Ternary Complexes in Solution. Comparison of the Coordination Tendency of Some Polybasic Oxygen Acids toward the Binary Complexes of Cu(II) and Adenosine 5′-Mono-, 5′-Di-, and 5′-Triphosphate

H. A. Azab,* A. M. El-Nady, Ahmed Hassan, and R. S. A. Azkal

Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt

Potentiometric equilibrium measurements have been made at (25 ± 0.1) °C and ionic strength I = 0.1 mol dm^{-3} KNO_3 for the interaction of adenosine 5′-mono-, 5′-di-, and 5′-triphosphate (AMP, ADP, and ATP) and Cu(II) with biologically important secondary ligand acids (malic, maleic, succinic, tartaric, and oxalic acids) in a 1:1:1 ratio and the formation of various 1:1:1 mixed ligand complex species inferred from the potentiometric pH titration curves. Initial estimates of the formation constants of the resulting species and the acid dissociation constants of AMP, ADP, ATP, and secondary ligand acid have been refined with the SUPERQUAD computer program. Δ log K values are positive; i.e., the ternary complexes are found to be more stable than the corresponding binary complexes. In some Cu(II) ternary systems studied the interligand interactions or some cooperativity between the coordinate ligands, possibly H bond formation, has been found to be most effective in deciding the stability of the ternary complexes formed in solution. Stabilities of mixed ligand complexes increase in the order AMP < ADP < ATP. With respect to the secondary ligands, the formation constants of the mixed ligand complexes decrease in the following order: succinic > malic > maleic > tartaric > oxalic.

Introduction

Metal ion complex formations are among the prominent interactions in nature (*1–3*), and the polybasic oxygen acid residues are important metabolic intermediates in biological systems, while ribonucleotides adenosine 5′-mono, 5′-di-, and 5′-triphosphates (AMP, ADP, and ATP) are equally important as substrates for many enzymic reactions (*4–7*). Ternary complexes of transition divalent metal ions with AMP, ADP, and ATP and other secondary ligands such as the catechols, ethanolamines, 2,2′-bipyridyl, ethylenediamine, pyrocatecholate, biogenic amines, 1,10-phenanthroline, tyrosine, phenylalanine, glycine, histidine, imidazole, ammonia, and aliphatic dipeptides have been investigated using several techniques (*8–22*) (pH—potentiometric, spectrophotometric, and calorimetric). For an improved understanding of the driving forces leading to mixed ligand complexes of the type Cu(II)–nucleotide–polybasic carboxylic acids (Cu(II)–NU–CA), where nucleotide = AMP, ADP, or ATP and carboxylic acid = malic, maleic, succinic, tartaric, or oxalic acid, have been investigated by potentiometric pH titrations to determine the stability constants of the complexes formed, as these systems mimic many biological reactions which may involve ribonucleotides–metal ion–metabolic intermediate interaction.

Experimental Section

Materials and Solutions. Adenosine 5′-monophosphoric acid disodium salt $C_{10}H_{12}N_5Na_2O_7P\cdot H_2O$ ($Na_2AMP\cdot H_2O$), adenosine 5′-diphosphoric acid disodium salt $C_{10}H_{13}N_5Na_2O_{10}P_2\cdot 2H_2O$ ($Na_2ADP\cdot 2H_2O$), and adenosine 5′-triphosphoric acid disodium salt $C_{10}H_{14}N_5Na_2O_{13}P_3\cdot 3H_2O$ ($Na_2ATP\cdot 3H_2O$) were purchased from Sigma Chemical Co. and were used without purification. The amount of free phosphates initially present in the nucleotides was determined (*23*). It was found to be 2% for ATP and 3% for ADP and AMP. To account for this and to prepare metal ion nucleotide solutions of exactly a 1:1 ratio, we also determined, by potentiometric pH titrations, the molecular weight of these nucleotides. $Cu(NO_3)_2\cdot 6H_2O$, nitric acid, NaOH, and the carboxylic acids (malic, maleic, succinic, tartaric, and oxalic acids) were of pa grade. The concentration of NaOH used for the titrations was determined by titration with a solution of potassium hydrogen phthalate (Merck AG). The concentrations of the metal ion stock solutions were determined with ethylenediaminetetraacetic acid (EDTA).

