Thermodynamics of Metal Complexes with Ligand–Ligand Interaction, Simple and Mixed Complexes of Copper(II) and Zinc(II) with Adenosine 5'-Triphosphate and L-Tryptophan or L-Alanine

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Thermodynamic parameters for the formation of simple copper(II) and zinc(II) complexes with adenosine 5'-triphosphate (ATP), L-tryptophanate (trpO), and L-alaninate (alaO) were determined by means of potentiometric and calorimetric measurements. Detailed analysis of the potentiometric data allowed reliable values of stability constants to be obtained. For the first time, the thermodynamic functions for the $[M_2(ATP)]$ species are also reported. On the basis of $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ changes, the structure, in aqueous solution, of the main species present in the M–ATP systems are discussed. The $\Delta G^{\ominus}$, $\Delta H^{\ominus}$, and $\Delta S^{\ominus}$ values for the $[M(ATP)L]^{3-}$ mixed-ligand complex (L = amino-acid anion) were also determined. The larger positive $\Delta H^{\ominus}$ and the less positive $\Delta S^{\ominus}$ accompanying formation of $[M(ATP)(trpO)]^{3-}$ compared with $[M(ATP)(alaO)]^{3-}$ are considered to indicate the presence of ligand–ligand interaction in the former complex. Further support for this is gained by comparing the differences between the $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ values for formation of $[Cu(ATP)(trpO)]^{3-}$ and $[Zn(ATP)(trpO)]^{3-}$ and those for $[Cu(ATP)(alaO)]^{3-}$ and $[Zn(ATP)(alaO)]^{3-}$. The presence of the stacking interaction is also discussed in relation to the geometric requirements of the metal ions.

The presence of an interaction between two ligands bound to the same metal ion, in solution, has been established by means of spectroscopic and thermodynamic studies.[1-3] Ligand–ligand electrostatic interactions between oppositely charged groups in the side chains of co-ordinated amino-acids have been invoked to explain the stereoselectivity observed in the formation of ternary complexes.[4,5] An enhancement of stability due to an interaction of the aromatic moieties through the formation of a metal bridge between two different ligands has also been reported.[6,7] Owing to the important role that mixed complexes with stacking interactions play in a large number of biological processes,[8-11] some properties of these systems have been investigated. In particular, the stoicheiometry, stability, and molecular conformations of NTP–M–L complexes (NTP = nucleoside triphosphate; L = ligand with 'aromatic' substratum such as 2,2'-bipyridyl, tryptophan, or catecholamines) have been studied.[12-19]

Due to the difficulty of obtaining definite information on the bonding characteristics of complexes in solution and because thermodynamic studies can be a useful tool for understanding the nature of the metal–ligand interactions,[20-25] we felt that it would be interesting to determine all the thermodynamic functions involved in the formation of ternary complexes of copper(II) or zinc(II) with ATP (ATP = adenosine 5'-triphosphate) and biofunctional ligands. For the latter we chose two L-amino acids, namely L-tryptophan (trp) and L-alanine (ala), because the comparison between the stability constants of their ternary complexes with ATP and some transition-metal ions has been considered useful to show the presence of the stacking interaction in these systems.[26,27]

Calorimetric studies of stacking interactions between organic molecules have been published,[28,29] but prior to this work no $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ values of metal complexes having this so-called 'secondary' bonding have been reported. Recently, the $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ values accompanying the interaction between caffeine and bis(1,10-phenanthroline)copper(II) have been published.[30] In that work the mixed complex is of M–A–B type, and not of the A–M–B type as reported here.

Moreover, we have carried out a detailed potentiometric and calorimetric study of simple copper(II) and zinc(II) complexes of ATP and of the two amino-acids under the same conditions used for the mixed complexes, *i.e.* 25 °C and $I = 0.1$ mol dm^{-3} ($NaClO_4$ or KNO_3). These measurements, while useful for obtaining homogeneous data for both simple parent complexes, were, on the other hand, indispensable for ATP systems owing to (*i*) the large spread in stability constant values,[19] (*ii*) the species models, and consequently,[19,31] (*iii*) the unreliable enthalpy and entropy values.[32] Furthermore our results were corrected by taking into account the complexation of ATP with Na^+ and K^+.[33]

Experimental

Chemicals.—Adenosine 5'-triphosphate was a Merck product; its purity was checked by two independent methods, alkalimetric titrations and determination of the inorganic phosphate content.[34] A rather high (5—9%) water content was detected; the inorganic phosphate was always lower than 3%, samples containing more than 3% being rejected. L-Tryptophan and L-alanine were Serva products. Their purity was determined alkalimetrically and was always higher than 99%. The salts $Zn(ClO_4)_2$ and $Cu(ClO_4)_2$ were prepared from zinc oxide and copper(II) basic carbonate $[CuCO_3 \cdot Cu(OH)_2 \cdot 2H_2O]$ respectively by adding a slight excess of $HClO_4$. The concentration of stock solutions was determined by titration with ethylenediaminetetra-acetate (edta). The excess of $HClO_4$ was determined by Gran plot and by ACBA computer program (see *Calculations*). Stock solutions of HNO_3 and KOH or NaOH were prepared by diluting the more concentrated contents of ampoules (Merck or C. Erba); their concentrations were determined by titrations with tris(hydroxymethyl)methylamine (tham) and potassium hydrogenphthalate, respectively. Cross titrations were also performed to check the results. Stock

Table 1. Experimental details of potentiometric measurements, at 25 °C and I = 0.1 mol dm^{-3}

System	Analytical concentrations in the titration vessel (mmol dm^{-3}) [a]	Titrant concentration (mol dm^{-3})	No. of titrations [b]
H^+ + amino-acids	$c_L = c_H$ = 1—10	c_{OH} = 0.1	4
	c_L = 1—10, c_H = 0	c_H = 0.25	3
Cu^{2+} or Zn^{2+} + amino-acids	$c_L = c_H$ = 2—7.5, c_M = 1—5, c_M/c_L = 1—0.25	c_{OH} = 0.1	6
Cu^{2+} or Zn^{2+} + ATP	$c_{ATP} = \frac{1}{2} c_H$ = 4—10, c_M = 2.5—10, c_M/c_{ATP} = 1—0.25	c_{OH} = 0.1	6
	$c_{ATP} = \frac{1}{2} c_H$ = 50—60, c_M = 7.5—15	c_{OH} = 0.5	4
	$c_{ATP} \neq c_M$ = 4—6		
	c_M = 3—4, c_H = 0—7	$c_{Na_4(ATP)}$ = 0.025	3
Cu^{2+} or Zn^{2+} + ATP + amino-acids	$c_M = c_{ATP} = c_L = \frac{1}{3} c_H$ = 0.75	c_{OH} = 0.005	3
	$c_M = \frac{1}{2} c_{ATP} = \frac{1}{2} c_L$ = 2, c_H = 4	c_{OH} = 0.1	2
	c_M = 6, c_L = 4, c_H = 4	$c_{Na_4(ATP)}$ = 0.025	3

[a] L = Amino acid. [b] 25—40 Points for each titration curve.

solutions of $NaClO_4$ were prepared according to Holmes and Williams.[35] Potassium nitrate (Fluka) was employed as received. All solutions were prepared with twice distilled water. Grade A glassware was employed throughout. The ionic strength was adjusted to 0.1 mol dm^{-3} by adding KNO_3 or $NaClO_4$.

