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Copper(II), Nickel and Uranyl Complexes of Some Amino Acids[1]

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The formation constants of copper(II) and nickel complexes of hydroxyproline, asparagine and N,N-dimethylglycine have been obtained. The pK_2 of asparagine and the formation constants of its complexes are independent of the optical configuration. The nickel complexes of serine methyl ester and glycine methyl ester are equally stable, indicating that the serine ester coördinates to the metal ion through the amino group only. At *p*H 1.90, uranyl ion forms a 1:1 complex with glycine. In the *p*H region 3.0 to 4.3, the uranyl ion does not complex with glycine, hydroxyproline, asparagine, N,N-dimethylglycine and serine, only hydrolysis of the uranyl ion occurs.

Introduction

As part of a general program of investigation of metal interaction with amino acids and peptides,[2] this paper presents the formation constants of the copper(II) and nickel complexes of some amino acids. A study of these complexes is of importance in biological chemistry, in that the accumulation of sufficient data on amino acid complexes with metal ions may contribute to a better understanding of the types of linkages involved in metal–protein interactions.

Sillén, Sutton and co-workers have pioneered in the investigation of the hydrolysis of metal ions.[3] They have found that for uranyl ion,[3b,c] the chief products of metal ion hydrolysis are the polynuclear complexes of the form $UO_2((OH)_2UO_2)_n^{2+}$ or, which is equivalent, $UO_2(OUO_2)_n^{2+}$. In view of this, it is of interest to determine whether uranyl ion interacts with the amino acids or not.

Experimental

Materials.—U-233 was obtained in the form of uranyl nitrate solution containing 4 *M* nitric acid and was purified of possible α-emitting decay products by ether extraction just prior to use. Pulse height analysis showed the purity of the U-233 isotope to be 99 ± 1%. The 2-theonyltrifluoroacetone (TTA) was obtained from the Dow Chemical Co. It was very light yellow in color and homogenous in appearance, and was used without further purification. It was stored in the dark over phosphorus pentoxide. For the cation exchanger, Dowex-50, 8% cross-linked, 100–200 mesh, was used. Stock solution of copper(II) nitrate was analyzed by addition of excess of KI and titration of the liberated iodine; nickel nitrate by precipitation with dimethylglyoxime; uranyl nitrate concentration was determined by precipitating with NH_4OH, igniting and weighing as U_3O_8. The amino acids were purchased from Nutritional Biochemical Corps., Cleveland, Ohio.

Procedure.—Measurements of *p*H and polarographic half-wave potentials were carried out in the manner described by the preceding paper.[2a]

In solvent extraction experiments for the uranyl complex of glycine, the stock solution of glycine was 0.7493 *M* containing 0.5619 *M* $HClO_4$, *p*H 1.88. The aqueous phase, *p*H 1.90, was prepared to contain (a) U-233 in concentration of the order of 10^{-6} *M*, (b) glycine in varying concentrations and (c) NaCl in varying concentrations to maintain the ionic strength at 0.45. The benzene phase, containing TTA, previously had been hydrated by shaking with an equal volume of aqueous solution containing 0.44 *M* NaCl and 0.01 *M* $HClO_4$. Ten ml. portions of aqueous and benzene phases were then placed in glass-stoppered Erlenmeyer flasks and shaken at 27° for three hours, a period of time which had been found to be more than sufficient for equilibration. At the end of the shaking period, duplicate aliquots of the benzene phases were taken for counting. The liquid was deposited directly on steel planchets, dried by overhead heating with an infrared bulb, and assayed using a proportional α-counter. The samples were infinitely thin.

In ion-exchange experiments for the uranyl complex of acetate, the acetate stock solution was 1.004 *M* acetic acid and 0.2009 *M* sodium acetate. The aqueous phase, *p*H 3.96, was prepared to contain (a) U-233 in concentration of the order of 10^{-6} *M*, (b) acetate in varying concentrations and (c) NaCl in varying concentrations so as to maintain the ionic strength constant at 0.16. Two hundred mg. of the resin and 25 ml. of the aqueous phase were then placed in erlenmeyer flasks and shaken at 27° for three hours. After equilibration, a measured volume of supernate was removed from each flask, and the α-activity was assayed in the manner shown above.

