Molecular Clusters in Water Protein Solutions in the Presence of Heavy Metal Ions

G. P. Petrova¹, Yu. M. Petrusevich² and A. N. Evseevicheva¹

¹ Moscow State University, Faculty of Physics, Department of Molecular Physics, Moscow, Russia
² Moscow State University, Faculty of Physics, Department of Low Temperatures, Moscow, Russia

Abstract. For the first time, formation of macromolecular protein clusters in the presence of heavy metal ions were observed by the light scattering method. Conditions of the formation and destruction of such clusters were investigated. The clusters mass has a maximum value at the isoelectric point of the protein and increases with the ionic strength of the solution. Formation of clusters in the presence of toxic heavy metals in living cells is believed to have an essential physiological meaning. The molecular mechanisms of such clusters formation are discussed.

Key words: Molecular clusters — Proteins — Heavy metal ions — Light scattering

Introduction

The interaction of protein macromolecules in water solution is determined by electrostatic forces between charged biopolymers and small ions. Herein the general ion concentration will be termed ionic strength. A solution containing ions should as a whole remain electrically neutral.

The interaction of macroions in solution, which along with the low molecular solvent also contains the third component - a strong electrolyte, has been considered in Scatchard’s theory (Scatchard 1946). The expression for the second virial coefficient \( B \) (in decomposition for the free energy) according to this theory can be written as:

\[
B = \frac{V_1}{M_2^2} \left( \frac{Z^2}{4m_3} + \frac{\beta_{22}}{2} - \frac{\beta_{33}^2 m_3}{4 + 2\beta_{33} m_3} \right)
\]

where \( V_1 \) is the specific volume of the solvent, \( Z \) is the macroion charge, \( M_2 \) is its weight and \( m_3 \) is the concentration of salt ions.

Correspondence to: Dr. Yu. Petrusevich. Department of Low Temperature, Physical Faculty, Moscow State University, Vorobyevy Gory, Moscow 119 899, Russia. E-mail: asl@phys.msu.su
Parameters $\beta_{22}$, $\beta_{23}$ and $\beta_{33}$, being derivatives of activity coefficients, characterize various interactions between ions in the solution: the effect of the excluded volume and interaction between charges of various macromolecules ($\beta_{22}$), interaction between macromolecules and salt ions ($\beta_{23}$), and interaction only between salt ions ($\beta_{33}$).

According to formula (1), the intermolecular interaction coefficient changes with the growth of the total charge of the protein according to the parabolic law $\sim Z^2$ (Donnane effect) with a minimum at point $Z \sim 0$ (isoelectric point) (Tanford 1961). The coefficient $\beta_{22}$ is usually small in comparison with the other terms in formula (1). At large salt concentrations the term containing factor $\beta_{24}$ can sufficiently exceed (in absolute value) the other two terms and parameter $B$ can become negative.

A very effective method for the determination of the interaction coefficient $B$ and of the weight of the macromolecules is the Rayleigh light scattering method (Stass 1956). As it was proved by Debye (1946) for molecular solutions it enables to connect the experimentally measured coefficient of solution-induced light scattering $R_90$ with the virial expansion for osmotic pressure $\Pi$

$$\frac{cHK}{R_90} = \frac{1}{RT} \frac{d\Pi}{dc} = \frac{1}{M} + 2Bc +$$

(2)

where $c$ is concentration, $H = \frac{2\pi^2 n_0^2 (\frac{dn}{d\lambda})^2}{\lambda^4 N_A}$ is the so-called solution constant (Debye constant), $R$ is the gas constant, $\lambda$ is the wavelength of the exciting light, $n_0$, $n$ are refractive indexes of the pure solvent and solution, respectively, $M$ is the mass weight of the macromolecule, $A$ is the Cabannes factor (Cabannes 1929).

