An Investigation of the Interaction of Uranium with Some Biocomplexones
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[English Abstract]
An Investigation of the Interaction of Uranium with Some Biocomplexons

S. Chevari, D. Zikhner

The authors commit to paper the results of determining the complex-formation of uranium and the stability of the compounds forming with C_{17} RNA and DNA amino acids, as well as with albumin-protein. The data derived may be utilized for the exact selection of substances accelerating the elimination of uranium from the human organism.

The first *in vitro* experiments studying the complex formation of uranium in different bio-systems investigated the influence of uranium compounds on proteins, nucleic acids, ferments, etc., since the discovery of specific ferments, amino acids, hormones, etc., which are highly sensitive to uranium compounds would open the path to the understanding of the mechanism of chronic uranium poisoning and would significantly ease the search for therapeutic drugs. Numerous investigations on this topic are present in the scientific literature (A.L. Downs), but the data are purely qualitative. Based on these data, it can be speculated that the processes of biological catalysis can be perturbed in the presence of uranium, and therefore, changes in the synthesis of ferments, hormones, etc. are possible.

Our experiments were carried out with the purpose of studying the complex formation ability of uranium *in vitro* with respect to some bio-systems that play an important role in living organisms: amino acids, nucleic acids (RNA and DNA), and also the albumin protein. The need for studying the interaction of uranium compounds with proteins is based on the premise of the significant role of plasma proteins in the processes of uranium distribution in the organism. The experiments were carried out with the purpose of quantitative analysis for the process.

Naturally, the direct translation of relationships in artificial chemical systems to those [in] living matter is exceptionally difficult, especially when studying relatively simple compounds; however, the quantitative estimation of their interactions in living organisms seems to remain relatively close.

The great complex formation ability of uranium has been known for a long time. Such reactions are governed to a large degree by the closeness of the energy levels of the 5f and 6d electrons (I. I. Chernov), the interaction of which can change under the influence of different factors and in particular depends on the compounds reacting with uranium. Almost all compounds of U^{6+} contain not the U^{6+} ion but the uranyl group, UO_2. The latter has a linear structure, is chemically stable, and during reactions carried out under normal conditions is incorporated into the compounds intact (I. I. Cherniaev). It should be mentioned that the uranyl ion has high proclivity to hydrolysis in water solutions, leading to formation of aqua-hydroxyl compounds of various compositions ($[UO_2(HO_2)]^{2+}$, $[UO_2OH]^{+}$, etc.). In this form, uranium has lower ability of complex formation with other compounds because the conversion [substitution?] of the hydroxide into a complex is either too slow or completely suppressed. The pH of the start of the precipitation of uranyl hydroxide can be calculated using the formula: $pH = 3.85 + 0.65 \log C$ (A. P. Vinogradov), where C is the concentration of the uranium salt. For reactions that occur at conditions favoring the hydrolysis of uranium, this reaction must be taken into account, especially in cases of high concentrations of the latter.

There are many methods for determination of stability constants of complexes (A. A. Greensburg), out of which we have chosen the method of cation exchange. As a cation-exchange resin we used KY-2 (made in USSR). The experiments were carried out at 25 ± 1°C in solutions with ionic strength of 0.1 and concentration of uranium of $2 \cdot 10^{-5}$ M. The concentration of the compounds was different depending on the purpose of the experiment. 100 mL of the solution under investigation were placed in contact with 50 mg of the resin by shaking on a special machine. To avoid the adsorption of the amino acids and proteins on the resin, the resin was used in its Na-form, so that the dissolved Na^+ ions prevent the adsorption of amino acids because of the usual competition with Na^+ (E. M. Savitskaia). In the case of amino acids, the pH was maintained in the range 4.0 – 4.5 in order to avoid the uncontrolled hydrolysis of the uranyl-ions. For nucleic acids and albumin, considering the isoelectric points (Straub), pH was maintained at about 6 and concentration of uranium in these cases was decreased to $2.28 \cdot 10^{-6}$

M. In the preliminary experiments, it was determined that the equilibrium between the uranium in solution and that on the resin is established during 2 hours. The observed decrease in the adsorption of uranium in the presence of the compounds under investigation can be explained with the transition of free uranium ions into complexes. The distribution (partition) coefficient of uranium between the phases was calculated using the formula (Poccomu):

$$D = \frac{C_{init} - C_{eq}}{C_{eq}} \cdot \frac{V}{m}$$

where D is the distribution coefficient; C_{init} is the initial concentration of uranium; C_{eq} is the equilibrium concentration of uranium; V is the volume of the solution (in mL) and m is the mass of the resin (in g).

The amount of uranium in solution was determined spectrophotometrically with the help of the reagent Arsenazo III (Zsoldos and Csovari), and in the cases of lower concentrations, the radiometric method was used (Zsoldos *et. al.*).