Apparatus. Potentiometric pH measurments were made on solutions in a double-walled glass vessel using a Beckman Model 4500 digital pH meter with a precision of ±0.1 mV. The potentiometric system was connected to a glass electrode (Metrohm 1028) connected to a double junction reference electrode (Orion 9020). The titrant was delivered by an Amel 882 dispenser, readable to 1 μL. The measurement cell was kept at a temperature constant within ± 0.1 °C, and a magnetic stirrer was used. Purified nitrogen was bubbled through the solutions during titrations.

Procedure. The test solution was titrated with standard CO_2-free potassium hydroxide. The electrodes were calibrated, in both the acidic and alkaline regions, by titrating 0.01 mol dm^{-3} nitric acid with standard potassium hydroxide under the same experimental conditions. Carbonate-free KOH was standardized against standard potassium hydrogen phthalate with the aid of a Gran plot.

The concentration of free hydrogen ion, C_{H^+}, at each point of the titration is related to the measured emf, E°, of the cell by the Nernst equation:

$$E = E^\circ + Q \log C_{H^+} \quad (1)$$

where E° is a constant which includes the standard potential of the glass electrode and Q is the slope of the glass electrode response. The value of E° for the electrode was determined from a Gran plot derived from a separate titration of nitric acid with standard KOH solution under the same temperature and medium conditions as for the test solution titration. The results so obtained were analyzed by the nonlinear least-squares computer program ESAB2M (*24*) to refine E° and the autoprotolysis constant of water, K_w.

In order to avoid hydrolysis prior to the potentiometric measurements, samples of the nucleotides were weighed out as the solid and added to the reaction vessel just prior to performing the titration. The solutions titrated can be

0021-9568/93/1738-0502$04.00/0

represented according to the folloiwing scheme: HNO_3 (a); HNO_3 + nucleotide (b); HNO_3 + nucleotide + Cu(II) (c); HNO_3 + polybasic carboxylic acid (d); HNO_3 + polybasic carboxylic acid + Cu(II) (e); HNO_3 + nucleotide + polybasic carboxylic acid + Cu(II) (f). A constant ionic strength was obtained with 0.1 mol dm^{-3} KNO_3, and the total volume was kept constant at 50 cm^3.

Results and Discussion

To calculate the initial estimates of the stability constants of the ternary complexes of Cu(II) with AMP, ADP, ATP, and malic, maleic, succinic, tartaric, or oxalic acid, the following equations were used:

$$\mathrm{Cu(II)(NU)} + \mathrm{CA} \rightleftharpoons \mathrm{Cu(II)(NU)(CA)} \quad (2)$$

$$K^{\mathrm{Cu(II)(NU)}}_{\mathrm{Cu(II)(NU)(CA)}} = \frac{[\mathrm{Cu(II)(NU)(CA)}]}{[\mathrm{Cu(II)(NU)}][\mathrm{CA}]} \quad (3)$$

[I = 0.1 mol dm^{-3} (KNO_3), 25 °C]

$$\mathrm{Cu(II)} + \mathrm{NU} \rightleftharpoons \mathrm{Cu(II)(NU)} \quad (4)$$

$$K^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)(NU)}} = \frac{[\mathrm{Cu(II)(NU)}]}{[\mathrm{Cu(II)}][\mathrm{NU}]} \quad (5)$$

$$\mathrm{Cu(II)} + \mathrm{CA} \rightleftharpoons \mathrm{Cu(II)(CA)} \quad (6)$$

$$K^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)(CA)}} = \frac{[\mathrm{Cu(II)(CA)}]}{[\mathrm{Cu(II)}][\mathrm{CA}]} \quad (7)$$

where CA = polybasic carboxylic acids (malic, maleic, succinic, tartaric, and oxalic acid) and NU = nucleotide (AMP, ADP and ATP). In addition the protonation and complexation reactions of the free phosphate initially present in solutions have been included in the calculations to get better conditional stability constants. The overall stability constant $\beta^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)(NU)(CA)}}$ may be represented by eq 8.

