Equilibrium Study on Mixed Ligand Complexes of UO_2^{2+} and Th^{4+} with EDTA as Primary Ligand and Various Nucleosides and their Bases as Secondary Ligands

K. RAMALINGAM and C. R. KRISHNAMOORTHY*

Department of Chemistry, Indian Institute of Technology, Madras 600 036, India

Received July 7, 1982

Formation constants of mixed ligand complexes of UO_2^{2+} and Th^{4+} with EDTA and various nucleosides, purines and pyrimidines such as adenosine, guanosine, cytidine, uridine, adenine, 2,6-diaminopurine, 8-azaadenine, cytosine, thymine and uracil in a 1:1:1 ratio were determined in aqueous solution at 35 °C by potentiometric equilibrium measurements. The acid dissociation constants of the above mentioned secondary ligands were determined at 35, 45 and 55 °C together with the thermodynamic parameters involved in their ionization reactions. Formation constants of 1:1 binary complexes of UO_2^{2+} and Th^{4+} with the above secondary ligands are also reported at 35 °C. All the measurements were made in aqueous solution and μ = 0.1 M (KNO_3). Both enthalpy and entropy factors involved in the dissociation reactions of secondary ligands were found to be favourable for the first dissociation reactions. There was a good correlation between the basicity of secondary ligands and stability of their 1:1 binary complexes determined. In general the binary complexes of Th^{4+} showed greater stability in comparison to the corresponding UO_2^{2+} complexes. Excepting the case of adenosine and cytidine, formation of protonated ternary complex in the early buffer region followed by dissociation to normal complex was visualized. In all the cases the binary complexes were more stable than their corresponding ternary complexes. Among the secondary ligands, thymine, uracil and uridine showed no measurable interaction with both the metal ions in binary and ternary complex systems. Cytosine and cytidine also showed very weak interactions with the UO_2–EDTA system.

Introduction

The intrinsic role of metal ions in the conformation of DNA and RNA and related molecules has drawn the attention of researchers to the study of metal complexes of nucleotides, nucleosides and their bases [1–5]. Though earlier studies have been made on the metal complexes of purines, pyrimidines, nucleosides and their biologically important derivatives, only very few systematic data are available in these lines, especially in aqueous solution [6–8]. Similarly mixed ligand complex formation interested researchers because this offers an alternative to hydrolysis and olation reactions of metal ions. The metal ions UO_2^{2+}, Th^{4+} used in this investigation have the high coordination number of eight, enabling them to accommodate additional secondary ligands and thereby readily forming mixed ligand complexes. The choice of hexadentate EDTA as primary ligand is due to its tendency to form stable metal complexes even in high acidic conditions [9] and it can also leave two 'vacant' sites on these metal ions for the binding of secondary ligands. Various nucleosides, purines and pyrimidines such as adenosine, guanosine, cytidine, uridine, adenine, 2,6-diaminopurine, 8-azaadenine, cytosine, thymine and uracil are chosen as secondary ligands in this study. Thus the present investigation is the first attempt to study the various equilibria involved in the formation of binary and ternary complexes of UO_2^{2+} and Th^{4+} with the above mentioned biologically important ligands in aqueous solution.

Experimental

Reagents

Anhydrous nucleosides and their bases obtained from Sigma Chemicals (U.S.A.) were analytically pure. Their purity was further checked by potentiometric titration with standard sodium hydroxide. Fresh solid nucleosides were weighed out for each titration to avoid any possible hydrolysis or photochemical decomposition. Stock solution of pure EDTA (BDH) was prepared and estimated volumetrically. Stock solutions of analytically pure Th^{4+} and UO_2^{2+} nitrates (BDH) were prepared and

*Author to whom correspondence should be addressed.

0020-2693/82/0000-0000/$02.75

© Elsevier Sequoia/Printed in Switzerland

TABLE I. Acid Dissociation Constants of Secondary Ligands $\mu = 0.1\ M$ (KNO_3). All the constants are accurate to ±0.02 pk unit.