On the basis of the acquired data for amino acids, the stability constants of the complexes were calculated by the method of Fronaeus using the following equation:

$$\frac{D_0 - D}{D} \cdot \frac{1}{[A]} = \beta_1 + \beta_2[A] + ...\beta_n[A]^{n-1}$$

where D_o is the distribution coefficient of uranium in the absence of the ligand under investigation; [A] is the concentration of the ligand; β_1, β_2,…, β_n are the stability constants of the forming complexes.

The concentrations of free ligands were determined as follows:

$$[A] = \frac{C_0}{B}$$

$$B = 1 + \frac{[H^+]}{K_1} + \frac{[H^+]^2}{K_1 \cdot K_2} ... \frac{[H^+]^n}{K_1 ... K_n}$$

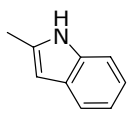
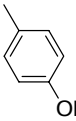
where C_o is the initial concentration of the amino acid; $[H^+]$ is the concentration of H^+ ions; K_1, K_2, …, are the dissociation constants of the carboxylic ions of the acid (Zsoldos, *et. al.*). By applying this formula, we found that at the pH of the experiment, the concentration of the ligands is equal to that of the amino acid.

The experimental and calculated data for the amino acids are given in Table 1 and reflect the above discussion.

To determine the composition of the uranium complexes with amino acids, the following relation was plotted and is shown in the figure:

$$\beta = \lim \frac{D_o - D}{D} \cdot \frac{1}{[A]}$$

From the figure, it is clear that $\frac{D_o - D}{D} \cdot \frac{1}{[A]} \approx const$. This means that, under the experimental conditions, uranium and amino acids form complexes with 1:1 molecular ratio between the ligand and the uranyl ion, such as $[UO_2A]^+$. A comparison of the stability constants of the uranyl-amino acid complexes shows the influence of the different functional groups in the chain of the amino acid on the strength of the coordination bond (this bond is formed by the lone pair of the amino group, NH_2). The influence of the functional groups can be explained by the shift of the electron cloud towards or away from the NH_2 group (Lowry). The functional groups of the amino acids can be ordered by their influence on the stability of the coordination bond in the complex in the following series:

-COOH > =NH > -NH_2 > -OH >  >  >  >  >  > -SH > -S—R.

The nucleic acids and albumin-protein have very complex structures, containing larger amount of functional groups. In this case the calculation of the stability constants by the method of Fronaeus is quite difficult since the calculation of the concentration of free ions is not possible. Therefore, for the calculations of the complexes of uranium with nucleic acids and albumin, a different method was suggested. The following equilibrium is established in the system:

$$[R\text{-}UO_2]_{resin} \rightleftharpoons [R^{2-}] + [UO_2^{2+}] + [A^{2n-}] \rightleftharpoons [A(UO_2)_n],$$

where $[A^{2n-}]$ is the concentration of the ligand in equilibrium with the complex; $[A(UO_2)_n]$ is the concentration of the complex. It is equal to:

$$[A(UO_2)_n] = [UO_2^{+2}]_{init} - [UO_2^{+2}]_{free} - [R - UO_2]_{resin} = \frac{[UO_2^{+2}]_{eq}}{n}$$

The equilibrium (stability) constant of the formation of the complex is:

