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THE ROLE OF HEAVY METALS IN THE FORMATION OF PROTEIN CLUSTERS IN AQUEOUS SOLUTIONS

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The formation of macromolecular protein clusters in the presence of heavy metal ions was discovered using the Rayleigh scattering technique. The conditions attending the formation and destruction of such clusters are studied. It is demonstrated that the mass of the clusters is a maximum at the isoelectric point of the protein and increases with the increasing of ionic strength of the solution. The formation of protein clusters in the presence of toxic heavy metal ions in living cells is of substantial physiological and ecological importance. The molecular mechanism is discussed whereby such clusters are formed.

Ecologists are well aware of the high toxicity of a number of heavy metals such as mercury, lead, tin, thallium, tellurium, chromium, and many others. So low a concentration of these metals in air as some 10^{-2} mg/m^3 is toxic to man. When in aqueous solutions, mercury is toxic to man in a concentration of 5×10^3 mg/l, and nickel, lead, titanium, bismuth, and chromium, in a concentration of 0.1 mg/l. The toxicity of heavy metals such as cesium and rubidium is little known, and it is these metals that are the subject of this study.

Some chemical and biophysical mechanisms are known whereby heavy metals affect the functioning of living cells and organisms, for example, the poisoning of enzymes, disturbance of membrane penetrability and electron transport, the blocking of nervous conductivity, and development of chain free-radical processes. At the same time, there exist purely physical mechanisms whereby charged heavy metal ions interact with biological macromolecules, proteins in the first place. The interaction results include the formation of protein clusters, anomalous molecular motion of charged biopolymers, and anomalous sorption of heavy metal ions on the surface of lipoproteins and biological membranes.

This paper considers the physical processes in solutions that are associated with the formation of protein clusters in the presence of heavy metal ions.

The interaction of protein macromolecules in a water solution is governed by the electrostatic forces developing between the charges of the macroions and those of the low-molecular ions surrounding them, the total concentration of ions in the solution being its ionic strength. An ion-containing solution as a whole remains electronegative. The interaction of macroions in a solution containing, in addition to the low-molecular solvent, a third component in the form of a strong electrolyte was theoretically analyzed by Scatchard [1]. According to this theory, the expression for the second virial coefficient B (in the expansion for the free energy) can be written as

$$B = \frac{V_1}{M_2^2}\left(\frac{Z^2}{4m_3} + \frac{\beta_{22}}{2} - \frac{\beta_{23}^2 m_3}{4 + 2\beta_{33} m_3}\right). \quad (1)$$

Here V_1 is the specific volume of the solvent, Z is the charge of the macroion, M_2 is its mass, and m_3 is the concentration of the salt ions. The parameters β_{22}, β_{23}, and β_{33} which are the derivatives of the

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activity coefficients characterize the various interactions among the ions in the solution: the effect of the excluded volume and interaction between the charges of various macroions (β_{22}), the interaction between the macroions and salt ions (β_{23}), and the interaction between the salt ions alone (β_{33}).

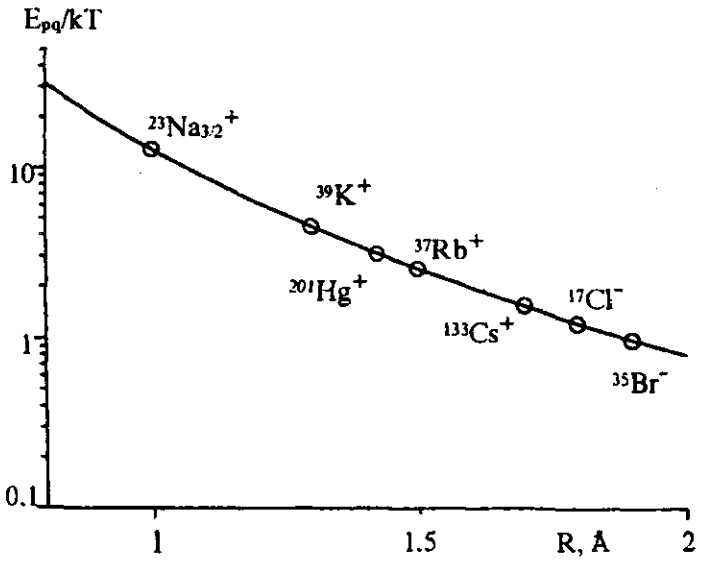

Fig. 1

Relative interaction energy of a charged ion-water dipole as a function of the ionic radius.

