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Absrtact

Gadoteric acid and gadodiamide molecules have gadolinium-containing chelate structures that are used in magnetic resonance tomography (MRT). [1]. Over the past two decades, they were considered safe and effective for medical imaging. However, recent results indicate the presence of gadolinium in the skin and soft tissues in patients with renal insufficiency, even with the current hemodialysis [2]. Nephrogenic systemic fibrosis (NSF), which was described in 1997, is a recently discovered rare disease of unknown etiology that affects patients with renal insufficiency. The development of NSF was directly linked to the influence of gadolinium-containing magnetic resonance contrast agents in 2006 [3].

Introduction

This work is devoted to the study of main blood protein - albumin and its interaction with gadolinium-containing chelates. In this regard, it became interesting to study the nature of molecular motion and intermolecular interactions in water solutions of biological macromolecules (albumin). First was identified the physical mechanism of toxicity of gadolinium ions belonging to the chelated structure of gadoteric acid on serum albumin.

Albumin

Firstly albumin water solutions were studied. Serum albumin is globular protein [4] which performs transport function in organism. It is readily soluble in water and easily diffuses. Hydrodynamic behavior of these molecules is well described by the model of ellipsoid with axial ratio of about 150/50/50 Å. Electrically neutral albumin contains about a hundred pairs of positive and negative charges.

At our experiment we used bovine serum albumin (BSA) Sigma Aldrich.

Experimental results

By using the photon correlation spectroscopy following experimental results were performed:
1) The dependencies of translation diffusion coefficient $(D_t(pH))$ for the pure albumin water solution (Fig. 1, curve 1), for albumin water solutions with the addition of gadoteric acid (Fig. 1, curve 2) and albumin water solutions with the addition of ferric chloride (Fig. 1, curve 3). These dependences $D_t(pH)$ are similar in character and values, and have a form close to parabolic.

2) When adding two impurity substances in albumin water solutions (gadoteric acid and ferric chloride) translational diffusion coefficient (D_t) decreases. As the ionic strength of ferric chloride at a fixed concentration of gadoteric acid in albumin solution decreases the D_t increases (Fig. 2).

3) The changes of the dynamic parameters of serum albumin under the influence of gadodiamide molecules in water solutions were studied (Fig. 3).

4) The dynamic processes of destruction of serum albumin dipole clusters (formed by adding of cesium ions) under heating were investigated (Fig. 4).
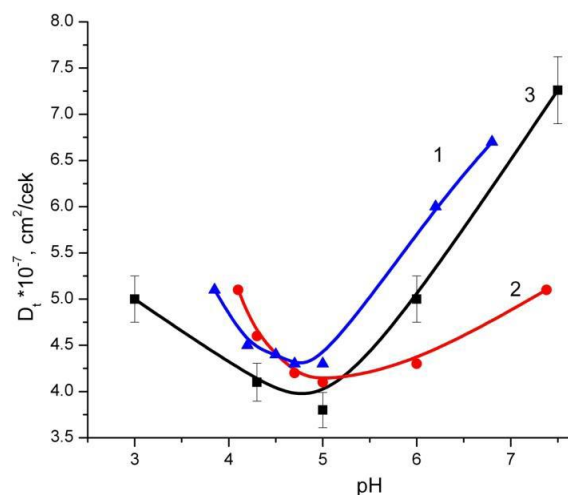

Figure 1: Dependence of the translational diffusion coefficient on the pH in water solutions of pure BSA (curve 1), with the addition of gadoteric acid (I=0,05 mmol/ml) (curve 2), with the addition of salts of ferric chloride $FeCl_3$ (I=0,004 mol/L) (curve 3).
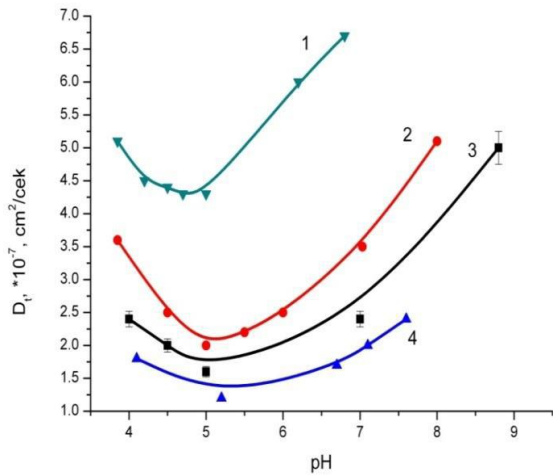
Figure 2: Dependence of the translational diffusion coefficient on the pH in water solution of pure BSA (curve 1) and BSA water solutions with the addition of two impurity substances - gadoteric acid (I=0,05 mmol/ml) (curve 2, 3, 4) and salts of ferric chloride $FeCl_3$- I=0,001 mol/L (curve 2), I=0,002 mol/L (curve 3), I=0,004 mol/L (curve 4).
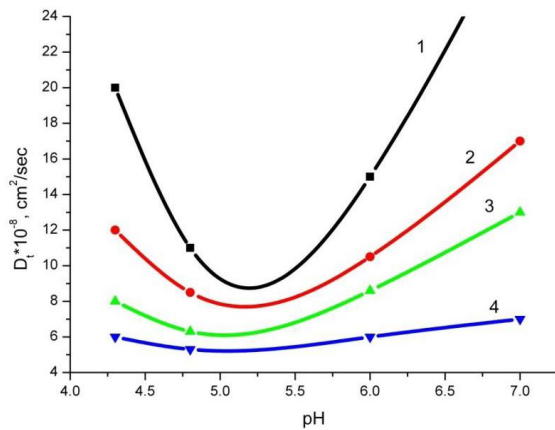