Potentiometric Measurements.—Two different sets of equipment were used to perform the pH-metric measurements: (*a*) a Radiometer PHM 52B potentiometer equipped with Ingold electrodes, the titrations being carried out manually and the titrant delivered with a Radiometer ABU 12 motorized burette; (*b*) an Orion 801 A meter equipped with an EIL glass and an Ingold saturated calomel electrode, the potentiometer being connected with an Amel timer-printer (model 882) controlling the addition of titrant delivered from an Amel digital dispenser (model 232) and the titrations being performed automatically.

The titration cells were equipped with a jacket connected to a Colora thermostat bath (model NB/DS 997) so that the temperature was kept at 25.0 ± 0.2 °C. All solutions were maintained under an atmosphere of inert nitrogen, which had been saturated with water by bubbling it through a solution of the same ionic strength and temperature as that being titrated. The solutions were magnetically stirred. The electrode couples were standardized on the pH ≡ −log c_{H^+} scale by titrating HNO_3 (0.01—0.005 mol dm^{-3}) with standard NaOH or KOH at 25 °C and I = 0.1 mol dm^{-3} (KNO_3 or $NaClO_4$). Solutions (25—100 cm^3) containing, in turn, a ligand (protonation), a ligand and a metal ion (binary complex formation), and a metal and two ligands (ternary complex formation) were titrated with standard NaOH or KOH until a precipitate was observed. Some titrations were performed on solutions containing, in turn, the metal or the metal and the amino-acid with Na_4(ATP). The solutions were freshly prepared to avoid hydrolysis of ATP. Experimental details are reported in Table 1.

Calorimetric Measurements.—The calorimetric measurements were performed at 25.000 ± 0.001 °C employing an LKB model 8700 precision calorimeter, and a 100-cm^3 LKB model 8726-1 titration vessel, equipped with a standard resistance (50 Ω) and a 2 000-Ω thermistor. Changes in thermistor resistance during the titration were recorded by means of a Leeds and Northrup model Speeddomax W recorder. Several series of electrical calibrations were carried out to test the reproducibility of the calorimetric system, and the standard deviation of the mean value of the heat equivalent, ε_v, was always better than 0.1%.

The titrant was delivered from a Radiometer motorized burette (model ABU 12B, 2.5 or 25 cm^3) or a Metrohm motorized burette (model Dosimat E 415, 10 cm^3). In order to check the accuracy of the calorimeter, tham solutions were titrated with HCl and $HClO_4$ solutions were neutralized with standard NaOH; in both cases good agreement with results of other investigators was achieved.[36,37]

In order to determine the formation heats the following measurements were performed.

(*a*) Protonation heats (for the amino-acids): the ligand solutions (80—95 cm^3), up to 95% neutralized with NaOH or KOH, were titrated with standard $HClO_4$ or HNO_3.

(*b*) Binary-complex formation heats: solutions (80—95 cm^3) containing the ligands were titrated with a metal standard solution. Titrations of the metal with ligand solutions were also performed.

(*c*) Ternary-complex formation heats: the same procedure as in (*b*) was used, the titrants being the metal ion or the ATP solution.

The heat equivalent, ε_v, was determined by electrical calibration after each addition of titrant. The reaction heats, corrected for the dilution heats determined in separate experiments, were calculated considering 1 cal = 4.184 J.

Experimental details are reported in Table 2.

Calculations.—The calculations concerning the E° of the electrode system, the purity of the ligands, their protonation constants, and the determination of the excess of HNO_3 or $HClO_4$ in the metal-ion stock solutions were performed by the least-squares computer program ACBA,[38] which minimizes the error-squares sum in the titre.

For the determination of the excess of acid in the metal-ion stock solutions, only points in the acidic range were taken into consideration, *i.e.* before hydrolysis occurs, whereas for the ligands the standardization of the electrode couple was performed in the pH range 2.75—10.5; in the latter case E° and pK_w were simultaneously refined. (We obtained pK_w = 13.73 ± 0.02 and 13.75 ± 0.02 in $NaClO_4$ and KNO_3 respectively at 25 °C and I = 0.1 mol dm^{-3}.) The standard deviation in the titre was always lower than 0.005 cm^3.

The calculations of the formation constants for both binary and ternary complexes were performed by means of two least-squares computer programs: (*i*) SCOGSB,[39] which minimizes the error-squares sum in titre; (*ii*) MINIQUAD 76A,[40] which minimizes the error-squares sum in the analytical concentrations. In performing these calculations, we took into consideration two problems; (*a*) as potassium and sodium ions form weak complexes with ATP^{4-}, a four mass-balance equation system for ATP binary complexes and a five mass-

Table 2. Experimental details of calorimetric measurements, at 25 °C and I = 0.1 mol dm^{-3}

System	Analytical concentrations in the titration vessel (mmol dm^{-3}) [a]	Titrant concentration (mol dm^{-3})	No. of titrations [b]
H^+ + amino-acids	c_L = 5—10, c_H = 0	c_H = 0.15	5
Cu^{2+} or Zn^{2+} + amino-acids	c_L = 4—7.5, c_H *ca.* 0	c_M = 0.2	5
Cu^{2+} or Zn^{2+} + ATP	c_{ATP} = 4—7.5, c_H = −0.5—4	c_M = 0.2	4
	c_M = 3—5, c_H = 0—2	$c_{Na_4(ATP)}$ = 0.05	4
	c_{ATP} = 20—50, c_H = 4—10	c_M = 0.25	2
Cu^{2+} or Zn^{2+} + ATP + amino-acids	$c_{ATP} = c_L$ = 5—10, c_H = 5—20	c_M = 0.1—0.25	4
	c_{ATP} = 1.5, c_L = 7.5—25, c_H = 5—25	c_M = 0.25	5
	$c_M = c_L$ = 5—15, c_H = 2—10	$c_{Na_4(ATP)}$ = 0.05	5

[a] L = Amino-acid; M = Cu^{2+} or Zn^{2+}. [b] 10—20 points for each titration curve.

Table 3. $\Delta G°$, $\Delta H°$, and $\Delta S°$ values * for the protonation of ATP at 25 °C and I = 0.1 mol dm^{-3}

Reaction	$-\Delta G°$/ kcal mol^{-1}	$-\Delta H°$/ kcal mol^{-1}	$\Delta S°$/ cal K^{-1} mol^{-1}
$H^+ + ATP^{4-} \rightleftharpoons HATP^{3-}$	9.69	0.2	32
$2H^+ + ATP^{4-} \rightleftharpoons H_2ATP^{2-}$	15.25	3.8	38

* Corrected for Na^+- and K^+-ATP complex formation, see ref. 33.

balance equation system for ATP ternary complexes were employed, using as input data also the concentration of the alkali-metal ions and the formation constants of their ATP complexes,[33] (*b*) since the solutions, in some cases, are quite concentrated, the ionic strength was greater than 0.1 mol dm^{-3}, without KNO_3 or $NaClO_4$ having been added, consequently a modified version [41] of MINIQUAD was used in which changes in ionic strength were taken into account. When performing the calculations with SCOGSB, the standard deviation was always <0.01 cm^3 whereas the standard deviation in the analytical concentration was always <1% when using MINIQUAD.