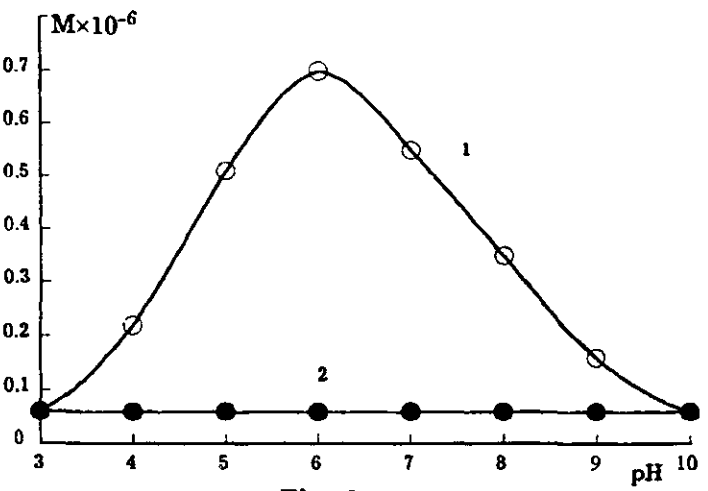

Fig. 2

Mass of scattering particles in a serum albumin solution as a function of its pH value: (*1*) in the presence of the salt CsCl at an ionic strength of $\mu = 0.1$ mole/l; (*2*) in the absence of the salt.

According to formula (1), the intermolecular interaction coefficient varies with the rise of the total charge of the protein by a parabolic law, in proportion to Z^2 (Donnan effect), and has a minimum at the point $Z \cong 0$ (isoelectric point) [2]. The coefficient β_{22} is usually small in comparison with the other terms in formula (1). At high salt concentrations in the solution the term containing the coefficient β_{23} may materially exceed (in absolute value) the first two terms, and the parameter B may become negative.

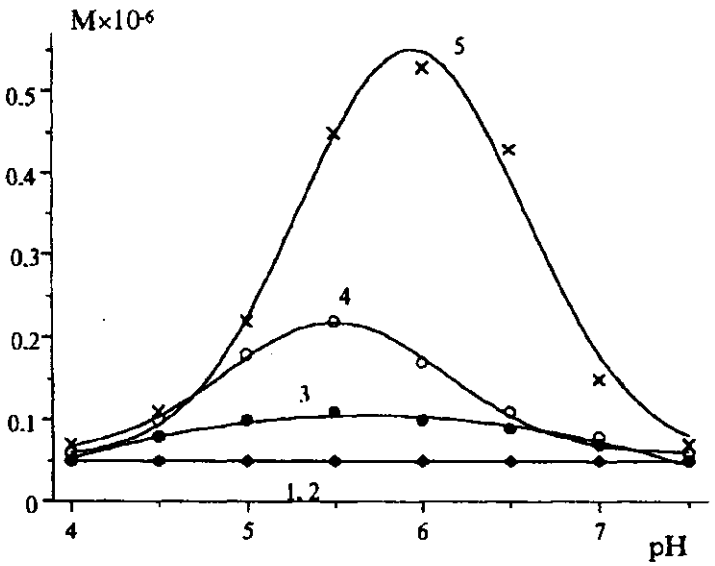

Fig. 3

Mass of scattering particles in an ovalbumin solution as a function of its ionic strength: (*1*) ovalbumin + water, $\mu = 0.001$ mole/l; (*2*) ovalbumin + water + NaCl, $\mu = 0.1$ mole/l; (*3*) ovalbumin + water + CsCl, $\mu = 0.01$ mole/l; (*4*) ovalbumin + water + CsCl, $\mu = 0.1$ mole/l; (*5*) ovalbumin + water + CsCl, $\mu = 0.2$ mole/l.