$$K = \frac{[A(UO_2)_n]}{[A^{2n-}][UO_2^{2+}]_{free}^n}$$

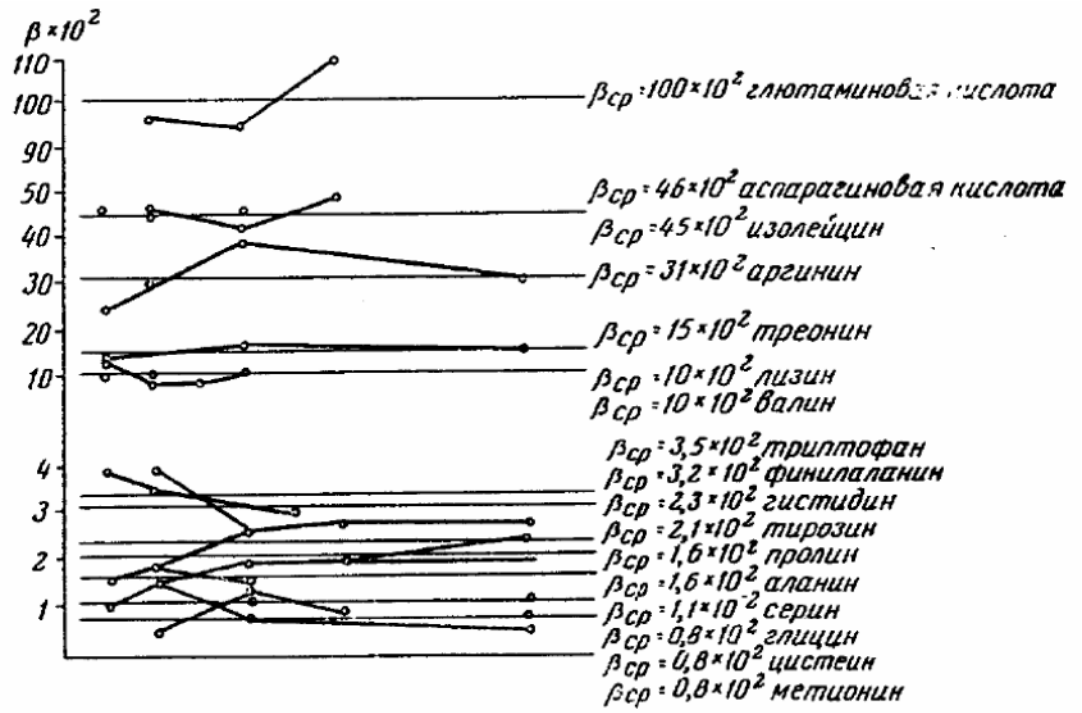
Table 1

Table 1 Caption

Ligand	Concentration (in M)	Distribution coefficient	β	$\beta_{avg} = K_{stab}$
Glycine	$2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$ $6 \cdot 10^{-3}$	2700 1960 2000	$0.5 \cdot 10^{2}$ $1.3 \cdot 10^{2}$ $0.8 \cdot 10^{2}$	$0.8 \cdot 10^{2}$
L-Alanine	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$ $10 \cdot 10^{-3}$	2700 2200 1690 1000	$0.1 \cdot 10^{3}$ $0.15 \cdot 10^{3}$ $0.19 \cdot 10^{3}$ $0.20 \cdot 10^{3}$	$0.16 \cdot 10^{3}$
L-Valine	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $3 \cdot 10^{-3}$ $4 \cdot 10^{-3}$	1200 1020 630 350	$1.4 \cdot 10^{3}$ $0.95 \cdot 10^{3}$ $0.92 \cdot 10^{3}$ $1.1 \cdot 10^{3}$	$1.0 \cdot 10^{3}$
L-Isoleucine	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$	530 310 105	$4.65 \cdot 10^{3}$ $4.50 \cdot 10^{3}$ $4.50 \cdot 10^{3}$	$4.5 \cdot 10^{3}$
L-Serine	$4 \cdot 10^{-3}$ $1 \cdot 10^{-2}$	2700 2200	$0.11 \cdot 10^{3}$ $0.11 \cdot 10^{3}$	$1.1 \cdot 10^{2}$
L-Cysteine	$4 \cdot 10^{-3}$ $1 \cdot 10^{-2}$	2250 1700	$0.8 \cdot 10^{2}$ $0.8 \cdot 10^{2}$	$0.8 \cdot 10^{2}$
L-Methionine	$2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$ $1 \cdot 10^{-2}$	2300 2260 2200	$1.5 \cdot 10^{2}$ $0.7 \cdot 10^{2}$ $0.36 \cdot 10^{2}$	$0.8 \cdot 10^{2}$
L-Phenylalanine	$2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$	1660 1520	$0.4 \cdot 10^{3}$ $0.25 \cdot 10^{3}$	$0.32 \cdot 10^{3}$
L-Arginine	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$ $1 \cdot 10^{-2}$	860 500 170 94	$2.5 \cdot 10^{3}$ $3.0 \cdot 10^{3}$ $3.9 \cdot 10^{3}$ $3.0 \cdot 10^{3}$	$3.1 \cdot 10^{3}$
L-Lysine	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$	1400 900 580	$1.1 \cdot 10^{3}$ $1.1 \cdot 10^{3}$ $1.0 \cdot 10^{3}$	$1.0 \cdot 10^{3}$
L-Tyrosine	$4 \cdot 10^{-3}$ $6 \cdot 10^{-3}$ $1 \cdot 10^{-2}$	1720 1210 845	$0.19 \cdot 10^{3}$ $0.20 \cdot 10^{3}$ $0.25 \cdot 10^{3}$	$0.21 \cdot 10^{3}$
L-Tryptophan	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $5 \cdot 10^{-3}$	2100 1780 1230	$0.4 \cdot 10^{3}$ $0.35 \cdot 10^{3}$ $0.30 \cdot 10^{3}$	$0.35 \cdot 10^{3}$
L-Proline	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$	2580 2180 1800	$0.16 \cdot 10^{3}$ $0.18 \cdot 10^{3}$ $0.16 \cdot 10^{3}$	$0.16 \cdot 10^{3}$
L-Threonine	$1 \cdot 10^{-3}$ $2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$	1250 710 500	$1.4 \cdot 10^{3}$ $1.6 \cdot 10^{3}$ $1.5 \cdot 10^{3}$	$1.5 \cdot 10^{3}$
L-Histidine	$2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$ $6 \cdot 10^{-3}$ $1 \cdot 10^{-2}$	2300 1540 1100 840	$1.5 \cdot 10^{2}$ $2.5 \cdot 10^{2}$ $2.8 \cdot 10^{2}$ $2.6 \cdot 10^{2}$	$2.3 \cdot 10^{2}$