Investigations of water solutions of various proteins with the help of this method have shown that the mass weight of protein macromolecules in the solution with a net charge (determined by pH) remains practically constant. The dependence of the interaction coefficient (parameter $B$) on solution pH is nonlinear with a minimum at the isoelectric point in concordance with formula (1). If the ionic strength of solution increases, i.e., if the salt (e.g., NaCl) concentration increases, there is more complex formation with the participation of Na$^+$ and Cl$^-$ ions. Around each charged protein molecule there is a layer of counterions, which shields Coulomb’s type interactions. The value $B$ decreases with the increasing salt concentration $m$; however the parabolic dependence of $B$ on pH remains.

These effects were observed experimentally. For lysozyme at large ionic strength a sign reversion was observed for $B$, as determined by the rise of the third term in Scatchard’s formula (1).

With the increasing NaCl concentration in the solution a shift was observed in the minimum of the curve $B = f(\text{pH})$ from the isoelectric point towards smaller pH values (positive surface charge), as e.g., for serum albumin (Edsall et al. 1950) and
γ-globulin (Petrova and Petrucevich 1987, 1994, Petrova et al 1990) The shift of the minimum of $B$ to the positive direction of $Z$ (smaller pH value) was explained by Edsall by absorption of Cl$^{-}$ ions to the albumin surface.

The ionic radius for Cl$^{-}$ is equal $1.75 \times 10^{-1}$ nm, and for Na$^{+}$ is equal $0.8 \times 10^{-1}$ nm. So Cl$^{-}$ ions have a stronger binding to the protein surface than Na$^{+}$ ions. Ions of heavy metals have greater ionic radii (Cs $1.65 \times 10^{-1}$ nm and Ce $1.7 \times 10^{-1}$ nm) and are unable to retain bound water. Consequently, they could form so-called Coulomb's complexes on the protein surface as the result of the binding with the negative charge of the macromolecule. We may therefore expect a difference in the adsorption process between heavy ions Cs$^{+}$ and Ce$^{+}$ and the light one Na$^{+}$ on the protein surface.

Materials and Methods

Water solutions of albumin (serum albumin, lactalbumin, and ovalbumin, all from Serva Heidelberg, Germany) were investigated in the presence of CsCl, RbCl and Ce(SO$_4$)$_2$ salts with various values of ionic strength.

The experiments were carried out using He-Ne laser and photo-electric registration of scattered light, and $125\text{I}$ LKB Wallac lumimeter equipped with a special light source and a filter $l = 454$ nm with an optical fiber for the registration of light scattering.

![Graph](image)

Figure 1. The dependence of coefficient $B$ on pH(Z) for HSA water solution in the presence of CsCl at the ionic strength $\mu = 0.1$ (mol/l).
Figure 2. The dependence of coefficient $B$ on pH(Z) for water solution of ovalbumin in the presence of CsCl at various ionic strengths 1 ovalbumin + water ($\mu = 0.001$), 2 ovalbumin + water + CsCl ($\mu = 0.01$), 3 ovalbumin + water + CsCl ($\mu = 0.1$), 4 ovalbumin + water + CsCl ($\mu = 0.2$)

Results

Figs 1 and 2 show dependencies of the second virial coefficient $B$ on pH(Z) for serum albumin and ovalbumin in the presence of various concentrations of CsCl. For comparison, Figs 3 and 4 represent dependencies of $B$ on pH for the same proteins but in the presence of various concentrations of NaCl (the data for serum albumin is from Edsall et al (1950), and those for ovalbumin are our own data. As indicated in Fig 4, the dependence $B$ on pH sharply changes in the presence of CsCl.

Curve $B$ on pH (Fig 2) shows maximum of $B$ rather than minimum almost at the isoelectric point. With the increasing ionic strength a shift was observed of the maximum value of $B$ towards higher values of pH.

Scattered particles mass was calculated from experimental data of the scattering coefficient for various protein concentrations and various ionic strengths (Figs 5 and 6, Tables 1 and 2).
Figure 3. The dependence of coefficient $B$ on pH($Z$) for HSA water solution in the presence of NaCl at various ionic strengths.

