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Quantitative Studies of the Avidity of Naturally Occurring Substances for Trace Metals

4. A POTENTIOMETRIC METHOD FOR HIGHLY INSOLUBLE COMPLEXES, AND ITS APPLICATION TO COMPLEXES OF ADENINE ANION*

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Adenine, in spite of the 1:2-diamine structure involving the 6- and 7-positions, has little tendency to form complexes with the cations of heavy metals, as was shown quantitatively (Albert, 1953). However, by using less acidic solutions it has been found that the anion of adenine has a strong avidity for bivalent cations, which it binds between $N_{(7)}$ and the primary amino group (Harkins & Freiser, 1958).

(I)

In pursuing this observation, we found that the copper complex was so insoluble that it was necessary to work at a greater dilution than has been customary hitherto in potentiometric work. This necessitated refinements of technique and apparatus which it is believed will be generally useful for insoluble complexes.

This work has revealed that, contrary to what

had been supposed, copper binds the adenine anion by almost simultaneous addition of two equivalents, giving the 2:1 complex (I).

METHODS

Materials. Glycine (A.R.) was recrystallized from water and dried at 110° for an hour. Adenine (British Drug Houses Ltd.) was recrystallized from water twice and then found to be chromatographically homogeneous. It was recrystallized once more and dried at 110°. Solutions (5 mM) of these substances were prepared in boiled ion-free water. A part of these solutions was diluted in the titration vessel with ion-free water, added under nitrogen from a grade A burette, to yield the concentrations given in the tables.

Pure copper perchlorate and nickel perchlorate solutions were made by pouring a solution of barium perchlorate on to a column of cation-exchange resin (Amberlite IR 120) previously quantitatively converted into the appropriate metal salt. A barium-free fraction of the eluate was collected and standardized by both a gravimetric and an ion-exchange method. Further dilutions were made with boiled ion-free water.

Apparatus. The titration vessel was a 250 ml. tall beaker sealed into a water-jacket through which water from a thermostatically controlled bath was circulated, thus maintaining the solution in the beaker at 20.0 ± 0.02°. The beaker was closed with a rubber bung carrying the electrodes, a nitrogen inlet, a thermometer and a sealable inlet for the glass capillary of a micrometer syringe. The nitrogen inlet was arranged so that a stream of oxygen-free nitrogen (saturated with water) was directed across the surface of the solution.

The pH was measured to 0.002 pH unit with a Vibron electrometer (model 33B; Electronic Instruments Ltd.), complete with pH attachment, glass electrode (type GG 33)

* Part 3: Albert (1953).

and a saturated calomel electrode (type RJ 23). This instrument, when left under experimental conditions, was found to reproduce a buffer reading to 0.004 pH unit or less, after 24 hr., without readjustment. To prevent precipitation of potassium perchlorate on the sintered-glass barrier, the calomel electrode was placed in an outer jacket fitted with another sintered barrier and containing a solution of 1.60 M ammonium nitrate and 0.20 M sodium nitrate adjusted to pH 7 (Perrin, 1958). Ammonium ions passed through this barrier at 59 $\mu\text{g./hr.}$ This was decreased to 3.6 $\mu\text{g./hr.}$ by setting in agar 0.5 ml. of the bridge solution above the sinter. The components were assembled so that the level of the solution in the titration vessel was about 5 mm. below those in the outer jacket and the calomel electrode.

The titration was performed by adding 0.1 N-sodium hydroxide (carbonate free) in small portions from a micro-meter syringe. The solution was stirred magnetically during, and for 60 sec. after, each addition. The pH was measured at 60 sec. intervals until constant (usually after about 6 min. and after a drift of about 0.030 to higher pH). When precipitation began, a drift to lower pH occurred; at this point a titration was always discontinued because experience showed that the results obtained were variable and irrelevant.