Figure 3: Dependence of the translational diffusion coefficient of the pH in BSA water solutions under the influence of gadodiamide molecules. Curve 1 – concentration of gadodiamide I=0,002 mmol/ml; Curve 2 – I=0,01 mmol/ml; Curve 3 – I=0,05 mmol/ml; Curve 4 – I=0,1 mmol/ml. T=298K.
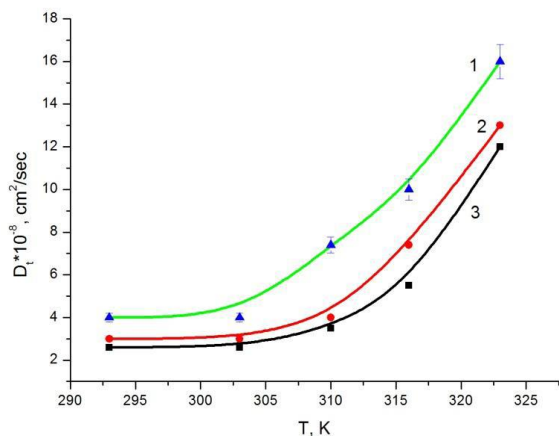

Fig. 4. The dependence of the translational diffusion coefficient of the temperature in BSA water solutions

(c=2.8 mg/ml) containing metal cesium ions. Curve 1 - I=0,01 mol / L, curve 2 - I=0,001 mol/L, curve 3 - I=0,0004 mol/L. (pH=4.8).

Discussion

In the study of the dynamic parameters of albumin molecules in pure water solution and in solutions with gadoteric acid and ferric chloride the nonlinear dependence of the D_t of the pH has a minimum near the isoelectric point of pH 4.8 (the minimum value of the surface charge of the protein molecule) (Fig. 1 , curves 1-3). Right and left to the isoelectric point D_t increases.

Previously [5], we have shown that in albumin water solutions, by Scatchard theory, the coefficient of intermolecular interaction has a minimum at the isoelectric point. D_t and the coefficient of molecular interaction depend on the charge of the protein in a similar way (albumin mass M in pure water solution is almost independent from pH).

When albumin influenced by gadoteric acid (Fig. 1, curve 2) parabolic dependence becomes flatter. In addition, the right side with respect to the isoelectric point of D_t decreases. First, it may be due to the fact that gadoteric acid conjugated with albumin macromolecules, and, second, gadoteric acid molecules have their electric charge.

The dependence of the albumin's D_t changes dramatically in albumin water solutions with the addition of two impurity substances – gadoteric acid and ferric chloride (Fig. 2, curves 2-4). At the fixed concentration of gadoteric acid, I = 0,05 mg / ml, with an increase of ionic strength of the ferric chloride $FeCl_3$ D_t of the particles of the solution decreases.

Gadolinium ions and iron in this case are the trivalent ions. In albumin water solutions containing gadoteric acid and ferric chloride salt $FeCl_3$, is possible replacement of gadolinium ions in the chelate structure of gadoteric acid with trivalent iron ions. Thus liberated gadolinium ions are free.

As shown previously in our laboratory, the presence of ions in solution with a large ionic radius leads to the predominance of the forces of dipole-dipole interactions between the molecules of the protein compared with the forces of the Coulomb repulsion and the formation of macromolecular complexes (dipole clusters) near the isoelectric point. Gd^{3+} ionic radius is much larger than that of the ions Fe^{3+}: R_{Gd3+}=0,938 Å (similar in size to the radius of the trivalent europium ion R_{Eu3+}=0,95 Å), R_{Fe3+}=0,64 Å. Large scattering particles have less mobility and larger hydrodynamic radius. This is the reason why the D_t when adding iron (Fig. 38, curve 3) is greater than the D_t when adding two impurity substances – gadoteric acid and ferric chloride (Fig. 2, curves 2-4).