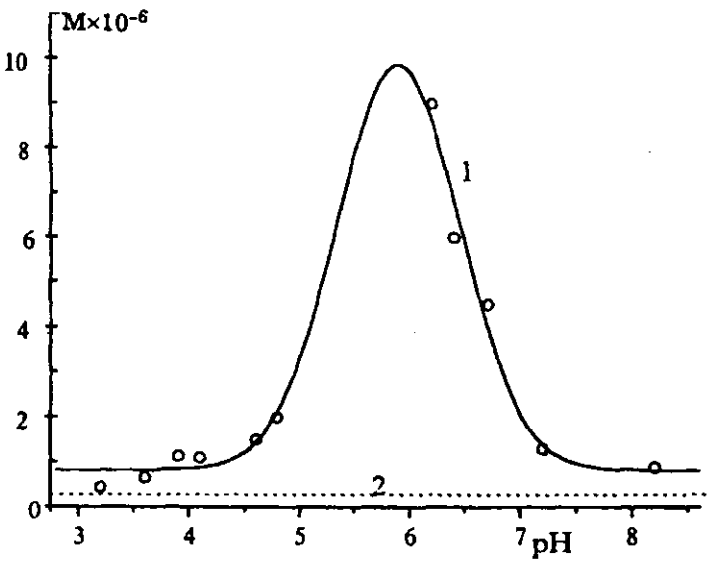

Fig. 4

Mass of scattering particles in a serum albumin solution as a function of its pH value at an ionic strength of $\mu = 0.1$ mole/l: (*1*) in the presence of the salt RbCl; (*2*) in the presence of the salt NaCl.

The Rayleigh scattering technique [3, 4] is a very efficient method to determine the interaction coefficient B and the mass of macromolecules. As shown by Debye [4], in the case of molecular solutions the experimentally measured scattering coefficient R_θ of the solution (θ is the scattering angle) can be related

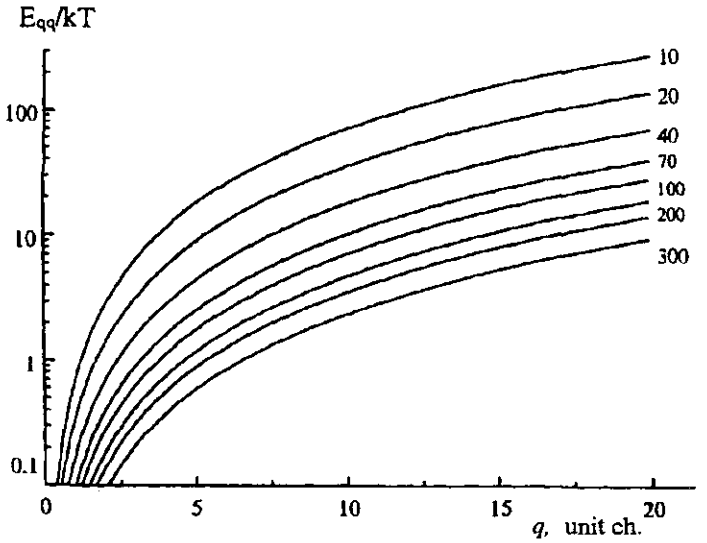

Fig. 5

Relative energy of charge-charge interactions between biopolymer macromolecules in aqueous solutions as a function of their elementary charge at different distances between them (distances in Angstrom units are indicated near the curves).

to the virial expansion for the osmotic pressure Π:

$$\frac{cHK}{R_\theta} = \frac{1}{RT}\frac{d\Pi}{dc} = \frac{1}{M} + 2Bc + \dots \tag{2}$$

where c is the concentration, $H = \dfrac{2\pi^2 n_0^2 (dn/dc)^2}{\lambda^4 N_A}$ is the so-called solution constant (Debye constant), R is the gas constant, λ is the wavelength of the exciting light, n_0 and n are the refractive indices of the pure solvent and solution, respectively, M is the mass of the macromolecule, and K is the Cabannes factor [5].

Investigations into the aqueous solutions of various proteins by this technique [6–9] have shown that the mass M of protein macromolecules in a solution is practically independent of changes in the surface charge of the protein (governed by the pH parameter). The relationship between the interaction coefficient (parameter B) and the pH value of the solution is nonlinear, with a minimum at the isoelectric point, which agrees with formula (1).