Secondary ligand	35 °C		45 °C		55 °C	
	pk_a	pk_{2a}	pk_a	pk_{2a}	pk_a	pk_{2a}
a. NUCLEOSIDES						
Adenosine	3.50	–	3.45	–	3.36	–
Guanosine	2.44	9.10	2.12	8.81	2.05	8.65
Cytidine	4.03	–	4.00	–	3.84	–
Uridine	8.94	–	8.80	–	8.63	–
b. PURINES						
Adenine	4.15	9.53	4.05	9.34	3.86	9.05
2,6-Diaminopurine	5.04	10.27	4.93	10.11	4.78	10.05
8-Azaadenine	2.73	5.99	2.70	5.91	2.68	5.81
c. PYRIMIDINES						
Cytosine	4.48	11.38	4.39	11.03	4.24	10.66
Thymine	9.46	–	9.29	–	9.16	–
Uracil	9.07	–	8.91	–	8.71	–

their concentrations determined by usual volumetric and gravimetric procedures [10]. Carbonate-free sodium hydroxide was prepared and standardised by titration with pure potassium acid phthalate

Procedure

Potentiometric titrations of the various ligands ($1.5 \times 10^{-3}\ M$) in the presence and absence of metal ions were carried out with standard sodium hydroxide solution. The ionic strength of the solution was maintained at 0.1 M (KNO_3). A stream of nitrogen was passed throughout the course of the experiment in order to exclude the adverse effect of atmospheric carbon dioxide. The reactants were equilibrated before commencing the titration and between each further addition of sodium hydroxide.

An Elico model L1-120 digital pH meter with a combined electrode was used to determine the hydrogen ion concentration. The electrode system was calibrated based upon the method described earlier [11].

Calculations

Acid dissociation constants of secondary ligands and their 1:1 binary complexes

Acid dissociation constants of the secondary ligands shown by following equilibrium expressions are calculated by a direct algebraic method [11]·

$$H_2A \overset{k_a}{\rightleftharpoons} HA + H^+ \qquad (1)$$

$$HA \overset{k_{2a}}{\rightleftharpoons} A + H^+ \qquad (2)$$

Also their thermodynamic parameters were calculated using standard mathematical expressions. The stability of 1:1 complex pertaining to the equilibrium (3) was calculated by the method developed by Martell [12].

$$M + A \overset{K_{MA}}{\rightleftharpoons} MA,\ K_{MA} = \frac{[MA]}{[M]\,[A]} \qquad (3)$$

For the 1·1:1 ternary systems the various complex formation equilibria assumed are given below·

normal complex, $ML + A \overset{K_{MLA}}{\rightleftharpoons} MLA$,

$$K_{MLA} = \frac{[MLA]}{[ML]\,[A]} \qquad (4)$$

protonated complex, $ML + HA \overset{K_{MLAH}}{\rightleftharpoons} MLAH$,

$$K_{MLAH} = \frac{[MLAH]}{[ML]\,[HA]} \qquad (5)$$

and protonated complex dissociating to give normal complex

$$MLAH \overset{K_{MLA}^{MLAH}}{\rightleftharpoons} MLA + H^+,\ K_{MLA}^{MLAH} = \frac{[MLA]\,[H^+]}{[MLAH]} \qquad (6)$$

and the respective equilibrium constants are calculated based on an earlier method [13] from the suitable

TABLE II. Thermodynamic Parameters Associated with the Dissociation Reactions of Secondary Ligands. $\mu = 0.1\ M\ (KNO_3)$