The heats of ligand protonation as well as of binary and ternary complex formation were calculated by means of the least-squares computer program DOEC,[42] which minimizes the error-squares sum in the reaction heat, Q. In this case also the changes in the ionic strength and the complex formation of alkali-metal ions with ATP were taken into consideration. The standard deviation in Q was always less than 0.05 cal.

In examining the potentiometric data, the use of statistical methods was necessary in order to choose the 'best' of the possible models. To this end the factor $R = (\Sigma\varepsilon_i/\Sigma x_i)^{\frac{1}{2}}$ was used, according to Vacca *et al.*,[43] where ε_i are the residuals of the variable x_i where $x = v$ (added titrant volume) and x = analytical concentrations for SCOGSB and MINIQUAD respectively.

Throughout the paper, errors are expressed as three times the standard deviation or as a range (maximum deviation from the mean).

In Table 3 the thermodynamic parameters of the protonation equilibria of ATP, reported elsewhere,[33] are listed.

Results

Potentiometry.—As is well known,[44-47] L-alanine and L-tryptophan exist as the protonated species, HL and H_2L^+, respectively; corresponding complexes, ML^+ and ML_2, are formed with both Cu^{2+} and Zn^{2+}. The values of the thermodynamic parameters for the H–M–amino-acid systems are given in Table 4.

Several models have been proposed for the Cu–ATP and Zn–ATP systems.[31,44-58] As regards the Cu–ATP system, we took into account the following species: $[Cu(ATP)]^{2-}$, $[Cu(ATP)H]^-$, $[Cu(ATP)H_2]$, $[Cu(ATP)_2]^{6-}$, $[Cu(ATP)_2H]^{5-}$, $[Cu(ATP)_2H_2]^{4-}$, $[Cu_2(ATP)_2]^{4-}$, $[Cu_2(ATP)_2H]^{3-}$, $[Cu_2(ATP)_2H_2]^{2-}$, $[Cu_2(ATP)]$, $[Cu_2(ATP)H]^+$, $[Cu(ATP)H_{-1}]^{3-}$, $[Cu(ATP)H_{-2}]^{4-}$, $[Cu(ATP)_2H_{-1}]^{7-}$, $[Cu_2(ATP)_2H_{-1}]^{5-}$, $[Cu_2(ATP)_2H_{-2}]^{6-}$ and $[Cu_2(ATP)H_{-1}]^-$; these, divided into different sets, were the same species taken into consideration by other authors. On the basis of the variance value obtained with both SCOGSB and MINIQUAD and of the statistical R factor,[43] the following set of species was found to be the 'best' one: $[Cu(ATP)]^{2-}$, $[Cu(ATP)_2]^{6-}$, $[Cu(ATP)H]^-$, $[Cu_2(ATP)]$, $[Cu_2(ATP)H]^+$, $[Cu(ATP)H_{-1}]^{3-}$, $[Cu_2(ATP)H_{-1}]^-$, and $[Cu_2(ATP)_2H_{-2}]^{6-}$. The species $[Cu(ATP)H_{-1}]^{3-}$ and $[Cu(ATP)H_{-2}]^{4-}$, proposed by some authors (see Table 5), were found not to exist under our experimental conditions.

As for the system Zn–ATP, the species $[Zn(ATP)]^{2-}$, $[Zn(ATP)_2]^{6-}$, $[Zn(ATP)H]^-$, $[Zn_2(ATP)]$, $[Zn_2(ATP)H]^+$, $[Zn(ATP)H_{-1}]^{3-}$, $[Zn_2(ATP)H_{-1}]^-$, $[Zn(ATP)H_2]$, $[Zn_2(ATP)_2]^{4-}$, and $[Zn_2(ATP)_2H]^{3-}$ were investigated. On the basis of the criteria outlined above for the Cu–ATP system, the 'best' model is represented by the first seven of these. In the calculations the hydrolysis of Cu^{2+} and Zn^{2+} was also taken into account.[59,60] Thus, we have not determined other dimeric species, recently hypothesized on the basis of spectroscopic studies.[61,*]

The species $[M_2(ATP)]$ have already been proposed for several metal ions [62-66] but their formation constants are very rarely reported (with the exception of approximate values).[31,64,66] The stability constants of the main species, *i.e.* $[M(ATP)]^{2-}$, are in relatively good agreement with those found by authors who used, as background, tetra-alkylammonium salts, which do not form complexes with ATP.

* Since these authors claimed the formation of a dimeric species, *i.e.* $[Zn_2(ATP)_2]^{4-}$, at concentrations lower than 0.7 mol dm^{-3}, we carried out some supplementary measurements, exploring the concentration range 2×10^{-2}—5×10^{-2} mol dm^{-3}, even when the data analysis from the previous experiments excluded the presence of species such as $[M_2(ATP)_2]^{4-}$. Once again our potentiometric data (twelve titrations; $2 \leq pH \leq 6$; 25 °C) confirmed the absence of dimeric species. It should be noted that our experimental conditions were not coincident with those of Sigel and co-workers.[61] In fact we were bound (*i*) to carry out the experiments at I = 0.3 mol dm^{-3} to avoid ionic strength changes, due to the relatively high ATP and zinc(II) concentrations, during the titration runs and (*ii*) to choose NEt_4Br instead of an alkali-metal salt, to minimize the influence of complexes of alkali-metal ions with ATP.

Table 4. Thermodynamic parameters for complex formation of H^+, Cu^{2+}, and Zn^{2+} with alanine and tryptophan at 25 °C and I = 0.1 mol dm^{-3}; standard deviations (3σ) are given in parentheses and values from ref. 44 are given in square brackets

Reaction	$-\Delta G^{\ominus}$/ kcal mol^{-1}	$-\Delta H^{\ominus}$/ kcal mol^{-1}	$\Delta S^{\ominus}$/ cal K^{-1} mol^{-1}
$H^+ + alaO^- \rightleftharpoons ala$	13.32(3) [13.21]	10.75(9) [10.8] [a]	8.6(3) [8.1] [a]
$2H^+ + alaO^- \rightleftharpoons Hala^+$	16.55(7) [16.34]	11.5(2) [11.5] [a]	16.9(7) [16.2] [a]
$H^+ + trpO^- \rightleftharpoons trp$	12.78(5) [12.72] [a]	10.68(11) [10.7] [b]	7.1(4) [6.8] [b]
$2H^+ + trpO^- \rightleftharpoons Htrp^+$	16.07(8) [15.92]	11.8(2) —	14.3(7) —
$Zn^{2+} + alaO^- \rightleftharpoons [Zn(alaO)]^+$	6.34(7) [6.24]	1.78(16) [1.5] [c]	15.3(6) [15.9(7)]
$Zn^{2+} + 2\ alaO^- \rightleftharpoons [Zn(alaO)_2]$	11.98(10) [11.72]	4.2(2) [4.3] [c]	26.1[[c] [24.9] [c]
$Zn^{2+} + trpO^- \rightleftharpoons [Zn(trpO)]^+$	6.41(7) [7.06] [d]	2.9(2) [4.10] [e]	11.8(7) [9.9] [e]
$Zn^{2+} + 2\ trpO^- \rightleftharpoons [Zn(trpO)_2]$	12.17(12) [13.45] [d]	5.8(3) [8.23] [e]	21.4(9) [17.5] [e]
$Cu^{2+} + alaO^- \rightleftharpoons [Cu(alaO)]^+$	11.15(4) [11.08]	5.47(9) [5.6] [b]	19.0(3) [18.4] [b]
$Cu^{2+} + 2\ alaO^- \rightleftharpoons [Cu(alaO)_2]$	20.39(6) [20.33]	12.0(2) [11.6] [b]	28.1(7) [29.3] [b]
$Cu^{2+} + trpO^- \rightleftharpoons [Cu(trpO)]^+$	11.31(7) [11.29] [d]	5.86(12) [5.5] [b]	18.3(4) [19.4] [b]
$Cu^{2+} + 2\ trpO^- \rightleftharpoons [Cu(trpO)_2]$	21.03(9) [21.09] [d]	13.4(2) [12.7] [b]	25.6(7) [28.2] [b]