$$\mathrm{Cu(II)} + \mathrm{NU} + \mathrm{CA} \rightleftharpoons \mathrm{Cu(II)(NU)(CA)} \quad (8)$$

$$\beta^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)(NU)(CA)}} = \frac{[\mathrm{Cu(II)(NU)(CA)}]}{[\mathrm{Cu(II)}][\mathrm{NU}][\mathrm{CA}]} = K^{\mathrm{Cu(II)(NU)}}_{\mathrm{Cu(II)(NU)(CA)}} K^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)(NU)}} \quad (9)$$

Formation constants and protonation constants were refined with the SUPERQUAD computer program (*25*). All the calculations were performed on an IBM XT 286 personal computer. The constants were refined by minimizing the error-square sum, U, of the potentials:

$$U = \sum W_i (E_{\mathrm{obs}} - E_{\mathrm{calc}})^2 \quad (10)$$

where E_{obs} and E_{calc} refer to the measured potential and that calculated from eq 1. The weighting factor W_i is defined as the reciprocal of the estimated variance of measurement:

$$W_i = 1/\sigma^2 = 1/[\sigma^2 + (\delta E/\delta V)\sigma^2] \quad (11)$$

where σ_E and σ_V are the estimated variances of the potential and volume readings, respectively. The quality of fit was judged by the values of the sample standard deviation, S, and the goodness of fit, X^2, (Pearson's test). At σ_E = 0.1 mV (0.001 pH error) and σ_V = 0.005 mL, the values of S in different sets of titrations were between 1.0 and 1.8, and X^2 was between 12.0 and 13.0. The scatter of residuals (E_{obs} - E_{calc}) vs pH was reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data.

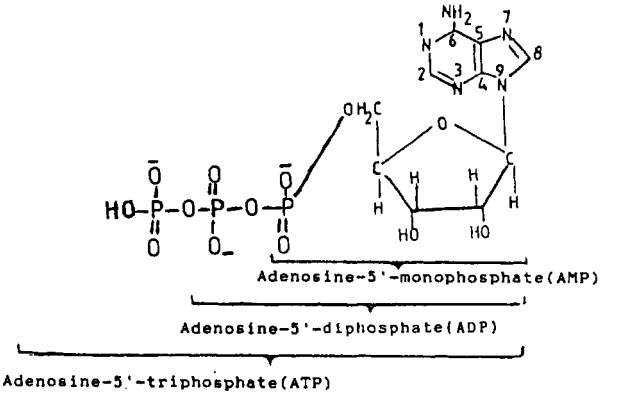

Figure 1. The structure of AMP, ADP, and ATP.

At the experimental pH values used in the calculation in this work the interfering effects of hydroxy complexes are negligble. Thus, the secondary ligand, CA, combines with the binary 1:1 Cu(II)(NU){[Cu(II)(AMP)], [Cu(II)(ADP)$^{1-}$], and [Cu(II)(ATP)]$^{2-}$} complex in a manner similar to its interaction with aquated metal ions $[Cu(H_2O)_6]^{2+}$ in solutions. Thus, the initial estimates of the stability constants of the ternary complexes formed in solution have been determined using the Rossotti and Irving formula (*26*). These values were then refined using the SUPERQUAD computer program (*25*). The determined acidity constants of malic (pK_{a_1} = 3.24 ± 0.02, pK_{a_2} = 4.73 ± 0.02), maleic (pK_{a_1} = 1.94 ± 0.02, pK_{a_2} = 5.77 ± 0.02), succinic (pK_{a_1} = 4.06 ± 0.02, pK_{a_2} = 4.86 ± 0.02), tartaric (pK_{a_1} = 2.82 ± 0.03, pK_{a_2} = 3.98 ± 0.02), and oxalic acids (pK_{a_1} = 1.15 ± 0.03, pK_{a_2} = 3.87 ± 0.03) and the stability constants of their binary Cu(II) complexes are in good agreement with those found in the literature (*27*). The two acid formation constant values for AMP (pK_{a_1} = 3.81 ± 0.03, pK_{a_2} = 6.24 ± 0.03), ADP (pK_{a_1} = 3.94 ± 0.03, pK_{a_2} = 6.38 ± 0.04), and ATP (pK_{a_1} = 4.05 ± 0.03, pK_{a_2} = 6.51 ± 0.03) and the stability constants of their Cu(II) complexes were determined from the titration curves, and the results were found to agree well with those reported in the literature (*27–30*).