Secondary ligand	ΔH_i (Kcal mol^{-1})	ΔG_i (Kcal mol^{-1})	ΔS_i (e.u.)
a. NUCLEOSIDES			
Adenosine	3.20 ± 0.20	4.93 ± 0.01	−5.60 ± 0.08
Guanosine	9.10 ± 0.30	3.45 ± 0.01	18.40 ± 0.90
	10.40 ± 0.70	12.83 ± 0.01	−7.90 ± 2.00
Cytidine	4.40 ± 0 20	5.68 ± 0 02	−4.30 ± 0 70
Uridine	7.30 ± 0.30	12.61 ± 0.01	−17.30 ± 0.70
b. PURINES			
Adenine	6.70 ± 0.06	5.85 ± 0.01	2.80 ± 0.20
	11.04 ± 0.08	13.43 ± 0.01	−7.80 ± 0.20
2,6-diaminopurine	6.00 ± 0.20	7.10 ± 0.02	−3.60 ± 0.80
	5.05 ± 0.05	14.49 ± 0.01	−30.60 ± 0.20
8-Azaadenine	1.08 ± 0.03	3.85 ± 0.01	−9.00 ± 0.10
	4.20 ± 1.00	8.45 ± 0.01	−13.80 ± 2.00
c. PYRIMIDINES			
Cytosine	5.64 ± 0.07	6.33 ± 0.01	−2.30 ± 0.30
	15.40 ± 0.20	15.97 ± 0.01	−1.90 ± 0.30
Thymine	6.90 ± 0.20	13.34 ± 0.01	−20.80 ± 0.30
Uracil	8.30 ± 0.20	12 79 ± 0.01	−14.50 ± 0 30

TABLE III. 1.1 Formation Constants for the Interaction of UO_2^{2+} and Th^{4+} with Secondary Ligands. t = 35 °C, $\mu = 0.1\ M$ (KNO_3).

Secondary ligand	$M = UO_2^{2+}$ log K	$M = Th^{4+}$ log K
Adenosine	2.9 ± 0.2	5.6 ± 0.2
Guanosine	3.1 ± 0.2	3.4 ± 0.2
Cytidine	3.5 ± 0.2	4.6 ± 0.2
Adenine	8.38 ± 0.03	10.30 ± 0.10
2,6-Diaminopurine	9.60 ± 0.02	11.98 ± 0.01
8-Azaadenine	4.20 ± 0.10	6.40 ± 0.10
Cytosine	3.70 ± 0 20*	5.50 ± 0.20*
	10.42 ± 0.01	12.40 ± 0.10

*Protonated complex.

TABLE IV. Formation Constants of 1:1:1 EDTA–M–Secondary Ligand. t = 35 °C, $\mu = 0.1\ M$ (KNO_3).

Secondary ligand	$M = UO_2^{2+}$ log K	$M = Th^{4+}$ log K
Adenosine	1.08 ± 0.08[a]	2 57 ± 0.08[a]
Guanosine	2.88 ± 0.03	2.81 ± 0.02
	−6.38 ± 0.04*	−5.67 ± 0.02*
Cytidine	–	2.85 ± 0.08[a]
Adenine	2.88 ± 0.05	3.21 ± 0.05
	−6.65 ± 0.05*	−5.88 ± 0.05*
2,6-Diaminopurine	3.01 ± 0.05	3.41 ± 0.05
	−6.57 ± 0.05*	−5 86 ± 0.05*
8-Azaadenine	3.18 ± 0.05	3.12 ± 0.05
	−5.91 ± 0.05*	−5.39 ± 0.05*
Cytosine	–	3.27 ± 0.05
		−5.90 ± 0.05*

*Constants for the formation of normal complexes by the dissociation of protonated complexes. [a]Normal complexes.

equations. Charges are omitted for simplicity. Where L = EDTA, A = Secondary ligand, M = UO_2^{2+} and Th^{4+}.

Results and Discussion

Acid Dissociation Constants of Secondary Ligands

Acid dissociation constants of the secondary ligands calculated by algebraic method are presented in Table I. Adenosine, guanosine cytidine, adenine, 8-azaadenine, cytosine were protonated by adding one equivalent of hydrochloric acid.

Thermodynamic parameters associated with the dissociation reactions of secondary ligands calculated from their corresponding temperature coefficient data are presented in Table II.