As the ionic strength of the solution grows higher, i.e., as the concentration of the dissolved salt, e.g., NaCl, is increased, more complex formations originate in the solution, with the participation of the ions Na^+ and Cl^-. In that case, a gegenion cloud is formed around the charged protein molecule, which shields the Coulomb interaction. The magnitude of B decreases with the increasing of the ionic strength μ, but the parabolic form of the dependence of B on pH remains. These effects were observed experimentally [6–9]. In the case of the protein lysozyme, the quantity B was found to change sign at a high ionic strength, which was governed by the rise (in absolute value) of the third term in Scatchard formula (1) [9].

As the concentration of the salt NaCl in the solution was increased, the minimum of the curves $B = f(\text{pH})$ was observed to shift from the isoelectric point toward lower pH values (a positive charge on the protein), as, for example, is the case with serum albumin [6] and γ-globulin [7]. The displacement of the minimum of B toward positive Z (lower pH values) was explained by Edsall and coworkers [6] by the binding of the chlorine ions (Cl^-) on the surface of the albumin molecule.

The ionic radius of Cl^- is 1.75 Å, and that of Na^+ is around 0.8 Å. For this reason, the chlorine ion binds stronger to the surface groups of the protein than Na^+. The ions of heavy alkali metals have a great mass and a great ionic radius (e.g., the ionic radius of cesium comes to 1.65 Å and that of rubidium is 1.47 Å), and so they retain but weakly their hydrate shell and thus can form on the surface of the protein

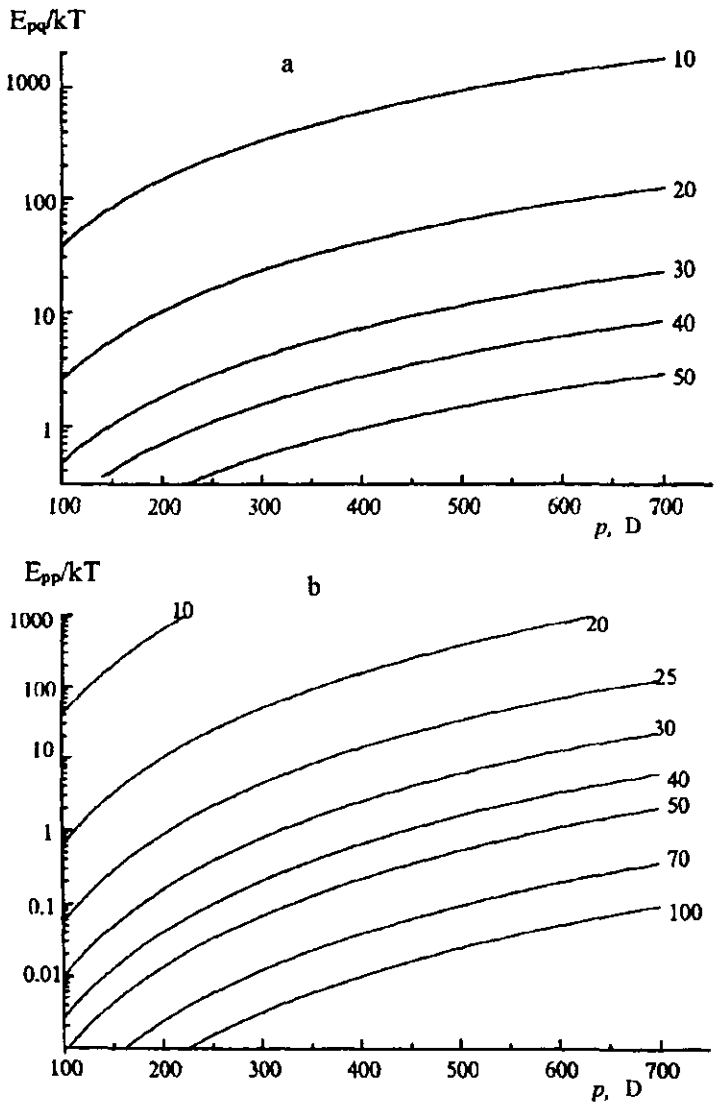

Fig. 6

Relative energy of dipole-charge (a) and dipole-dipole (b) interactions between biopolymer macromolecules in aqueous solutions as a function of their dipole moment at different distances between them (distances in Angstrom units are indicated near the curves). The curves of Fig. 6a were calculated for a macromolecule charge of $q = 10$ unit charges.

macromolecule a so-called Coulomb complex by combining with a negative counterion. In this connection, it might be expected that the processes of adsorption of the heavy ions Cs^+ and Rb^+ on the surface of a protein macromolecule will substantially differ from those of the light ion Na^+. That this is true is confirmed by the calculation results for the relative interaction energy between various charged ions and the dipole water molecule (Fig. 1).

