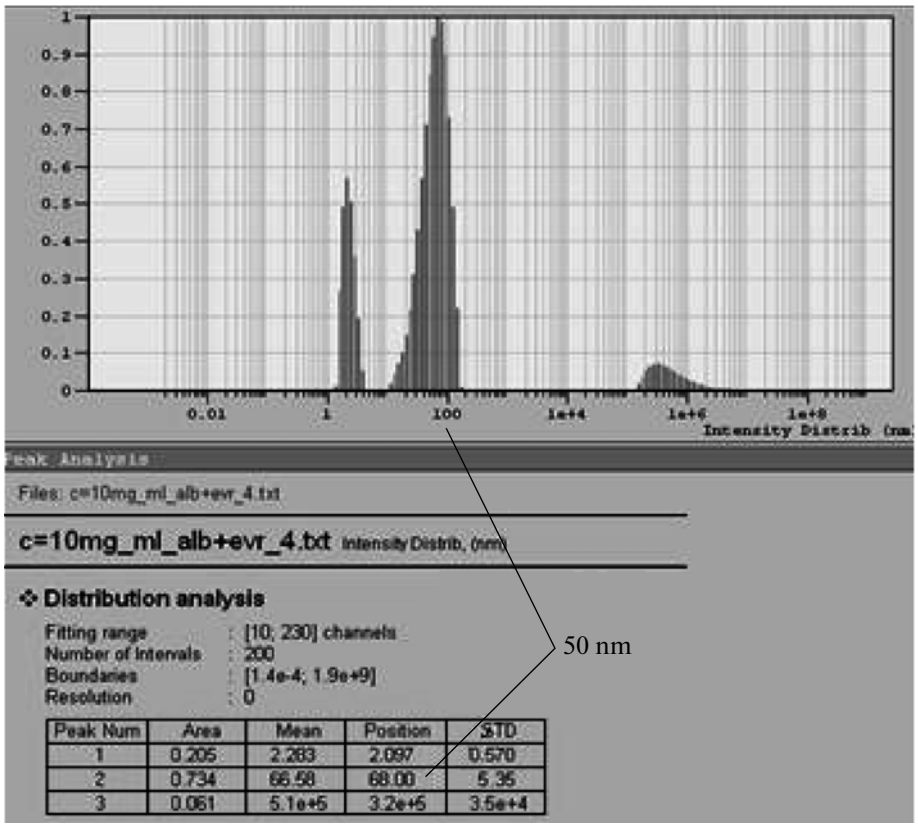

Fig. 8. Radius of nanoclusters in the albumin solution upon addition of $Eu(NO_3)_3$. The radius was detected by a correlation spectrograph.

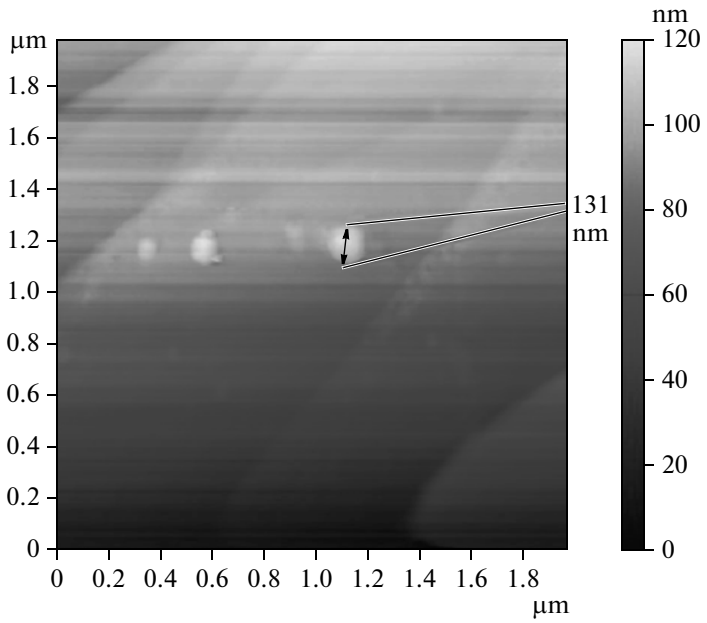

Fig. 9. A 2D image of the particles formed in a γ-globulin solution upon addition of KCl. The image is obtained with the use of AFM.

the use of AFM, agree well with the sizes of the same particles that were obtained with the use of a correlator; their diameters were about 100 and 138 nm in the albumin interactions with KCl and $Eu(NO_3)_3$, respectively. This can be clearly seen when comparing Figs. 5 and 6 with Figs. 7 and 8.

Figures 7 and 8 show the results of the computer processing of the correlation function [10]. The first peak corresponds to the spectral density of the intensity of the albumin molecules' Brownian motion (the particle size was about 2 nm). Not all molecules of this protein are bound with europium ions; therefore, a part of the albumin is present in the solution in the free form. The second peak corresponds to the scattering on clusters (particles with a size of about 68 nm) that formed as a result of albumin interaction with europium ions. The third, wide, peak describes the contribution from the dust particles present in the solution.

Images of nanoclusters formed during the interaction of the g-globulin with potassium ions were also obtained on the atomic-force microscope (Fig. 9).

The particle size was about 130 nm, which agrees well, within the margin of error, with the data from photon-correlation spectroscopy.

CONCLUSIONS

Macromolecular aggregation of major serum proteins was investigated in aqueous solutions that contained potassium and europium ions using the methods of dynamic light scattering and probe microscopy. Different parameters of the medium, such as the protein macromolecule concentration, the solution pH value, and the salt concentration, were varied.

As a result, a decrease of the particles' translation diffusion coefficient in the albumin and γ-globulin solutions upon addition of potassium ions was observed, which indicates the formation of dipole nanoclusters.

For the first time, images of nanoclusters that formed during the interaction of albumin with potassium and europium salts were obtained with the use of AFM. It was shown that the AFM method is a noninvasive method for protein molecule investigation, because the cluster sizes that were obtained using the methods of photon-correlation spectroscopy agree with the sizes of the same clusters that were obtained with the use of AFM.

It was shown that upon the addition of $Eu_3(NO_3)_3$ salt to the albumin solutions, the sizes of the scattering particles increase by approximately 20 times.

It was found that in the process of nanocluster formation in albumin solutions containing K^+ and Eu^{+++} ions saturation was observed (the particle size did not change) at an ionic force of about 0.001 mol/l, which corresponds to the MPC for these metals.

Thus, we discovered and explained the mechanism of the formation of nanosized dipole clusters in protein solutions containing different metal ions [2–4].

One can suppose that the formation of protein clusters in humans due to the influence of heavy metals leads to the malfunction of an entire set of enzyme reactions and transport and immune functions of blood and lymph, as well as causing structural changes of a set of tissues.

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