In the case of ADP and ATP the monoprotonated complexes, i.e., Cu(HADP) and Cu(HATP)$^-$, were taken into consideration. The calculated values log $K^{\mathrm{Cu}}_{\mathrm{Cu(HADP)}}$ = 2.61 and log $K^{\mathrm{Cu}}_{\mathrm{Cu(HADP)}}$ = 3.33 agree also favorably with the literature (*30*).

Figure 1 shows the structure of the nucleotides used in the present work.

Early researchers (*31–34*) found pK_{a_1} values of 3.5–4.2 to be associated with proton ionization from the protonated forms of AMP, ADP, and ATP. Calorimetric work (*35*) provides evidence that proton ionization from protonated adenine and adenosine is from the N_1H^+ group. The second proton ionization was attributed to the phosphate groups.

The purine bases have two high electron density centers which are possible sites for metal ion chelation, viz., C_6NH_2/N_7 and N_3–N_9. Chelation of Cu^{2+} by both sites has been suggested (*36–39*).

Potentiometric (*40, 41*), ^{31}P NMR (*40, 42*) and aqueous solution infrared absorption data (*42*) confirm the binding of Cu^{2+} to the phosphate portion of AMP, ADP, and ATP. These studies are in essential agreement that Cu^{2+} binds the available phosphate group in the mono-and dinucleotides but only the α-and β-phosphates in ATP. This latter behavior has been attributed to the square-planar stereochemical requirements of Cu^{2+}.

Table I. Formation Constant Values for the Binary Cu(II)–Nucleotide or –Carboxylic Acid Complexes and Those for the Mixed Ligand Complexes Cu(II)–Nucleotide–Carboxylic Acid at 25 °C and I = 0.1 mol dm^{-3} KNO_3

ligand	log $K^{Cu(II)}_{Cu(II)(Nu)}$ or log $K^{Cu(II)}_{Cu(II)(CA)}$	log $K^{Cu(II)(AMP)}_{Cu(II)(AMP)(CA)}$	log $K^{Cu(II)(ADP)}_{Cu(II)(ADP)(CA)}$	log $K^{Cu(II)(ATP)}_{Cu(II)(ATP)(CA)}$	log $\beta^{Cu(II)}_{Cu(II)(AMP)(CA)}$	log $\beta^{Cu(II)}_{Cu(II)(ADP)(CA)}$	log $\beta^{Cu(II)}_{Cu(II)(ATP)(CA)}$	Δ log K_1[a]	Δ log K_2[a]	Δ log K_3[a]
AMP	3.20 ± 0.02									
ADP	6.05 ± 0.03									
ATP	6.40 ± 0.03									
oxalic acid	4.04 ± 0.02	5.21 ± 0.03	5.76 ± 0.03	6.46 ± 0.03	8.41	11.81	12.86	1.17	1.72	2.42
succinic acid	2.98 ± 0.03	5.82 ± 0.02	6.35 ± 0.04	7.25 ± 0.04	9.02	12.40	13.65	2.84	3.37	4.27
tartaric acid	3.20 ± 0.01	5.55 ± 0.04	6.10 ± 0.02	6.88 ± 0.04	8.75	12.15	13.28	2.35	2.90	3.68
malic acid	4.22 ± 0.02	5.74 ± 0.04	6.28 ± 0.03	6.99 ± 0.03	8.94	12.33	13.39	1.52	2.06	2.77
maleic acid	3.42 ± 0.01	5.63 ± 0.02	6.18 ± 0.03	6.91 ± 0.04	8.83	12.23	13.31	2.21	2.76	3.49