Figure 4. The dependence of coefficient $B$ on pH($Z$) for water solution of ovalbumin in the presence of CsCl at various ionic strengths.
Figure 5. The dependence of the mass of scattering particles in HSA solution on pH(Z) in the presence of CsCl at ionic strengths 1 $\mu = 0.001$ (mol/l), 2 $\mu = 0.1$ (mol/l).

Figure 6. The dependence of the mass of scattering particles in the ovalbumin solution on pH(Z) in the presence of CsCl at various ionic strengths 1 ovalbumin + water ($\mu = 0.001$), 2 ovalbumin + water + CsCl ($\mu = 0.01$), 3 ovalbumin + water + CsCl ($\mu = 0.1$), 4 ovalbumin + water + CsCl ($\mu = 0.2$).
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Table 1 Parameters of the ovalbumin solution containing Ce(SO₄)₂, μ = 0.1

<table>
<thead>
<tr>
<th>B 10⁻⁵ sm³/m</th>
<th>M₀, g/m</th>
<th>M, g/m</th>
<th>M/M₀, g/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>45,000</td>
<td>550,000</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2 Parameters of the lactalbumin solution containing Ce(SO₄)₂, μ = 0.1

<table>
<thead>
<tr>
<th>B 10⁻⁵ sm³/m</th>
<th>M₀, g/m</th>
<th>M, g/m</th>
<th>M/M₀, g/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.4</td>
<td>15,500</td>
<td>320,000</td>
<td>22</td>
</tr>
</tbody>
</table>

Discussion

The essence of the newly found phenomenon is the mass increase in the presence of heavy metals nearby the isoelectric point of the protein. This phenomenon can be explained by formation of molecular clusters in solutions containing heavy metals. In this case, strong binding of heavy metals to the protein surface diminishes the net charge of the latter so that dipole-dipole interactions become the main interaction force. The latter are connected with anomalous big values of the dipole moment for proteins (about 400D for serum albumin and about 700D for lactalbumin).

The weak binding of water with heavy metal ions determines the ratio of the electrostatic energy, which depends on ionic radius and the thermal energy, kT

\[ W_q = \frac{q^2 p^2_\sigma}{12 \pi \varepsilon \eta^4_0} \frac{1}{kT} \]

where \( W_q \) is the energy of the electrostatic interaction of ions with water molecules, \( q \) is the heavy metal ion charge, \( p_\sigma \) is the dipole moment of the water molecule, \( \eta_0 \) is the distance between centers of the ion and the water molecule, \( \varepsilon \) is the dielectric permeability of water (approximately 80).

If the energy of the interaction \( W_q < kT \), then water molecules will not be retained on the ion surface, and ions can form electrostatic couples on the protein, completely compensating its net charge. In this case, the type of interaction of the albumin macromolecules will be gradually determined by dipole-dipole interaction forces. The energy of dipole-dipole interactions for protein molecules is determined by the ratio

\[ E_q = \frac{p^4}{6 \pi \varepsilon kT^16} \]
Where \( p \) the dipole moment of the macromolecule, \( l \) the minimum distance between dipoles. When this distance is about \( 3 \times 10^{-2} \) nm energy \( E_q \) may exceed the thermal energy \( kT \) almost 100 times.

In conditions of a zero net charge there will be a minimum of free energy; therefore protein molecules will have favourable conditions to form clusters. Proton macromolecules can come close to each other, and the distances between them may be extremely small and a macromolecular cluster is formed.

For a heavier protein (like serum albumin) the maximum mass number, \( M \) was more than 20 for ovalbumin it was more than 10 for lactalbumin the cluster mass number was about 20.

With the increasing total (negative or positive) net charge of a protein the Coulomb's forces of repulsion grow and clusters decay, the effective mass of the scattered particles is thus approximately equal to the molecular mass of the protein.

The obtained results are clearly of interest for the understanding of molecular processes occurring in living organisms in the presence of heavy metal ions.

References

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Final version accepted April 27, 1998