[a] I = 0. [b] I = 0.16 mol dm^{-3}. [c] I = 0.05 mol dm^{-3}. [d] 20 °C, I = 0.37 mol dm^{-3}. [e] I = 3 mol dm^{-3}.

The formation constants of the Cu–ATP and Zn–ATP systems, together with the more significant literature data pertinent to these systems, are listed in Table 5.

The formation constants of the mixed complexes are shown in Table 6, together with Δlog K values of Sigel and Naumann;[26] the latter are in good agreement with our values, even when the logβ values are considerably different. {The previous authors did not take into account the $[Na(ATP)]^{3-}$ and $[K(ATP)]^{3-}$ species; this leads to a marked lowering of the formation constants of $[Cu(ATP)]^{2-}$ and $[Zn(ATP)]^{2-}$ and of their mixed complexes with amino-acids.}

Calorimetry.—Table 7 shows the thermodynamic parameters of formation of the main simple ATP complexes. While the enthalpy and entropy changes of the simple complexes of copper(II) and zinc(II) with tryptophanate ($trpO^-$) and alaninate ($alaO^-$) agree with the literature values (Table 4), this is not the case for the simple ATP complexes. In fact, our data determined by direct calorimetry differ from those obtained by Van t' Hoff's method.[54] In our opinion, this marked difference {3.8 and 6.2 kcal mol^{-1} for $\Delta H^{\ominus}$ of $[Cu(ATP)]^{2-}$ and $[Zn(ATP)]^{2-}$ respectively} can be explained in terms of the different speciation of the M–ATP systems and the neglect of the contribution of K–ATP species to the formation heats.[33] In fact, neglecting some species and/or substituting them with others can significantly affect the constants of the other species and, moreover, this influence can vary with the temperature. Other reasons for the above discrepancy may be that the hydrolysis of ATP is temperature dependent, and that, in the case of log K values, the accuracy of which is ±0.02 log units, the error in $\Delta H^{\ominus}$ values is larger than 1 kcal mol^{-1}, as determined by measurements carried out at four different temperatures over a range of 40 °C.[67] In agreement with these conclusions, the value of $\Delta H^{\ominus}$ for $[Mg(ATP)]^{2-}$, determined by direct calorimetry,[68] differs by about 2 kcal mol^{-1} from that obtained using the temperature-dependence method.[54] Furthermore, the Mg–ATP system is far simpler than those reported here.

In Table 8 we report the thermodynamic parameters for the formation of mixed complexes.

Discussion

Simple Complexes.—The formation of the simple ATP complexes according to the equilibrium $M^{2+} + ATP^{4-} \rightleftharpoons [M(ATP)]^{2-}$ is enthalpically disfavoured but entropically stabilized. The $\Delta S^{\ominus}$ values are significantly lower than those determined for copper(II) and zinc(II) tripolyphosphate,[69] where three oxygen atoms are involved in the co-ordination, and nearly equal to those obtained for dicarboxylate metal complexes,[23,24,70] where two oxygen atoms are involved in the bonding to these bivalent cations. From these comparisons, we can suggest that two oxygen atoms of the nucleotide phosphate groups are engaged in co-ordination to Cu^{2+} and Zn^{2+}, in agreement with previous results regarding the involvement of β- and γ-oxygen atoms of phosphate groups in $[Cu(ATP)]^{2-}$ and $[Zn(ATP)]^{2-}$.[71] However, up to now, the main subject of debate has been the interaction of metal ions with the adenine ring.[72,73]

More than 20 years ago Szent-Gyorgyi [74] suggested that ATP may be folded so that metal ions co-ordinate simultaneously to phosphate oxygens and to adenine nitrogens N(7) and N(6); this is known as the back-bound structure. Our data do not agree with this model, which has recently been reproposed.[75,76] In fact, if copper(II) and zinc(II) were bound through two nitrogen atoms, the resulting $\Delta H^{\ominus}$ changes should be negative.[77] On the other hand, the positive enthalpy changes for $[Cu(ATP)]^{2-}$ and $[Zn(ATP)]^{2-}$ cannot indicate the sole interaction with β- and γ-phosphate groups, with the consequent formation of a six-membered chelate ring, which would also imply neutralization of the metal ion 2+ charge. When oxygen-donor ligands co-ordinate to copper(II) and zinc(II), the differences in the $\Delta H^{\ominus}$ values for complex

Table 5. log β Values with standard deviations (3σ) in parentheses reported in the literature for the systems Cu–ATP and Zn–ATP

	θ_c/°C	I/mol dm^{-3}	log β_{110} [a]	log β_{111}	log β_{11-1}	Other constants		Ref.
Cu^{2+}	25	0.1 KNO_3	6.13	9.65	−0.34	log β_{22-2} = 1.91	log β_{11-2} = −7.36	48
	22	0.1 KCl	5.77		−1.9			50
	20	0.1 KCl	5.82	9.75		log β_{120} = 7.7	log β_{112} = 12.4	51
	20	0.1 $NaClO_4$	6.30	10.23				52
	30	0.1 NMe_4Br	6.83	10.78				53
	0.4	0.1 KNO_3	6.42	9.88	−0.63	log β_{22-2} = 1.84	log β_{11-2} = −7.95	54
	12	0.1 KNO_3	6.20	9.74	−0.54	log β_{22-2} = 1.82	log β_{11-2} = −7.72	54
	40	0.1 KNO_3	5.97	9.53	−0.22	log β_{22-2} = 1.94	log β_{11-2} = −7.13	54
	25	0.1 $NaClO_4$	6.38		−1.5			55, 56
	25	0.12 NaCl	5.95					57
	25	0.1 KNO_3	5.83	9.45	−0.82	log β_{210} = 8.19	log β_{211} = 11.5 [b]	This work
	25	0.1 [c]	6.50(4)	10.02(6)	−0.3(1)	log β_{210} = 8.76(7)	log β_{211} = 12.1(2) [d]	This work
Zn^{2+}	25	0.1 KNO_3	4.85	9.20				48
	25	0.1 KCl	4.76	9.48				58
	22	0.1 KCl	4.80		−3.7			50
	20	0.1 KCl	4.75	9.28		log β_{120} = 6.16	log β_{112} = 12.54	51
	30	0.1 NMe_4Br	5.52	9.72				53
	0.4	0.1 KNO_3	5.00	9.37				54
	12	0.1 KNO_3	4.88	9.27				54
	40	0.1 KNO_3	4.71	9.10				54
	25	0.1 $NaClO_4$	5.21			logg β_{210} = 8.1		55
	25	0.12 NaCl	4.71					57
	25	0.1 KNO_3	4.66	8.62	−3.8	log β_{210} = 6.51	log β_{211} = 10.6 [e]	This work
	25	0.1 [c]	5.23(4)	9.22(6)	−3.2(1)	log β_{210} = 7.08(8)	log β_{211} = 11.2(2) [f]	This work