[a] $\Delta \log K_1 = \log K^{Cu(II)(AMP)}_{Cu(II)(AMP)(CA)} - \log K^{Cu(II)}_{Cu(II)(CA)}$. $\Delta \log K_2 = \log K^{Cu(II)(ADP)}_{Cu(II)(ADP)(CA)} - \log K^{Cu(II)}_{Cu(II)(CA)}$. $\Delta \log K_3 = \log K^{Cu(II)(ATP)}_{Cu(II)(ATP)(CA)} - \log K^{Cu(II)}_{Cu(II)(CA)}$.

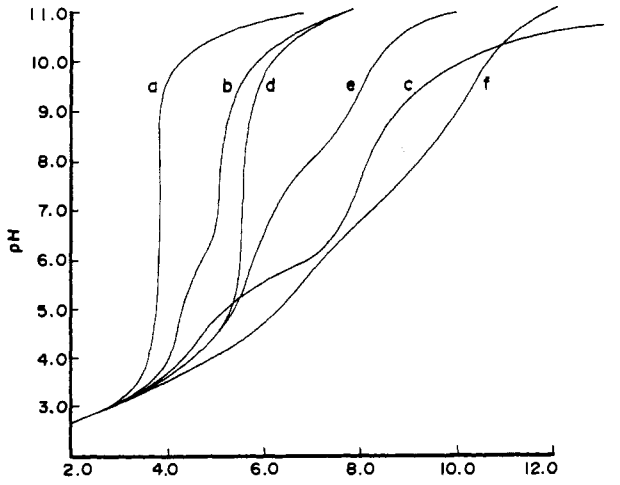

Figure 2. pH against the volume of 0.0495 mol dm^{-3} NaOH for the Cu(II)–AMP–oxalic acid system at 25 °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.0037 mol dm^{-3} HNO_3; (b) solution a + 1 × 10^{-3} mol dm^{-3} AMP; (c) solution b + 1 × 10^{-3} mol dm^{-3} Cu(II); (d) solution a + 1 × 10^{-3} mol dm^{-3} oxalic acid; (e) solution d + 1 × 10^{-3} mol dm^{-3} Cu(II); (f) solution e + 1 × 10^{-3} mol dm^{-3} AMP.

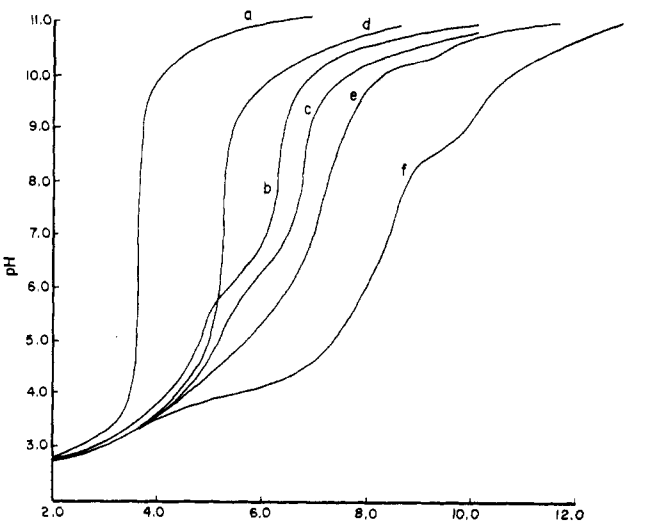

Figure 3. pH against the volume of 0.0495 mol dm^{-3} NaOH for the Cu(II)–ADP–malic acid system at 25 °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.0037 mol dm^{-3} HNO_3; (b) solution a + 1 × 10^{-3} mol dm^{-3} AMP; (c) solution b + 1 × 10^{-3} mol dm^{-3} Cu(II); (d) solution a + 1 × 10^{-3} mol dm^{-3} malic acid; (e) solution d + 1 × 10^{-3} mol dm^{-3} Cu(II); (f) solution e + 1 × 10^{-3} mol dm^{-3} ADP.