1:1 Binary complexes

A typical potentiometric titration curve is shown in Fig. 1. Potentiometric titrations of secondary

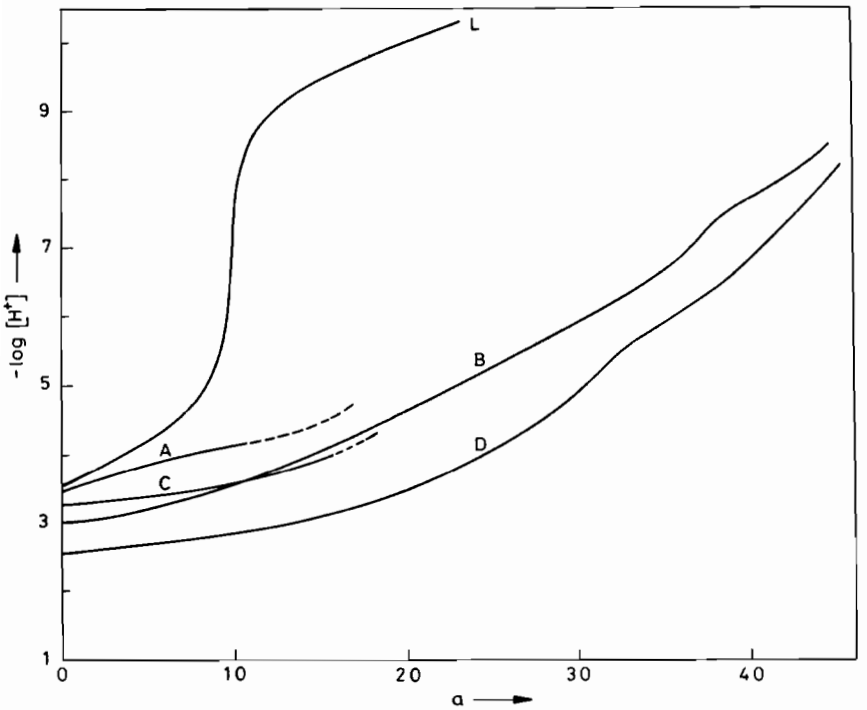

Fig. 1. Potentiometric titration curves of 1:1 binary and 1:1:1 ternary complexes involving UO_2^{2+} and Th^{4+} EDTA and adenine. L = free ligand (adenine), A = 1:1 UO_2^{2+}–adenine, B = 1:1·1 EDTA–UO_2^{2+}–adenine, C = 1:1 Th^{4+}–adenine, D = 1:1·1 EDTA–Th^{4+} adenine. a = no. of mol of base added per mol of ligand, t = 35 °C, μ = 0.1 *M* (KNO_3).

ligands in the presence of UO_2^{2+} and Th^{4+} in a 1:1 ratio could not be completed because of the separation of solid phase. However the stability of 1:1 complexes calculated in that region of the titration curve well ahead of the precipitation point are presented in Table III. In all the cases the actual metal–ligand interaction was confirmed by checking the possibility of metal hydrolysis using the titration procedure described earlier [13]. The above treatment showed very weak interaction in the case of uracil, thymine and uridine with both the metal ions.

1:1:1 Ternary complexes

In the case of ternary complexes, the region a = 0 to 2 in the titration curves (Fig. 1) corresponds to the dissociation of two protons from EDTA. Various equilibria involving the formation of normal, protonated and the protonated complex dissociating to normal complex were mathematically analysed. On this basis in the cases of adenosine with both the metal ions and cytidine with Th^{4+} ion the normal complex was assumed to be formed in the region a = 2 to 3 and the constants are calculated. In all other cases the formation of a protonated complex in the region a = 2 to 3 and its dissociation to normal complex in the region a = 3 to 4 was seen to be important and the constants calculated are given in Table II. The horizontal addition procedures [13] showed the absence of any significant interaction in the cases of thymine, uracil, uridine with both the M–EDTA systems, and also in the cases of cytidine, uridine with UO_2–EDTA system.