Visual comparison value of D_t at the isoelectric point (pI 4,8) are shown in Figure 5.
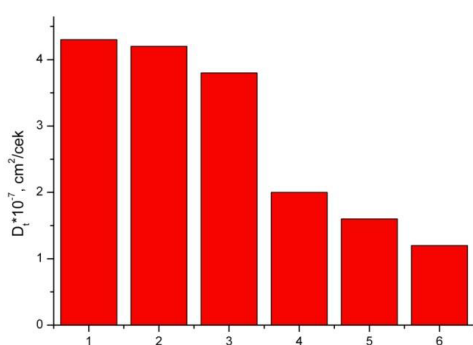

Figure 5: Comparison of translational diffusion coefficient values (pH 4.8) for the pure BSA water solutions (1), with the addition of gadoteric acid (I=0,05 mol/L) (2), with the addition of ferric chloride salt (I=0,004 mol/L) (3), followed by a fixed concentration of gadoteric acid I=0,05 mg/ml with different ionic forces FeCl$_3$ - I=0,001 mol/L (4), I=0,002 mol/L (5), I=0,004 mol/L (6)

Since the total charge at the isoelectric point (pI=4,8) on the albumin macromolecules surface (close to spherical particles) has a zero charge, the processing of data (Fig. 5) by the Stokes-Einstein relation:

$$D_t = \frac{kT}{6\pi\eta R},$$

where R is hydrodynamic radius, T – temperature, k – Boltzmann constant, η - viscosity

allows to calculate the hydrodynamic radius.

Recent studies [6] showed that gadolinium particles were found in 4 of 13 samples taken from seven patients with disease of the NSF, and iron ions were in all these samples.

Figure 3 shows the dependence of translational diffusion from the pH in albumin water solutions containing gadolinium chelates. pH - dependence of the translational diffusion coefficient varies with the growth of the net charge of the protein molecules on the surface, has a minimum at the isoelectric point (pH 4.8). In addition, if the concentration of gadolinium chelates complexes is big dependence D_t(pH) becomes flatter.

In comparison with the mobility of BSA in pure water solution (Fig. 1, curve 1) the addition of gadodiamide translational diffusion coefficient significantly reduces (Fig. 3).

In Figure 3 is shown that near the isoelectric point with increasing concentration of gadolinium chelates D_t decreases.

Comparison of the features of molecular mobility at the influence of two different gadolinium chelates

Compared with the value of albumin translational diffusion coefficient in water solutions containing gadoteric acid (Fig. 1), the coefficients of D_t in albumin solution containing gadodiamide greatly reduces (Fig. 3). As an example, at a concentration of gadoteric acid I = 0,05 mg / ml ratio D_t was $4,2 \times 10^{-7}$ cm^2/sec, which coincides with the value of D_t for the pure albumin water solution. At the same concentration in gadodiamide solution translational diffusion coefficient is equal to $6,1 \times 10^{-8}$ cm^2/sec (decreasing in the order).

This may be due to the fact that the gadodiamide solution there are a number of free gadolinium ions, which are formed under the influence of protein clusters.

In contrast to gadodiamide, gadolinium ions from the gadoteric acid structure more strongly associate with their chelate complexes.

Dynamic process of destruction of the dipole clusters albumin (BSA + Cs) with heating:

Comparing the relative energy of the interaction of a charged ion and dipolar water molecules with the thermal energy, it can be concluded that the metal ions which are heavy and have large ionic radii (eg, ionic radius of cesium is 1,67 Å), will keep hydrated shell weaker than the ions with small ionic radii (eg, ionic radius of sodium is 0,8 Å). Ions with large radii more closely associated with the negatively charged groups on the protein surface and may form the so-called Coulomb complex with total hydration shell on the protein molecule. In this case the ions fully compensate the surface charge of the protein.

In this case, the main interaction between macromolecules is dipole-dipole interaction, and not Coulomb repulsion.

Conclusions

1. Optical method (DLS) revealed the formation of dipole clusters of albumin in water solutions containing gadolinium having chelate complexes of gadodiamide. As the concentration of gadodiamide increases D_t decreases. This may be due to the fact that in the gadodiamide solution exist determined number of free gadolinium ions.

2. First is showed that the use of gadodiamide to improve contrast imaging in studies using magnetic resonance tomography is not safe for the organism. There is a risk of some diseases, which has important meaning for medicine.

3. There was obtained by photon correlation spectroscopy method, that in albumin water solutions containing cesium ions, D_t increases when heated.

Observable increase in D_t is observed at the temperature range 303-323 K, which is probably due to the destruction of the dipole protein nanoclusters.

References

[1]   A. V. Zubarev, V. E. Gazhonova, E.V. Zaytseva, E.V. Perepadya, and D.V. Salnikov, "Diagnostic possibilities of magnetic resonance, ultrasound and x-ray angiography in evaluation of renal vessels", Med. visualization. № 1, 106-121 (2003)

[2]   S. E. Cowper, H. S. Robin, S. M. Steinberg et al., "Scleromyxoedema-like cutaneous dieases in renal-dialysis patients", Lancet., 356, 1000-1001 (2000)

[3]   T. Grobner, "Gadolinium - a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis?", Nephrol Dial Transplant., 21, 1104-1108 (1945)

[4]   A.K. Wright, M.R Thompson. "Hydrodynamic structure of bovine serum albumin determined by transient electric birefringence" // J. biophys. 1975, 15, P. 137-141

[5]   S.E. Cowper, H.S. Robin, S.M. Steinberg, et al. "Scleromyxoedema-like cutaneous dieases in renal-dialysis patients" // Lancet. 2000, 356, P. 1000-1001

[6]   W.A High., R.A Ayers., J. Chandler, et al. "Gadolinium is detectable within the tissue of patients with nephrogenic systemic fibrosis" // J Am Acad Dermatol. 2007, 56