Calculations. Values of $\bar{n}$ (average number of molecules bound by one atom of metal) and $[L^-]$ (concentration of free chelating species, formerly written $[\text{Sc}]$), were calculated as before (Albert, 1952). These were inserted in equation (1) (Irving & Rossotti, 1953). This is an equation to a straight line and can give results of higher precision than the similar equations used in earlier parts of this series, particularly when $\log K_1 - \log K_2$ lies in the range 0.5–2.5.

$$\frac{\bar{n}}{(\bar{n}-1)[L^-]} = \frac{(2-\bar{n})[L^-]}{(\bar{n}-1)} \cdot \beta_2 - K_1, \quad (1)$$

where K_1 is the stability constant of the 1:1 complex and β_2 (formerly written K_2) is the overall stability constant of the 2:1 complex (e.g. 1). K_2 , the constant governing the equilibrium between the 1:1 and the 2:1 complexes, is given by:

$$\log K_2 = \log \beta_2 - \log K_1 \quad (2)$$

K_1 and β_2 were evaluated from equation (1) by the method of least squares.

When the addition of two equivalents of ligand (to one of

the metal) is not stepwise but simultaneous, equation (3) gives consistent results whereas (1) fails to do so (Albert, 1953):

$$\log \beta_2 = \log \bar{n} - \log (2 - \bar{n}) - 2 \log [L^-] \quad (3)$$

The pK_a values of 4.25 ± 0.01 (basic) and 9.83 ± 0.04 (acidic) were found at 20° (5 mm) and used in these calculations.

For the copper concentrations used in this work, the pH regions are such that hydrolysis of the cupric ion is negligible (Dr D. D. Perrin, personal communication; cf. Perrin, 1960).

RESULTS

The refined technique was worked out with glycine, which has a moderately soluble copper salt. It was titrated against alkali at 0.5 mm, with glycine-copper ratios varying from 2:1 to 10:1. The need for the higher ratio arose from the realization that with adenine it would be necessary to work at very low copper concentrations to avoid precipitation. At such low concentrations it was desirable to increase the ligand-metal ratio to force the equilibrium sufficiently far to enable the slope of the formation curve to be studied.

The figures for glycine, given in Table 1, were calculated from equations (1) and (2) as described. To obtain the spread values for the last two columns of Table 1, the mean value of K_1 was substituted in equation (1) and β_2 was solved for ten individual values of $\bar{n}$ and L . Similarly, the mean value of β_2 , originally obtained was used to give 12 results for K_1 . The first three dilutions in Table 1 gave results which show excellent agreement among one another, thus justifying the use of high ligand-metal ratios. The fourth experiment, where the increased ionic strength corresponds to that obtained in earlier work with 10 mm-glycine (Albert, 1950), shows good agreement, thus justifying titration in dilute solution.

The titration of adenine with nickel (ratio 10:1) by this technique gave $\log K_1$ of 4.37 ± 0.06 , as shown in Table 2. Precipitation prevented higher

Table 1. *Determination of the stability constants of the glycine-copper complexes in water at low concentrations of copper*

Concn. of glycine was 0.5 $\times$ mm in a total volume of 100 ml. Temp. 20°.

Concn. of Cu ²⁺ ions (mm)	Ionic strength (approx.)	$\log K_1$	$\log \beta$	$\log K_2$
0.25	10 ⁻⁴	8.57 $\pm$ 0.02	15.70 $\pm$ 0.04	7.13 $\pm$ 0.03
0.125	10 ⁻⁴	8.60 $\pm$ 0.02	15.74 $\pm$ 0.05	7.13 $\pm$ 0.03
0.05	10 ⁻⁴	8.61 $\pm$ 0.02	15.74 $\pm$ 0.05	7.13 $\pm$ 0.05
0.05	10 ⁻³	8.34 $\pm$ 0.03	15.38 $\pm$ 0.06	7.05 $\pm$ 0.05
(NaClO ₄)				
10 ⁻² M*-Glycine				
5.0	10 ⁻²	8.47	15.38	6.91

* Albert (1950).

Table 2. *Determination of the stability constants of two metal complexes of the adenine anion*

Concn. of adenine, 0.5 mM, and Ni²⁺ ions, 50 μM, in a total volume of 100 ml. Temp., 20°.