[a] The indexes refer to the reaction $pM + qATP + rH \rightleftharpoons [M_p(ATP)_qH_r]$. [b] log β_{21-1} = 3.3, log β_{22-2} = 0.65 (this work). [c] Values corrected for K–ATP complex formation. [d] log β_{21-1} = 3.9(2), log β_{22-2} = 1.2(1), log β_{120} = 8.5(3) (this work). [e] log β_{21-1} = −0.5 (this work). [f] log β_{21-1} = 0.1(1), log β_{120} = 7.1(3) (this work).

Table 6. log β and Δlog K * values for the formation of $[M(ATP)L]^{3-}$ ternary complexes (M = Cu^{2+} or Zn^{2+}, L = L-tryptophanate or L-alaninate) at 25 °C and I = 0.1 mol dm^{-3}; values from ref. 26 are given in square brackets

Reaction	log β (3σ)	Δlog K (3σ)
$Cu^{2+} + ATP^{4-} + alaO^- \rightleftharpoons [Cu(ATP)(alaO)]^{3-}$	12.94(7)	−1.74(10) [−1.53]
$Cu^{2+} + ATP^{4-} + trpO^- \rightleftharpoons [Cu(ATP)(trpO)]^{3-}$	13.35(10)	−1.45(15) [−1.30]
$Zn^{2+} + ATP^{4-} + alaO^- \rightleftharpoons [Zn(ATP)(alaO)]^{3-}$	9.18(7)	−0.70(10) [−0.80]
$Zn^{2+} + ATP^{4-} + trpO^- \rightleftharpoons [Zn(ATP)(trpO)]^{3-}$	9.90(6)	−0.03(9) [−0.18]

* $\Delta\log K = \log \beta^{M}_{M(ATP)L} - \log K^{M}_{M(ATP)} - \log K^{M}_{ML}$.

Table 7. Thermodynamic parameters with standard deviations (3σ) in parentheses for the formation of Cu–ATP and Zn–ATP species, at 25 °C and I = 0.1 mol dm^{-3}; the values were corrected for the formation of Na- and K–ATP complexes

Reaction	$-\Delta G^\circ$/kcal mol^{-1}	ΔH°/kcal mol^{-1}	ΔS°/cal K^{-1} mol^{-1}
$Cu^{2+} + ATP^{4-} \rightleftharpoons [Cu(ATP)]^{2-}$	8.86(5)	0.84(7)	32.5(3)
$Cu^{2+} + 2\,ATP^{4-} \rightleftharpoons [Cu(ATP)_2]^{6-}$	11.6(3)	−1.7(6)	32(2)
$Cu^{2+} + ATP^{4-} + H^+ \rightleftharpoons [Cu(ATP)H]^-$	13.66(8)	−3.7(5)	33(2)
$2\,Cu^{2+} + ATP^{4-} \rightleftharpoons [Cu_2(ATP)]$	11.94(10)	5.1(2)	57(1)
$Zn^{2+} + ATP^{4-} \rightleftharpoons [Zn(ATP)]^{2-}$	7.13(5)	3.9(2)	37(1)
$Zn^{2+} + 2\,ATP^{4-} \rightleftharpoons [Zn(ATP)_2]^{6-}$	9.7(4)	1.2(6)	37(2)
$Zn^{2+} + ATP^{4-} + H^+ \rightleftharpoons [Zn(ATP)H]^-$	12.57(8)	0.9(1)	45(1)
$2\,Zn^{2+} + ATP^{4-} \rightleftharpoons [Zn_2(ATP)]$	9.65(11)	6.9(5)	56(2)

formation of the two metal ions are small.[70,78] For example, when dealing with the tripolyphosphate (tri) complexes,[69] the ΔH° is 4.9 kcal mol^{-1} for $[Cu(tri)]^{2-}$ and 6.3 kcal mol^{-1} for $[Zn(tri)]^{2-}$; a similar difference is found for malonate complexes.[70] In our complexes the difference in the formation enthalpy between $[Cu(ATP)]^{2-}$ and $[Zn(ATP)]^{2-}$ is about 3 kcal mol^{-1}, similar to that observed with the amino-acid ligands (see also Table 4) in which the donor atoms are nitrogen and oxygen. We believe that this difference indicates that the metal ion somehow interacts with the adenine ring,

Table 8. Thermodynamic parameters with standard deviations (3σ) in parentheses for mixed-ligand complex formation at 25 °C and I = 0.1 mol dm^{-3}

Reaction	$-\Delta G^{\circ}$/kcal mol^{-1}	$-\Delta H^{\circ}$/kcal mol^{-1}	ΔS°/cal K^{-1} mol^{-1}
$Cu^{2+} + ATP^{4-} + alaO^{-} \rightleftharpoons [Cu(ATP)(alaO)]^{3-}$	17.64(9)	2.5(3)	51(1)
$Cu^{2+} + ATP^{4-} + trpO^{-} \rightleftharpoons [Cu(ATP)(trpO)]^{3-}$	18.22(14)	5.5(3)	43(2)
$Zn^{2+} + ATP^{4-} + alaO^{-} \rightleftharpoons [Zn(ATP)(alaO)]^{3-}$	12.52(9)	−4.0(8)	55(3)
$Zn^{2+} + ATP^{4-} + trpO^{-} \rightleftharpoons [Zn(ATP)(trpO)]^{3-}$	13.50(8)	5.9(8)	25(3)

probably at N(7). The overall enthalpy change is the result of the exothermic contribution of the N(7) co-ordination, counterbalanced by the endothermic contributions due to the co-ordination of the phosphate oxygens and the folded semi-back-bound structure that is energetically disfavoured.

At first sight it seems that this conclusion only corroborates previous assertions based mainly on n.m.r. measurements of the copper(II) and zinc(II) complexes.[79] Owing to the high concentrations of ATP or extreme M : ATP ratios used in that investigation, caution should be employed in considering the structure proposed. In particular, in the case of copper(II),[15,79-82] the structure of $[Cu(ATP)]^{2-}$ has been inferred on the basis of ^{1}H and ^{31}P n.m.r. investigations in which the ATP : M ratio was *ca.* 10 : 1. Under the experimental conditions used, $[M(ATP)_2]^{6-}$ might also be formed; this possibility, suggested in some reports,[83-86] has now a quantitative basis in the findings reported here. Bearing in mind the limits of extrapolation, *i.e.* to ionic strengths and viscosity changes of very concentrated solutions, it can reasonably be assumed that in those investigations the complexed ATP exists mainly as $[Cu(ATP)_2]^{6-}$, in which the metal ion has been suggested to interact with the adenine ring.[86] Also in the case of zinc(II), most of the n.m.r. data were obtained at high concentrations (0.1 [79] or 0.5—0.9 mol dm^{-3} [75]), where the speciation of the system has not yet been determined experimentally, and furthermore, the existence of polymeric species has been hypothesized.[61] Therefore the structural conclusions inferred from such data cannot be assumed as unambiguous proof of a zinc(II) interaction with N(7) of the adenine ring, even when our calorimetric results indicate the presence of this interaction. (Our conclusion is reinforced by considerations [86,87] of the degree of confidence of n.m.r. investigations of metal ATP complexes with respect to the bonding details.)