Results

(A) Copper(II) and Nickel Complexes of Amino Acids.—The stepwise formation constants for equilibria of the type $MA_{n-1} + A = MA_n$ are designated by the expression $k_n = (MA_n)/(MA_{n-1})(A)$, and were determined by means of the Bjerrum method.[4] Table I summarizes the formation constants of the Cu(II) and nickel complexes obtained at an ionic strength of 0.15; the values in columns 1 and 2 refer to the initial concentrations of the divalent metal ion and the amino acid, respectively. For the serine ester complexes there are indications that higher order complexes exist. However, only values of log k_1 and log k_2 are recorded, because of the hydrolysis of the ester at higher *p*H values. Included as reference in Table I are Basolo's values for the complexes of N,N-dimethylglycine[5] at $u = 0.14$.

The formation constant of the Cu(II) complex of hydroxyproline also was determined by means of the polarographic method. The half-wave potential of a solution consisting of 5.00×10^{-4} *M* $Cu(NO_3)_2$, 0.15 *M* KNO_3, 0.200 *M* hydroxyproline, 0.100 *M* KOH, was determined to be −0.382 v. at 25°. By using equation (7) of the preceding paper,[2a] and taking $(E_{1/2})_{s,Cu^{++}} = 0.014$ v. and $p = 2$, we have calculated for the Cu(II) complex of hydroxyproline log $k_f = \log k_1k_2 = 15.4$.

(B) Uranyl Complexes of Glycine and Acetate.—Table II lists the solvent-extraction results with the uranyl complex of glycine at *p*H 1.90.

(1) This investigation was supported by National Science Foundation Grant No. G1926 at D.U. and by Atomic Energy Commission Contract No. AT (40-1)-2005 at C.B.C.

(2) (a) N. C. Li and M. C. M. Chen, THIS JOURNAL, **80**, 5678 (1958); (b) N. C. Li and E. Doody, *ibid.*, **76**, 221 (1954).

(3) (a) L. G. Sillén, *Acta Chem. Scand.*, **8**, 299, 318 (1954); (b) S. Ahrland, S. Hietanen and L. G. Sillén, *ibid.*, **8**, 1907 (1954); (c) J. Sutton, *J. Chem. Soc.*, Suppl. Issue No. 2, S275 (1949).

(4) J. Bjerrum, "Metal-Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

(5) F. Basolo and T. T. Chen, THIS JOURNAL, **76**, 953 (1954).

Table I

Formation Constants of Cu(II) and Nickel Complexes

M(II), M	Amino acid, M	pK	log k_1	log k_2
Cu	Hydroxyproline	9.47		
0.05	0.01		8.34	
.03	.01		8.36	
.02	.01		8.41	
.01	.02		8.22	6.96
.02	.02		8.33	
		Av.	8.33 ±0.05	
Ni				
0.02	0.02		5.94	
.01	0.02		5.89	4.84
Cu	Asparagine[a]	8.71[b]		
0.01	0.02		7.78	6.35
Ni				
0.01	0.02		5.58	4.38
Cu	N,N-Dimethyl-glycine	9.80		
0.01	0.02		7.26	6.27
			7.30[5]	6.35[5]
Ni				
0.01	0.02		4.77	3.70
			4.82[5]	3.78[5]
Ni	Serine methyl ester	7.10		
0.01	0.04		2.37	1.98

[a] Log k_a has the same value (±0.05) for D-, L- or DL-asparagine. [b] pK = 8.71 ± 0.03 for D-, L-, or DL-asparagine.

The assumption is made that the cationic species of glycine, CH_2–(NH_3^+)–COOH, does not interact with uranyl ion, so that at pH 1.90, the only ligand is the isoelectric glycine, HG. In Table II, K_d is

Table II

Solvent-extraction Results with the Uranyl Complex of Glycine, u = 0.45, pH 1.90, 25°

(Glycine) = (HG)[a] × 10^2, M	$1/K_d$	k_f
0	2.50[b]	
1.01	3.13	25
2.89	4.54	28
4.76	5.83	28
6.63	6.72	25
8.51	8.45	28
10.38	9.64	28
12.25	11.15	28
	Av.	27

[a] In calculating the concentration of glycine, HG, the value of pK_1 is taken to be 2.32. [b] This is $1/K_d°$, and is obtained by extrapolation of the $1/K_d$ *vs.* (HG) linear plot at the constant pH of 1.90.

the distribution coefficient of uranyl ion between the organic phase containing TTA as a chelating agent, and the aqueous phase, that is

$$\frac{1}{K_d} = \frac{\%\text{ U-233 in aq. phase}}{\%\text{ U-233 in org. phase}} \times \frac{\text{ml. org. phase}}{\text{ml. aq. phase}}$$

k_f is the formation constant of the uranyl–glycine complex and is calculated from the equation[6]

$$k_f = \frac{(K_d°/K_d) - 1}{(\text{HG})} \qquad (1)$$

(6) N. C. Li, W. M. Westfall, A. Lindenbaum, J. M. White and J. Schubert, This Journal, **79**, 5864 (1957).