On the basis of the observed lack of reaction (from pH titration data) between Cu^{2+} and adenosine and the increased stability of Cu^{2+} complexes in the order AMP < ADP < ATP, the suggestion was made that Cu^{2+} did not react with the base moiety of ATP (*41*). However, proton NMR studies have demonstrated binding of Cu^{2+} to the N_7 positions of the adenine base in dAMP (*40*).

Berger and Eichhorn (*44*) conclude that, in general, Cu^{2+} can bind to multiple sites on the adenine base, with preference for a given site influenced by molecular associations which in the different AMP isomers are governed by the position of the phosphate on the ribose. In the case of 2′-AMP a chelate involving N_3 and a phosphate group of the same molecule is proposed. It is expected that Cu^{2+} ions would interact more strongly with the electron donor groups of the purine base of mononucleotides than they do with the phosphate groups.

Differences in the nature of binding sites for Cu^{2+} ions on purine nucleotides have been characterized by different g values and hyperfine splittings, as measured by the EPR spectra of the complexes formed under conditions close to physiological ones (*45*). Thus, there is lack of agreement as to the assignment of the site of coordination. Our opinion on this point will be discussed later.

In Figures 2–4 representative sets of experimental titration curves obtained according to the sequence described in the experimental section for the different Cu(II)–NU–CA systems studied are displayed. It is observed that the Cu(II)–NU titration curves (c) diverge from the nucleotide curve (b) in the lower pH range (pH ≃ 3.5), denoting the formation of the Cu(II)–NU complex. Generally, the complex titration curves show an inflection after addition of 2 mol of base per 1 mol of the nucleotide (AMP, ADP, or ATP). This indicates the simultaneous dissociation of two protons from AMP while in the case of ADP and ATP the complex species Cu(HADP), Cu(ADP), Cu(HATP)$^-$, and Cu(ATP) have been formed in solution. Cu(II)–NU are quite stable up to high pH values; i.e., they have no tendency to form hydroxy complexes. With respect to the titration curves of the Cu(II)–carboxylic acid binary complex solutions studied, one may deduce that these complexes begin to form at pH > 4.0. Generally, for all Cu(II)–carboxylic acid complexes studied precipitation occurred at pH > 10.5. In all cases no calculations have been performed beyond the precipitation point; hence, the hydroxy species likely to be formed after this point could not be studied.

For the titration curves of the ternary systems studied (Cu(II)–NU–CA) one observes that the C and F are well separated at a pH of 4.3. This behavior reveals that in these pH ranges coordination of the secondary ligand, carboxylic acid, with Cu(II)–NU starts.

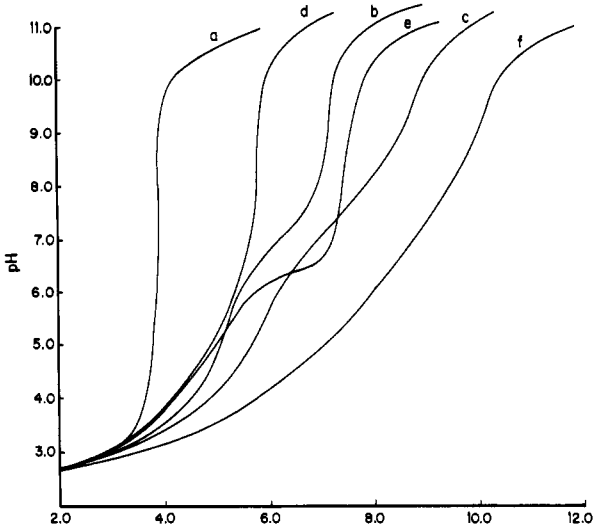

Figure 4. pH against the volume of 0.0495 mol dm^{-3} NaOH for the Cu(II)–ATP–succinic acid system at 25 °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.0037 mol dm^{-3} HNO_3; (b) solution a + 1 × 10^{-3} mol dm^{-3} ATP; (c) solution b + 1 × 10^{-3} mol dm^{-3} Cu(II); (d) solution a + 1 × 10^{-3} mol dm^{-3} succinic acid; (e) solution d + 1 × 10^{-3} mol dm^{-3} Cu(II); (f) solution e + 1 × 10^{-3} mol dm^{-3} ATP.