The trend in ΔH° values observed for the $[M(ATP)]^{2-}$ species remains unchanged also for the $[M(ATP)H]^{-}$ complexes. This suggests that, in the protonated species, the same donor atoms are involved in the bonding to copper(II) and zinc(II) as in the non-protonated species. The protonation site is probably the N(1) atom of the adenine ring; this is supported by the values of the enthalpy changes for protonation according to the equilibrium $[M(ATP)]^{2-} + H^{+} \rightleftharpoons [M(ATP)H]^{-}$; $\Delta H^{\circ} = -2.9$ and -4.5 kcal mol^{-1} for Zn^{2+} and Cu^{2+}, respectively. In fact, the protonation of N(1) of ATP is accompanied by a negative ΔH° value as can be seen from the value for the second protonation step reported in Table 3. This explanation of the thermodynamic data is in agreement with that previously suggested for $[M(ATP)H]^{-}$ species.[85]

The formation of $[M_2(ATP)]$ complexes is enthalpically disfavoured but entropically favoured. This indicates that the second metal ion is bound to the phosphate oxygen. The difference in the enthalpy changes for the zinc(II) and copper(II) complexes is less than that found in the case of $[M(ATP)]^{2-}$ species, suggesting that no interaction of the second metal ion with nitrogen-donor atoms is present in $[M_2(ATP)]$ complexes. This disagrees with the conclusion of Glassman *et al.*[65] who, on the basis of n.m.r. results, proposed the interaction of Zn^{2+} with N(1) and/or N(3) in $[Zn_2(ATP)]$, but is in accordance with some more recent hypotheses.[31]

The ΔH° values for formation of the bis-ATP complexes according to the equilibrium $[M(ATP)]^{2-} + ATP^{4-} \rightleftharpoons [M(ATP)_2]^{6-}$ are -2.5 and -2.7 kcal mol^{-1} for copper(II) and zinc(II), respectively. These negative values suggest that in $[M(ATP)_2]^{6-}$ a nitrogen atom of the nucleotide ring is involved in the co-ordination, in agreement with the model currently accepted in which the metal ion is co-ordinated to the triphosphate chain of one ATP molecule and to the adenine ring of the other.[83,85] Nevertheless, assuming that the same donor atoms are co-ordinated in both cases to the metal ion, it is rather surprising that $[Cu(ATP)_2]^{6-}$ and $[Zn(ATP)_2]^{6-}$ show nearly equal enthalpy changes. This behaviour can be explained bearing in mind that, in these complexes, base stacking between the two ATP molecules also occurs.[86] The thermodynamic parameters for the self-association of ATP have recently been determined:[88] $\Delta H^{\circ} = -5.1$ kcal mol^{-1}; $\Delta S^{\circ} = -13.0$ cal K^{-1} mol^{-1} at pH = 8.7. On the basis of our calorimetric results it must be concluded that such an interaction is occurring in $[Zn(ATP)_2]^{6-}$, and accounts for the exothermic ΔH° value of the bis complex. As regards $[Cu(ATP)_2]^{6-}$, owing to the uncertainties in the co-ordination number and therefore about the influence of solvation on the thermodynamic properties, base stacking cannot be excluded. Probably, the different geometric requirements of the two metal ions account for the observed behaviour.

The trend in thermodynamic values for formation of mono and bis complexes of the amino-acids is as expected on the basis of previous results.[89] The ΔH° and ΔS° values give no useful information towards understanding of the difference between the ΔG° values of the first and second complexation steps of copper(II) with tryptophan compared to alanine. On the basis of our calorimetric measurements, it is not possible to infer the presence either of an interaction of the metal ion with the aromatic ring of trp or of attractive interactions between side chains in a bis complex.[90] These interactions have been hypothesized to explain the enhanced second stability constants for some aromatic amino-acid complexes of Cu^{2+} on the basis of spectroscopic and structural investigations.[91,92]

Mixed Complexes.—The mixed-ligand complex formation of copper(II) with both the investigated amino-acids is enthalpically and entropically favoured as shown in Table 8. The negative enthalpy changes indicate that the amino-acids are acting as bidentate ligands. The endothermic contribution due to the co-ordination of the oxygen atoms of the ATP and trp or ala is counterbalanced by the bonding of the nitrogen atom of the amino-acid. The ΔH° values exclude the possibility that in the ternary complexes also the N(7) of the nucleotide is involved in co-ordination to Cu^{2+}; ΔS° values reinforce this assertion, the positive values being larger than found for the simple ATP complex and similar to those

reported for the mono-tripolyphosphate complex,[69] at least as regards the ala system. The chromophore around copper(II) should therefore be of NO_3 type. Also, in the zinc(II) ternary complexes, the same donor atoms should be involved in the bonding, as previously proposed,[27] yet the thermodynamic parameters for the formation of the two zinc(II) complexes are different. The $[Zn(ATP)(alaO)]^{3-}$ complex shows a positive entropy change, nearly equal to that found for $[Cu(ATP)(alaO)]^{3-}$, and an endothermic enthalpy change more positive than that resulting from the sum of the exothermic amino-acid and the endothermic ATP contributions; this is in accord with co-ordination of the ATP by means of oxygen atoms only. On the contrary, $[Zn(ATP)(trpO)]^{3-}$ is more enthalpically and less entropically stabilized with respect to what might have been expected. Furthermore, even if the $\Delta G^{\ominus}$ value for the zinc(II) mixed-ligand complex of ATP and trp is lower than that of the corresponding copper(II) complex, the enthalpy change is the same. This behaviour is clearly anomalous. It is noteworthy that, whereas Zn^{2+} and Cu^{2+} do not show very different thermodynamic parameters when interacting with ala or with trp, their $[M(ATP)(trpO)]^{3-}$ complexes involve marked differences in enthalpy and entropy changes when compared with those for formation of $[M(ATP)(alaO)]^{3-}$.

If the above data are compared to those for the stacking interaction of ATP and trp molecules[93] ($\Delta G^{\ominus} = -1.74$ kcal mol^{-1}, $\Delta H^{\ominus} = -7.02$ kcal mol^{-1}, and $\Delta S^{\ominus} = -17.7$ cal K^{-1} mol^{-1}), the observed differences are easily interpretable and provide evidence for the existence of such an interaction in the ternary complexes. The effect of this so-called 'secondary' bond results in a more favourable enthalpy and in a less favourable entropy contribution for $[M(ATP)(trpO)]^{3-}$ with respect to $[M(ATP)(alaO)]^{3-}$ species. As for the zinc(II) complexes, the difference is more pronounced in comparison with what is found for copper(II). The formation of $[Zn(ATP)(trpO)]^{3-}$ is about 10 kcal mol^{-1} more exothermic and 30 cal K^{-1} mol^{-1} lower with respect to $[Zn(ATP)(alaO)]^{3-}$. Bearing in mind that in the latter complex the stacking interaction is not possible, this difference can be assumed to be an index of the extent of stacking interaction. The thermodynamic quantities are more negative in comparison with those corresponding to the association of the ligands alone,[93] and can be explained by taking into account that in the complex the negative charges of the ligands are partly neutralized by the positive charge of the metal ion. In the complex, owing to bridging by the metal ion of the charged ends of the two molecules, the two ligands are more stable with respect to molecular association, and this involves an unfavourable entropy contribution.