The use of this equation assumes that the positively charged uranyl–glycine complex remains in the aqueous phase and that the uncharged TTA complex of uranyl exists predominantly in the benzene phase.[7]

The uranyl complexes of acetate were investigated by the use of U-233 in conjunction with the ion-exchange method, in the manner described by Li, *et al.*[6] The formation constants of the acetate complexes are: k_1 = 240 and $K_3 = 2.4 \times 10^6$, where K_3 is the concentration equilibrium constant for the reaction at u = 0.16 and 25°

$$UO_2^{++} + 3Ac^- = UO_2Ac_3^-$$

These values are in excellent agreement with the values found by Ahrland,[8] who obtained for the acetate complexes at u = 1.0, 20°

$$k_1 = 240,\ K_3 = 2.2 \times 10^6$$

(C) *p*H Titrations of Uranyl Solutions.—Numerous pH titrations were carried out for solutions containing different concentrations of uranyl and different molar ratios of uranyl and amino acid, sufficient KNO_3 being added in each case to maintain ionic strength at 0.16. The stock solution of uranyl nitrate always contained equal molar amounts of HCl, and only freshly prepared solutions of uranyl and amino acids were used. Titration data for a 1:1 uranyl–hydroxyproline mixture are given in Table III; column 3 lists values of

Table III

Titration of Uranyl-Hydroxyproline Mixture

30 ml. solution containing 0.01 M $UO_2(NO_3)_2$, 0.01 M HCl, 0.12 M KNO_3, 0.01 M hydroxyproline, titrated with 0.0978 M KOH

KOH, ml.	pH	Z
3.40	3.58	0.14
3.60	3.67	.20
3.80	3.73	.26
4.00	3.80	.32
4.20	3.86	.38
4.40	3.92	.45
4.60	3.98	.51
4.80	4.03	.57
5.00	4.09	.63

Z, where Z is the number of protons split off per uranyl ion

$$Z = \frac{(\text{NaOH}) - (\text{HCl}) + (\text{H}^+) - (\text{OH})^-}{T_m}$$

and T_m is the total concentration of uranyl nitrate. Table IV summarizes some of the results obtained, and it is seen that the values of (log T_m + 2 pH) are practically the same, at given values of $Z/2$, independent of the concentration of uranyl and hydroxyproline, and independent of whether serine or proline is present or not.

Discussion

From the data in Table I it is seen that for asparagine the dissociation constant and the formation constants of its copper and nickel complexes are independent of the optical configuration. Asparagine contains but one asymmetric carbon

(7) (a) R. A. Day, Jr., and R. W. Stoughton, *ibid.*, **72**, 5662 (1950); (b) R. A. Day, Jr., and R. M. Powers, *ibid.*, **76**, 3895 (1954).

(8) S. Ahrland, *Acta Chem. Scand.*, **5**, 199 (1951).

TABLE IV

pH TITRATIONS OF URANYL, $u = 0.16$, 25°

	0.01 M hydroxyproline and 0.01 M UO_2^{++}	0.05 M UO_2^{++}	0.01 M serine and 0.01 M UO_2^{++}	UO_2^{++}[a]
$Z/2$	log T_m + 2 pH		log T_m + 2 pH	
0.04		4.82		
.05		4.91		
.06		5.01		5.01
.07	5.11	5.11	5.15	..
.08	..	5.18	..	5.14
.10	5.29	5.25	5.31	5.25
.13	5.41		5.43	..
.16	5.54		5.56	..
.19	5.66		5.68	..
.22	5.78		5.80	5.79
.26	5.90		5.92	5.94
.29	5.99		6.01	..
.32	6.11		6.13	6.15
.38			6.31	6.35
.48			6.64	6.65

[a] Containing no amino acid.

atom, and the absence of any effect of optical configuration on the dissociation of the substituted ammonium group is in agreement with that found by Ellenbogen[9] for the dissociation of glycylalanine and alanylglycine. On the other hand Ellenbogen[9] found that for a compound having two asymmetric carbon atoms, as for example alanylalanine, the LD form has a higher pK_2 than the LL. It would be interesting to study the effect of optical configuration on complex formation constants for such compounds, and we plan to pursue this study immediately.