Dissociation constants of secondary ligands determined in this study agree with those reported earlier [7, 8, 14–22] though different experimental conditions were employed. ΔH for the first dissociation step is less negative in comparison to the second one for secondary ligands investigated, which indicates that the second proton dissociation is difficult. The entropy changes are also not favourable for the second dissociation. The combined enthalpy and entropy effect is understandable as the increased negative charge on the ligand makes the second dissociation reaction more difficult.

Binary complexes of Th^{4+} secondary ligands are more stable than the corresponding UO_2^{2+} as seen from the values of formation constants given in Table III. The stability of the 1·1 binary complexes of both UO_2^{2+} and Th^{4+} could be well correlated with the basicity of the respective ligands, a plot of which is shown in Fig. 2. The stability of binary complexes of the purine, pyrimidine bases studied here decrease in the order cytosine > 2,6-diaminopurine > adenine > 8-azadenine. The increased stabi-

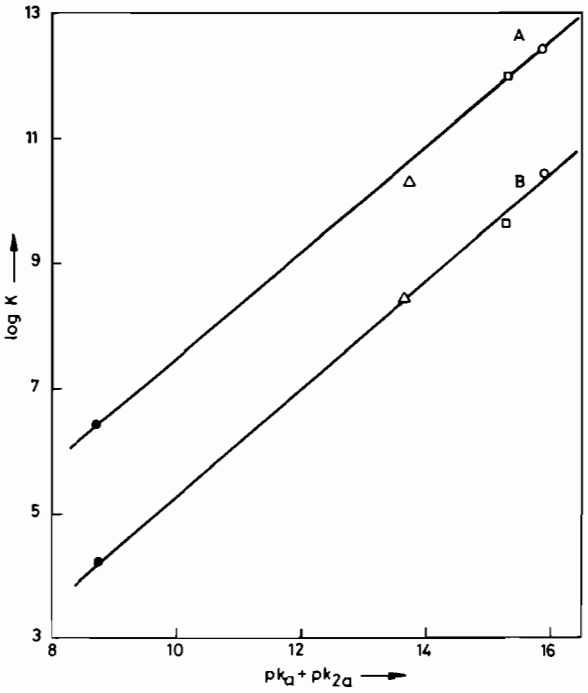

Fig. 2. Linear plot of stability of 1:1 binary complexes (log K) against the basicity of ligands (sum of pk_a and pk_{2a}). A = binary system of Th^{4+}, B = binary system of UO_2^{2+}, • = 8-azaadenine, △ = adenine, □ = 2,6-diaminopurine, ○ = cytosine. t = 35 °C, μ = 0.1 *M* (KNO_3).

lity of cytosine metal complexes may be due to the less steric environment resulting in the complexation process. In the case of nucleosides, the stability of 1:1 binary complexes decreases in the order cytidine > guanosine > adenosine. Binary complexes of nucleosides are much less stable than the corresponding bases as evident from the constants given in Table III. The presence of sugar residue imposes steric hindrance in nucleosides for their complexation with metal ions and reduces the overall basicity. The combined effect lowers the stability of metal complexes of nucleosides considerably.