Vol. of 0.01N-NaOH (ml.)	pH	log [L ⁻]	$\bar{n}$	log K ₁
0	6.516			
0.100	7.691	8.5515	0.122	4.59
0.150	7.979	8.8352	0.154	4.43
0.200	8.146	8.9981	0.182	4.35
0.250	8.268	9.1159	0.210	4.31
0.300	8.342	9.1855	0.264	4.37
0.350	8.414	9.2531	0.308	4.41
0.400	8.496	9.3308	0.326	4.35
0.500	8.622	9.4480	0.384	4.35
0.600	(pptn)	—	—	—

Adenine 0.10 mM and Cu²⁺ ions 7.5 μM (total volume 100 ml.)

Vol. of 0.01N-NaOH (ml.)	pH	log [L ⁻]	$\bar{n}$	log K ₁ (attempted)	log β
0	6.259	8.4182	0.201	6.98	14.11
0.010	6.359	8.5164	0.292	7.10	14.20
0.020	6.447	8.6018	0.381	7.20	14.17
0.030	6.519	8.6698	0.509	7.35	14.19
0.040	6.579	8.7260	0.646	7.54	14.22
0.050	6.633	8.7762	0.753	7.70	14.23
0.060	6.684	8.8227	0.881	—	14.25
0.070	6.727	8.8623	0.982	—	14.26
0.080	(pptn)	—	—	—	—

values of $\bar{n}$ than 0.38 being attained, even in more dilute solutions, and hence no values of log β and log K₂ could be found.

For the titration of adenine with copper, more dilute solutions were found necessary to avoid precipitation of the copper-adenine complex, which is very poorly soluble. Because of the greater avidity for copper, an $\bar{n}$ value as high as 0.98 was reached without precipitation, as shown in Table 2. When no consistent value for log K₁ could be calculated from equation (1), it was realized that most of the copper cations were simultaneously adding two equivalents of adenine anions. The use of equation (3) confirmed this, and the value log β₂ = 14.22 ± 0.05 was obtained. Even at very low values of $\bar{n}$, little evidence of 1:1 addition could be found.

Qualitative experiments showed that the adenine anion has considerable avidity for all the bivalent cations of the first-transition series of metals.

DISCUSSION

The results obtained with glycine show that potentiometric titration can be adapted for a far greater dilution than has hitherto been used in the determination of the stability constants of metal complexes (see Table 1).

It was shown (Albert, 1953) that the neutral molecule of adenine had even less metal-binding avidity than ammonia. Thus log K₁ for adenine-copper was 2.5, and no detectable combination with

cobalt could be found. This earlier work was carried out between pH 3 and 6. But in the present work, performed at higher pH values to furnish significant amounts of adenine anion, a strong avidity for bivalent cations was demonstrated. This avidity lies a little below that shown by the common amino acids. Thus log K₁ for adenine-nickel (4.37) may be compared with log K₁ for glycine-nickel (6.14), and log β₂ for adenine-copper (14.22) may be compared with log β₂ for glycine-copper (15.74).

Our adenine-copper results may be compared with some published figures: log K₁ (7.1), log K₂ (6.4), log β₂ (13.5) (Harkins & Freiser, 1958). These were obtained at concentrations (0.4 mM-adenine and 50 μM-copper) that produced precipitation throughout most of the titration, thus invalidating the results (the solubility product of [Cu²⁺] [L⁻]² is 10^{-19.1±0.1}). There is little stepwise addition of adenine anion to copper, and the two equivalents add simultaneously (for details, see Results section). Simultaneous addition, to which copper is particularly prone, has not previously been encountered in purines but has been demonstrated for riboflavin and several pteridines (Albert, 1953).

SUMMARY

1. The potentiometric technique for the determination of stability constants of metal complexes has been adapted for use in more dilute solutions than has been customary (in one example at 7.5 μM-

metal concentration). The procedure is useful for poorly soluble metal complexes.

2. Application of this technique to the adenine anion confirms that this species, unlike the neutral molecule of adenine, has a high avidity for bivalent cations.

3. Contrary to what has been thought, the 2:1 complex of the adenine anion and copper is formed simultaneously, and not stepwise.

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