Examination of the different formation constant values listed in Table I clearly reveals that the formation constant of the mixed ligand complexes increases in the order AMP < ADP < ATP. Though many studies in solution favored the phosphate group rather than the base as the primary metal binding site, the simultanous binding of Cu(II) ion to the N_7 site of the adenine residue (*46*, *47*) and phosphate may also be reported in the mixed ligand complexes formed in the present work. Thus, the Cu(II) bound to the base moiety may promote intramolecular base–phosphate interaction. Thus, the mixed ligands studied may be considered as relatively simple models from which information may be gained about the properties of nucleotides and their base moieties regarding the strength of their interactions with the important metabolic intermediates (polybasic oxygen acids), and even insight into the factors which influence this strength is thus becoming available.

With respect to the secondary ligands, the formation constants of the mixed ligand complexes decrease in the following order: succinic > malic > maleic > tartaric > oxalic. This behavior can be interpreted in terms of the basicities (Σ pK_{a_1} + pK_{a_2}) of the secondary ligand carboxylic acids used. It is well known that the increase in basicity of a ligand increases the stability of its metal complexes.

$\Delta \log K$, defined by eq 12, is a measure of the stability of the ternary complexes with respect to the binary complexes.

$$\Delta \log K = \log K^{Cu(II)(NU)}_{Cu(II)(NU)(CA)} - \log K^{Cu(II)}_{Cu(II)(NU)} \qquad (12)$$

In the case of Cu(II)–NU–CA systems, $\Delta \log K$ is found to be positive (Table I). The higher stability constant of Cu(II)–ATP–CA ternary complexes compared with the binary systems may be attributed to the interligand interactions or some cooperativity between the coordinate ligands, possibly H-bond formation.

Acknowledgment

Thanks are expressed to Prof. R. M. Izatt for providing H.A.A. with the SUPERQUAD computer program during his grant at the Department of Chemistry, Brigham Young University, Provo, UT.