Ligand	Concentration (in M)	Distribution coefficient	β	$\beta_{avg} = K_{stab}$
L-Aspartic Acid	$2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$ $6 \cdot 10^{-3}$	340 169 104	$4.6 \cdot 10^3$ $4.4 \cdot 10^3$ $4.8 \cdot 10^3$	$4.6 \cdot 10^3$
L-Glutamic Acid	$2 \cdot 10^{-3}$ $4 \cdot 10^{-3}$ $6 \cdot 10^{-3}$	149 78 46	$9.6 \cdot 10^3$ $9.4 \cdot 10^3$ $11.0 \cdot 10^3$	$10 \cdot 10^3$
$D_0 = 3000$				

Figure.



Зависимость констант стабильности аминокислотных комплексов урана от концентрации аминокислот.

The concentration of the free uranyl ions can be calculated using the formula:

$$[UO_2^{2+}]_{free} = \frac{[UO_2 - R]_{resin}}{D_0}$$

The equilibrium concentration of the ligand can be expressed as:

$$[A^{2n-}] = [A]_{init} - [A(UO_2)_n] = [A]_{init} - \frac{[UO_2^{+2}]_{eq}}{n}$$

Thus the equilibrium (stability) constant of the reaction can be expressed as:

$$K = \frac{[A(UO_2)_n]}{\left[\frac{UO_2 - R}{D_0}\right]^n \cdot \left[A_{init} - \frac{[UO_2^{+2}]_{eq}}{n}\right]}$$

In this equation the unknown quantity is n – the amount of uranyl ions attached to one molecule of albumin or nucleic acid. To solve the above equation, we need to consider the equilibrium with another concentration of the ligand. Thus, by analogy, the equilibrium (stability) constant of the reaction will be:

$$K_2 = \frac{[A(UO_2)_n]_2}{\left[\frac{UO_2 - R_2}{D_0}\right]^n \cdot \left[A_{2init} - \frac{[UO_2]_{2eq}}{n}\right]}$$

Since the two equilibrium constants must be equal, n can be determined by combining the above two equations. Knowing n allows the calculation of the stability constant. The data on the composition and the stability constants of the complexes of uranium with albumin and nucleic acids are shown in Table 2.

Table 2

Calculated data for albumin and nucleic acids

Ligand	Concentration (in M)	$[A(UO_2)_n] = [UO_2^{2+}]_{eq}$	$[UO_2^{2+}]_{free} = \left[\frac{UO_2 - R}{D_0}\right]$	n	K_{stab}	K_{avg}
Albumin-protein	$2.19 \cdot 10^{-6}$	$1.5 \cdot 10^{-6}$	$0.26 \cdot 10^{-9}$	1	$0.83 \cdot 10^{10}$	$0.95 \cdot 10^{10}$
DNA	$3.45 \cdot 10^{-6}$ $6.1 \cdot 10^{-6}$	$1.94 \cdot 10^{-6}$ $1.98 \cdot 10^{-6}$	$0.12 \cdot 10^{-9}$ $0.10 \cdot 10^{-9}$	1	$1.08 \cdot 10^{10}$ $0.48 \cdot 10^{10}$	$0.53 \cdot 10^{10}$
RNA	$12.2 \cdot 10^{-6}$ $3.5 \cdot 10^{-6}$ $8.08 \cdot 10^{-6}$	$2.17 \cdot 10^{-6}$ $1.69 \cdot 10^{-6}$ $2.07 \cdot 10^{-6}$	$0.037 \cdot 10^{-9}$ $0.19 \cdot 10^{-9}$ $0.07 \cdot 10^{-9}$	1	$0.58 \cdot 10^{10}$ $0.5 \cdot 10^{10}$	$0.5 \cdot 10^{10}$

As can be seen from the table, at the experimental conditions, uranium forms complexes with albumin-protein and nucleic acids in a 1:1 ratio.

The collected data on the stability of the complexes of uranium with amino acids, nucleic acids and albumin-protein could, to some extent, help in the search of therapeutic drugs that facilitate the acceleration of uranium excretion (elimination). The data can be used to account for the competition between introducible synthetic complexing agents and bioligands in the body.

In conclusion, we would like to thank our coworker Peter Oskarne for helping with the acquisition of the experimental material.

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