Potentiometric titration curves show that in the case of ternary complexes of Th^{4+}, there is much depression in pH in the region a = 2 to 3 compared to the corresponding UO_2^{2+} system, accounting for the higher stability of the protonated complexes of the former. The higher stability of binary complexes in comparison to their ternary complexes could be explained on the basis of steric and coulombic factors dominating in the latter case. In general Th^{4+} forms more stable complexes than UO_2^{2+}. Unlike binary systems no perfect correlation between the stability of the ternary complexes and the basicities of the secondary ligands involved could be made. The existence of various basic centres on the secondary ligands has led to conflicting literature statements regarding the metal ion binding sites in these ligands. These ligands can act as monodentate in their coordination to metal ions. Chelation is also a possible alternative [23–25]. When chelation is considered as the mode of binding of these ligands to metal ions, purines can afford to form more stable chelate, a five-membered ring involving the substituent at position 6, metal ion and N_7 or a less stable 4-membered ring chelate involving C_6 substitutent, N_1, whereas metal chelation in pyrimidines exclusively involves a less stable 4-membered ring. In the present investigation it is noticed that both the purines and pyrimidine bases and nucleosides have almost identical interaction towards metal ions which indicates that the mode of binding to metal ions in both these types of ligands are similar and also may not involve chelation. Thereby both purine and pyrimidine bases and nucleosides may be acting as monodentate, the actual binding site may be located on the pyrimidine ring which is common to both. However, in order to know the nature of binding in these complexes and the actual binding sites in these ligands more detailed structural information is required and attempts on these lines are in progress in our laboratory.

Acknowledgement

One of the authors (K.R.) is thankful to the C.S.I.R., New Delhi for the award of a research fellowship.

References

1 M. J. McCall and M. R. Taylor, *Acta Cryst., B32,* 1687 (1976).
2 R. Ettorre, *Inorg. Chim. Acta, 25,* L9 (1977).
3 T. J. Kistenmacher, D. J. Szalda and L. G. Marzilli, *Acta Cryst., B31,* 2416 (1975).
4 B. L. Kindberg and E. L. Amma, *Acta Cryst., B31,* 1492 (1975).
5 M. R. Taylor, *Acta Cryst., B29,* 884 (1973).
6 T. R. Harkins and H. Freiser, *J. Am. Chem. Soc., 80,* 1132 (1958).
7 M. M. Taquikhan and C. R. Krishnamoorthy, *J Inorg. Nucl. Chem., 33,* 1417 (1971).
8 M. M. Taquikhan and C. R. Krishnamoorthy, *J. Inorg. Nucl. Chem., 36,* 711 (1974).
9 G. Schwarzenbach and H. Ackerman, *Helv. Chim. Acta, 30,* 1798 (1947).
10 A. I. Vogel, 'A Textbook of Quantitative Inorganic Analysis', ELBS and Longman, 1975.
11 P. Rabindra Reddy, K. Venugopal Reddy and M. M. Taquikhan, *J. Inorg. Nucl. Chem., 40,* 1265 (1978).
12 S. Chabarek (Jr.) and A. E. Martell, *J. Am. Chem. Soc., 74,* 5052 (1952).
13 M. M. Taquikhan and Amjad Hussain, *Indian J Chem., 19A,* 44 (1981).

14 P. A. Levene and H. S Simms, *J. Biol. Chem., 65,* 519 (1925).
15 A. Albert, *Biochem. J , 54,* 646 (1953).
16 H. Reinert and R. Weiss, *Hoppe. Seyler's Z Physiol Chem., 350,* 1310 (1969).
17 B T. Suchorukow, V. I. Poltew and L. Blumenfeld, *Abh Deut. Akad Wiss. Berlin Kl. Med.,* 381 (1964).
18 P. Brookes and P. D. Lawley, *J. Chem. Soc ,* 1348 (1962).
19 R. A Alberty, R. M. Smith and R. M. Bock, *J. Biol Chem., 193,* 425 (1951).
20 J. J. Christensen, J. H. Rytting and R. M. Izatt, *J. Chem. Soc. B,* 1643 (1970).
21 J. J. Christensen, J. H. Rytting and R. M. Izatt, *J Phys Chem., 71,* 2700 (1967).
22 L. G. Bunville and S J. Schwalbe, *Biochemistry, 5,* 3521 (1966).
23 E. Slette and A. Apeland, *Acta Cryst , B31,* 2019 (1975).
24 C. R. Krishnamoorthy, *Ph.D Thesis,* IIT Madras (1973).
25 S M. Wang and N. C. Li, *J. Am. Chem. Soc , 90,* 5069 (1968).