The results for copper(II) are not as straightforward as in the case of the corresponding zinc(II) species, in agreement with spectroscopic measurements that pointed to a greater contribution to the stacking from the zinc(II) than from the copper(II).[26] It is evident that this different behaviour cannot be attributed to electronic characteristics of the metal ions because the latter are not bonded to the ligand moieties that interact. Probably, in the ternary complexes the different geometric requirements of the two metal ions place the purine and indole groups of ATP and trp in more or less suitable arrangements which result in the variation in the extent of interaction between the two rings. In the case of $[Cu(ATP)(trpO)]^{3-}$, it cannot be excluded that a different degree of solvent interaction of the metal ion with respect to the analogous zinc(II) complex contributes to the resulting thermodynamic parameters.*

In conclusion, on the basis of our results, we have found that the knowledge of all the thermodynamic parameters can be strongly diagnostic of the presence of ligand–ligand interaction in metal complexes. This is evident, for instance, in the case of zinc(II) mixed-ligand complexes where a $\Delta G^{\ominus}$ difference of less than 1 kcal mol^{-1} may result from a large difference in $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ contributions. Moreover, it is possible to foresee that the influence of the stacking interaction might have implications at a biological level. Preliminary results for other mixed-ligand complexes of ATP and biofunctional ligands such as histamine or phenylalanine seem to confirm the present findings.

* Recently, based on an approach similar to that of Mariam and Martin,[94] an intramolecular equilibrium between two isomeric complexes (*i.e.* between an 'open' and a 'closed' species in the ternary complexes [27,95,96] and between the semi-back-bound and the not-semi-back-bound species in the simple ones [61]) has been proposed to determine the degree of macrochelate formation in monomeric ATP complexes of Ni^{2+}, Co^{2+}, Mn^{2+}, and Mg^{2+}. We have not considered these equilibria in view of the conclusion that the related constants were only estimates.[96]

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References

1 O. Yamauchi, T. Sakurai, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 1776.
2 B. D. Fischer and H. Sigel, *J. Am. Chem. Soc.*, 1980, **102**, 2998 and refs. therein.
3 H. Sigel, in 'Advances in Solution Chemistry,' eds. I. Bertini, L. Lunazzi, and A. Dei, Plenum, New York, 1981, p. 149.
4 G. Brookes and L. D. Pettit, *J. Chem. Soc., Chem. Commun.*, 1975, 385.
5 L. D. Pettit and R. J. W. Hefford, in 'Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1979, vol. 9, p. 173.
6 C. F. Naumann and H. Sigel, *J. Am. Chem. Soc.*, 1974, **96**, 2750.
7 P. R. Mitchell and H. Sigel, *J. Am. Chem. Soc.*, 1978, **100**, 1564.
8 C. Helene, J. L. Dimicoli, and F. Brun, *Biochemistry*, 1971, **10**, 3802.
9 W. A. Brodosky and A. E. Shamoo, *Biochim. Biophys. Acta*, 1973, **291**, 208.
10 R. Thedford and D. B. Strauss, *Biochemistry*, 1974, **13**, 535.
11 K. S. Rajan, J. M. Davis, R. W. Colburn, and F. H. Jarke, *J. Neurochem.*, 1972, **19**, 1099.
12 J. Granot, *J. Am. Chem. Soc.*, 1978, **100**, 1539.
13 J. Granot, *J. Am. Chem. Soc.*, 1978, **100**, 2886.
14 Y. Fukuda, P. R. Mitchell, and H. Sigel, *Helv. Chim. Acta*, 1978, **61**, 638.
15 C. F. Naumann, B. Prijs, and H. Sigel, *Eur. J. Biochem.*, 1974, **41**, 209.
16 K. S. Rajan, A. Skripkus, G. E. Marks, and J. M. Davis, *Bioinorg. Chem.*, 1976, **6**, 93.
17 C. F. Naumann and H. Sigel, *FEBS Lett.*, 1974, **47**, 122.
18 J. J. Toulme, *Bioinorg. Chem.*, 1978, **8**, 319.
19 H. Sigel, *J. Inorg. Nucl. Chem.*, 1977, **39**, 1903.
20 S. Arhland, *Struct. Bonding (Berlin)*, 1973, **15**, 167.
21 A. M. Corrie and D. R. Williams, *J. Chem. Soc., Dalton Trans.*, 1976, 1068.
22 M. C. Lim and G. H. Nancollas, *Inorg. Chem.*, 1971, **10**, 1957.
23 G. Arena, R. Cali, E. Rizzarelli, S. Sammartano, R. Barbucci, and M. J. M. Campbell, *J. Chem. Soc., Dalton Trans.*, 1977, 581.
24 G. Arena, R. Cali, E. Rizzarelli, S. Sammartano, R. Barbucci, and M. J. M. Campbell, *J. Chem. Soc., Dalton Trans.*, 1978, 1090.
25 R. P. Bonomo, R. Cali, F. Riggi, E. Rizzarelli, S. Sammartano, and G. Siracusa, *Inorg. Chem.*, 1979, **18**, 3417.
26 H. Sigel and C. F. Naumann, *J. Am. Chem. Soc.*, 1976, **98**, 730.
27 P. R. Mitchell, B. Prijs, and H. Sigel, *Helv. Chim. Acta*, 1979, **62**, 1723.
28 P. R. Stoesser and S. J. Gill, *J. Phys. Chem.*, 1967, **71**, 564.