In this work, we used the Rayleigh scattering technique to study aqueous solutions of ovalbumin and serum albumin (SERVA) in the presence of the salts CsCl and RbCl at various protein surface charge and ionic strength values. The experiments were conducted with a setup using a He-Ne laser and a photoelectric scattered light detector as described in [7].

The experimental data on the scattering coefficient at various protein concentrations and various ionic strength values were used to calculate the intermolecular interaction coefficients B [10] and the masses of

the scattering particles (Figs. 2–4).

As follows from the literature data, in all of the protein solutions studied earlier in the presence of NaCl [6–9] the mass of the scattering particles is independent of the salt concentration. The new phenomenon discovered by us consists in that the mass of the scattering particles increases drastically in the presence of heavy metal salts, reaching a maximum in the region of the isoelectric point corresponding to the zero total charge on the surface of the protein macromolecule, the magnitude of this maximum growing higher with the increasing of the heavy metal salt concentration.

This effect can apparently be explained by the formation of molecular complexes (clusters) in these solutions. The approach of albumin macromolecules in neutral solutions is prevented by the charge q around 10 unit charges in magnitude that causes their mutual Coulomb repulsion. It can be seen from the theoretical curve of Fig. 5 that when the charge on the surface of protein macromolecules amounts to 10 unit charges, the Coulomb interaction energy proves to be an order of magnitude higher than the thermal energy at an intermolecular distance of some 70 Å. The effective reduction of the surface charge of protein macromolecules as a result of the strong binding of heavy metal ions to their charged surface groups results in that the main type of interaction between the macromolecules becomes their dipole-dipole interaction, because proteins possess abnormally great dipole moments ($P = 380$ D for serum albumin and 250 D for ovalbumin [2]).

The weak bond between water molecules and heavy alkali metal ions is governed by a relationship between their own electrostatic energy, which depends on the ionic radius, and the thermal energy kT:

$$E_{pq} = \frac{q^2 p_w^2}{12\pi\varepsilon r_0^4} \frac{1}{kT}.$$

Here E_{pq} is the electrostatic ion-water molecule interaction energy, q is the polarization charge of the heavy metal ion, p_w is the dipole moment of the water molecule, r_0 is the distance between the centers of the ion and water molecule, and ε is the dielectric constant of water (~ 80).

If the interaction energy $E_{pq} < kT$, water will not be retained on the surface of ions, so that the latter can form electrostatic couples on the surface of protein macromolecules, totally neutralizing their net charge. The character of interaction between albumin macromolecules in that case will be mainly controlled by the dipole-dipole and dipole-charge interaction forces (Figs. 6*a* and *b*). The dipole-dipole interaction energy of protein macromolecules is defined by the relation

$$E_{pp} = \frac{p^4}{6\pi\varepsilon kT l^6}.$$

Here p is the dipole moment of the macromolecule and l is the minimum distance between the dipoles. When the dipoles draw together as close as around 25 Å, the energy E_{pp} may exceed the thermal energy kT almost 100 times (Fig. 6*b*).

When the actual charge on the surface of the macromolecules tends to zero, conditions are created that favor their combining into complexes (minimum free energy is thus reached). The protein macromolecules may come very close together and form a macromolecular complex cluster.

For a heavier serum albumin, the maximum mass of such a cluster reaches some 60 molecular masses of the protein, and for ovalbumin, around 10 molecular masses.

As the total charge (negative or positive) on the surface of the protein molecules grows higher, the Coulomb repulsion forces rise, and the clusters break down, the effective mass of the scattering particles approaching the molecular mass of the protein (Figs. 2 to 4).

The result obtained is undoubtedly of interest for the understanding of the molecular processes occurring in living organisms under the effect of heavy metal ions.

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