The pK_2 of asparagine is one unit lower than that of glycine, and the log k_1k_2 value of Cu(II) or Ni(II) complex of asparagine is also one unit lower than the value for the corresponding metal complex of glycine.[10] This comparison indicates that the coördination sites of asparagine toward Cu(II) and Ni(II) are the same as those of glycine, namely, the amino and carboxylate groups, and that the amide group is not involved.

The log k_1 value of nickel complex of serine methyl ester is only 0.08 log unit lower than that of glycine methyl ester[10] and is of the order of the Ni complex of ammonia, while it is 3.6 log unit lower than that of glycine complex. The serine methyl ester therefore, like the glycine methyl ester, coördinates to the metal ion through the amino group only, and the hydroxyl group in serine probably is not involved in binding to the metal.

Our values for the N,N-dimethylglycine complexes are in good agreement with those of Basolo and Chen,[5] and demonstrate the steric effect of the methyl groups in lowering complex stability (glycine complex of Cu: log k_1k_2 = 15.10; glycine complex of Ni: log k_1k_2 = 10.92[10]). The metal ion concentrations used in Basolo's experiments were approximately 0.0025, while in our experiments the metal ion concentrations were 4-fold greater; the agreement in the formation constants indicates that these complexes are mononuclear.

The Cu(II) complexes of hydroxyproline were investigated by both pH and polarigraphic methods. For this amino acid, the metal ion concentration was varied 100-fold and the constancy of the formation constant over this wide metal ion concentration range demonstrates even more strikingly that the complexes are mononuclear.

In the solvent-extraction experiment for uranylglycine complex at pH 1.90, the value of k_f is essentially constant, independent of the concentration of glycine. In the concentration range studied, therefore, only a 1:1 complex with glycine is present in solution. The much greater stability of the uranyl–acetate complex over the uranyl–glycine complex is reasonable since the repulsion between the positively charged NH_3^+ group in glycine and the UO_2^{++} cation would be expected to decrease the affinity of the carboxylate ion for the same cation. The glycine complex was not investigated by the ion-exchange method, because the positively charged UO_2HG^{++} complex would be expected to be taken up by the cation exchanger.[6]

Ahrland, *et al.*,[3b] and Sutton[3c] have shown that the uranyl ion hydrolyzes to form polynuclear complexes of the general formula $UO_2(OUO_2)_n^{2+}$. Ahrland, *et al.*, found that in 1 M $NaClO_4$ medium at 20°, for uranyl concentration varying from 0.0014 to 0.0466 M, the data fall on a single curve of $Z/2$ *vs.* (log T_m + 2 pH), and that the data can be accounted for by assuming "mechanism IIIa," with the equations

$$UO_2^{2+} + H_2O + UO_2^{2+} = UO_2OUO_2^{2+} + 2H^+; \quad kk_0 = 10^{-6.05 \pm 0.1}$$

$$UO_2(OUO_2)_n^{2+} + H_2O + UO_2^{2+} = UO_2(OUO_2)_{n+1}^{2+} + 2H^+; \quad k = 10^{-6.35 \pm 0.1}$$

The data of Table IV fall on a curve of $Z/2$ *vs.* (log T_m + 2 pH), drawn with $k_0 = 2$, $k = 10^{-6.42}$. This means that in the pH region used (pH between 3.0 and 4.3), no complexation of uranyl ion with hydroxyproline and serine takes place; only hydrolysis of uranyl ion occurs. The titration data for uranyl solutions containing glycine, asparagine and N,N-dimethylglycine, in the pH region 3.0 to 4.3, are similar to those for uranyl solutions containing hydroxyproline and serine; therefore the same conclusion may be applied to these amino acids.

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(9) E. Ellenbogen, THIS JOURNAL, **74**, 5198 (1952).
(10) J. M. White, R. A. Manning and N. C. Li, *ibid.*, **78**, 2367 (1956).