29 M. G. Marenchic and J. M. Sturtevant, *J. Phys. Chem.*, 1973, **77**, 544.
30 A. Dei, A. Scozzafava, and G. Renzi, *Inorg. Chim. Acta*, 1981, **56**, 73.
31 H. Sigel and P. E. Amsler, *J. Am. Chem. Soc.*, 1976, **98**, 7390.
32 A. T. Tu and M. J. Heller, in ' Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1974, vol. 1, p. 2.
33 R. Calì, S. Musumeci, C. Rigano, and S. Sammartano, *Inorg. Chim. Acta*, 1981, **56**, L11.
34 J. B. Martin and D. M. Doty, *Anal. Chem.*, 1940, **21**, 965.
35 F. Holmes and D. R. Williams, *J. Chem. Soc. A*, 1967, 1256.
36 I. Grenthe, H. Ots, and O. Ginstrup, *Acta Chem. Scand.*, 1970, **24**, 1067.
37 D. J. Hale, R. M. Izatt, and J. J. Christensen, *J. Phys. Chem.*, 1963, **67**, 2605.
38 G. Arena, E. Rizzarelli, S. Sammartano, and C. Rigano, *Talanta*, 1979, **26**, 1.
39 R. Maggiore, S. Musumeci, E. Rizzarelli, and S. Sammartano, *Inorg. Chim. Acta*, 1976, **18**, 155.
40 A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53; P. Gans, A. Vacca, and A. Sabatini, *Inorg. Chim. Acta*, 1976, **18**, 237; A. Vacca, personal communication.
41 P. G. Daniele, C. Rigano, and S. Sammartano, *Ann. Chim. (Rome)*, 1980, **70**, 119; C. Rigano and S. Sammartano, unpublished work.
42 C. Rigano, E. Rizzarelli, and S. Sammartano, *Thermochim. Acta*, 1979, **33**, 211.
43 W. C. Hamilton, ' Statistics in Physical Sciences,' Ronald Press, New York, 1964; A. Vacca, A. Sabatini, and M. A. Gristina, *Coord. Chem. Rev.*, 1972, **8**, 45.
44 A. E. Martell and R. M. Smith, ' Critical Stability Constants,' Plenum, New York, 1974, vol. 1.
45 L. G. Sillen and A. E. Martell, ' Stability Constants of Metal-Ion Complexes,' *Special Publ.*, The Chemical Society, London, 1964, no. 17; 1971, no. 25.
46 D. D. Perrin, ' Stability Constants of Metal-Ion Complexes,' IUPAC Chemical Data Series No. 22, Pergamon, Oxford, 1979, part B.
47 J. J. Christensen, D. J. Eatough, and R. M. Izatt, ' Handbook of Metal Ligand Heats and Related Thermodynamic Quantities,' 2nd edn., Marcel Dekker, New York, 1975.
48 M. M. Taqui Khan and A. E. Martell, *J. Phys. Chem.*, 1962, **66**, 10.
49 R. M. Smith and A. E. Martell, ' Critical Stability Constants,' Plenum, New York, 1975, vol. 2.
50 H. Brintzinger, *Helv. Chim. Acta*, 1961, **44**, 935, 1199.
51 Y. Handschin and H. Brintzinger, *Helv. Chim. Acta*, 1962, **45**, 1037.
52 P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, 1964, **47**, 992.
53 D. D. Perrin and V. S. Sharma, *Biochim. Biophys. Acta*, 1966, **127**, 35.
54 M. M. Taqui Khan and A. E. Martell, *J. Am. Chem. Soc.*, 1966, **88**, 668.
55 H. Sigel, K. Becker, and D. B. Mc Cormick, *Biochim. Biophys. Acta*, 1967, **148**, 655.
56 H. Sigel, *Eur. J. Biochem.*, 1968, **3**, 530.
57 K. S. Rajan, S. Mainer, and J. M. Davis, *Bioinorg. Chem.*, 1978, **9**, 187.
58 G. Weitzel and T. Speer, *Z. Physiol. Chem.*, 1958, **313**, 212.
59 G. Arena, R. Calì, E. Rizzarelli, and S. Sammartano, *Thermochim. Acta*, 1976, **16**, 315.
60 C. F. Baes and R. E. Mesmer, ' The Hydrolysis of Cations,' Wiley, New York, 1976.
61 K. H. Scheller, F. Hofstetter, P. R. Mitchell, B. Prijs, and H. Sigel, *J. Am. Chem. Soc.*, 1981, **103**, 247.
62 C. Miller Frey and J. E. Stuehr, in ' Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1974, vol. 1, p. 52.
63 T. A. Glassman, J. Suchy, and C. Cooper, *Biochemistry*, 1973, **12**, 2430.
64 M. S. Mohan and G. A. Rechnitz, *Arch. Biochem. Biophys.*, 1974, **162**, 194.
65 T. A. Glassman, C. Cooper, G. P. P. Kuntz, and T. T. Swift, *FEBS Lett.*, 1974, **39**, 73.
66 C. Miller Frey and J. E. Stuehr, *J. Am. Chem. Soc.*, 1978, **100**, 139.
67 N. W. Please, *Biochem. J.*, 1954, **56**, 196.
68 J. P. Belaich and J. C. Sari, *Proc. Natl. Acad. Sci. USA*, 1969, **64**, 763.
69 G. Anderegg, *Helv. Chim. Acta*, 1965, **48**, 1712.
70 P. Amico, R. P. Bonomo, R. Calì, V. Cucinotta, P. G. Daniele, G. Ostacoli, and E. Rizzarelli, unpublished work.
71 R. B. Martin and Y. H. Mariam, ' Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1978, vol. 8, p. 57.
72 B. D. Fischer and R. Bau, *Inorg. Chem.*, 1978, **17**, 27.
73 M. Pezzano and F. Podo, *Chem. Rev.*, 1980, **80**, 366.
74 A. Szent-Gyorgyi, ' Bioenergetics,' Academic Press, New York, 1957.
75 J. A. Happe and M. Morales, *J. Am. Chem. Soc.*, 1966, **88**, 2077.
76 F. H. Jarke and K. S. Rajan, *J. Inorg. Nucl. Chem.*, 1978, **40**, 1719.
77 P. Paoletti, L. Fabbrizzi, and R. Barbucci, *Inorg. Chim. Acta Rev.*, 1973, **7**, 43.
78 G. H. Nancollas, ' Interactions in Electrolyte Solutions,' Elsevier, New York, 1966.
79 M. Cohn and T. R. Hughes, *J. Biol. Chem.*, 1962, **237**, 176.
80 H. Sternlicht, D. E. Jones, and K. Kustin, *J. Am. Chem. Soc.*, 1968, **90**, 7110.
81 H. Sigel, C. F. Naumann, and B. Prijs, *Eur. J. Biochem.*, 1974, **46**, 589.
82 I. Feldman and V. Wee, *Biochemistry*, 1974, **13**, 1836.
83 J. Bolard and G. Chottard, *Bioinorg. Chem.*, 1978, **8**, 185.
84 S. Fan, A. C. Storer, and G. C. Hammes, *J. Am. Chem. Soc.*, 1977, **99**, 8293.
85 J. Granot and D. Fiat, *J. Am. Chem. Soc.*, 1977, **99**, 70.
86 J. L. Bock, *J. Inorg. Biochem.*, 1980, **12**, 119.
87 M. S. Zetter, G. Y-S. Lo, H. W. Dodgen, and J. P. Hunt, *J. Am. Chem. Soc.*, 1978, **100**, 4430.
88 M. P. Heyn and R. Bretz, *Biophys. Chem.*, 1975, **3**, 35.
89 D. R. Williams, *J. Chem. Soc. A*, 1970, 1550.
90 R. B. Martin, in ' Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1979, vol. 9, p. 1.
91 P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc.*, 1980, **102**, 7906.
92 M. B. Hursthouse, S. A. A. Jayaweera, H. Milburn, and A. Quick, *J. Chem. Soc., Dalton Trans.*, 1975, 2569.
93 F. Morita, *Biochim. Biophys. Acta*, 1974, **343**, 674.
94 Y. H. Mariam and R. B. Martin, *Inorg. Chim. Acta*, 1979, **35**, 23.
95 J. B. Orenberg, B. E. Fischer, and H. Sigel, *J. Inorg. Nucl. Chem.*, 1980, **42**, 785.
96 H. Sigel, ' Conference Volume of the XXth International Conference on Co-ordination Chemistry,' ed. D. Banerjea, published by I.U.P.A.C. through Pergamon Press, Oxford, 1980, p. 23.

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