Literature Cited

(1) Eichhorn, G. L., Ed. *Inorganic Biochemistry*; Elsevier: New York, 1973; Vols. 1 and 2.
(2) Sigel, H., Ed. *Metal Ions in Biological Systems*; Marcel Dekker: New York, 1973–1982; Vols. 1–14.
(3) Wood, J. M. *Naturwissenschaften* **1975**, *62*, 357–364.
(4) Spiro, T. G. Phosphate Transfer and Its Activation by Metal Ions; Alkaline Phosphatase. Chapter 17 of ref 1.
(5) Cooperman, B. S. *Met. Ions Biol. Syst.* **1976**, *5*, 79–125.
(6) Mildvan, A. S. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1979**, *49*, 103–26.
(7) Sigel, H., Ed. Nucleotides and Derivatives: Their Ligating Ambivalency. 1979; Vol. 8 of ref 2.
(8) Colburn, R. W.; Mass, J. W. *Nature* **1966**, *208*, 37.
(9) Sigel, H.; Becker, W.; McCormick, D. B. *Biochim. Biophys. Acta* **1967**, *148*, 655.
(10) Chaudhudri, P.; Sigel, H. *J. Am. Chem. Soc.* **1977**, *99*, 3142.
(11) Rajan, K. S.; Mainer, S.; Davis, J. M. *Bioinorg. Chem.* **1978**, *9*, 187.
(12) Yatsimirskii, K. B.; Davidenko, N. K.; Manorik, P. A. *Dopov. Acad. Nauk Uukr RSR, Ser. B: Geol., Khim. Biol. Nauki* **1978**, *12*, 1111.
(13) Mohan, M. S.; Khan, M. M. T. *J. Coord. Chem.* **1979**, *8*, 207.
(14) Arena, G.; Call, R.; Cucinotta, V.; Musumeci, S.; Rizzareli, S.; Sammartano, S. *Congr. Naz. Chim. Inorg. (Atti)*, **1980**, *13*, 288.
(15) Bouisson, D. H.; Sigel, H. *Biochim. Biophys. Acta* **1974**, *43*, 343.
(16) Davidenko, N. K.; Manorik, P. A. *Zh. Neorg. Khim.* **1980**, *25* (2), 437.
(17) Saha, N.; Sigel, H. *J. Am. Chem. Soc.* **1982**, *104* (15), 4100.
(18) Manorik, P. A.; Davidenko, N. K. *Zh. Neorg. Khim.* **1983**, *28* (9), 2292.
(19) Werner, E. R.; Rode, B. M. *Inorg. Chim. Acta* **1984**, *91*, 217.
(20) Davidenko, N. K.; Raspopina, V. A. *Zh. Neorg. Khim.* **1986**, *31* (8), 2039.
(21) Matsuda, K.; Kanai, C.; Takahara, M.; Maki, M. *Nippon Kagoku Kaiski* **1985**, *4*, 698.
(22) Mahmoud, M. R.; Azab, H. A.; Hamed, M. M. A.; Mohamed, A. A. *Chem. Scr.* **1989**, *29*, 17–20.
(23) Buisson, D. H.; Sigel, H. *Biochim. Biophys. Acta* **1974**, *34*, 45–63.
(24) May, P. M.; Williams, D. R. In *Computational Methods for the Determination of formation constants*; Leggett, D. J., Ed.; Plenum Press: New York, 1985; pp 37–70.
(25) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195.
(26) Irving, H.; Rossotti, H. S. *J. Chem. Soc.* **1953**, 3397; **1954**, 2904.
(27) Sillen, L. G.; Martell, A. E. *Stability Constants of Metal ion complexes*; The Chemical Society, Burlington House: London, 1971.
(28) Khan, M. M. T.; Martell, A. E. *J. Am. Chem. Soc.* **1967**, *89*, 5585.
(29) Khan, M. M. T.; Martell, A. E. *J. Am. Chem. Soc.* **1966**, *88*, 668.
(30) Smith, R. M.; Martell, A. E., Chen, Y. *Pure Appl. Chem.* **1991**, *63*, 1015.
(31) Levene, P. A.; Simms, H. S. *J. Biol. Chem.* **1925**, *65*, 519.
(32) Taylor, H. F. W. *J. Chem. Soc.* **1948**, 765.
(33) Alberty, R. A.; Smith, R. M.; Bock, R. M. *J. Biol. Chem.* **1951**, *193*, 425.
(34) Beers, R. F.; Steiner, R. F. *Nature (London)* **1957**, *179*, 1076.
(35) Christensen, J. J.; Izatt, R. M. *J. Phys. Chem.* **1962**, *66*, 1030.
(36) Harkins, T. R.; Freiser, H. *J. Am. Chem. Soc.* **1958**, *80*, 1132.
(37) Albert, A. Biochem. J. **1953**, *54*, 646.
(38) Albert, A.; Serjeant, E. P. *Biochem. J.* **1960**, *76*, 621.
(39) Frieden, E.; Alles, J. *J. Biol. Chem.* **1957**, *230*, 797.
(40) Eichhorn, G. L.; Clark, P.; Becker, E. D. *Biochemistry* **1966**, *5*, 245.
(41) Khan, M. M. T.; Martell, A. E. *J. Phys. Chem.* **1962**, *66*, 10.
(42) Cohn, M.; Hughes, T. R. *J. Biol. Chem.* **1962**, *237*, 176.
(43) Brintzinger, H. *Biochim. Biophys. Acta* **1963**, *77*, 343.
(44) Berger, N. A.; Eichhorn, G. L. *Biochemistry* **1971**, *10*, 1847.
(45) Bemski,G.; Rieber,M.; Wust, M. *FEBS Lett.* **1971**, *14*, 117.
(46) Sigel, H.; Massoud, S. S.; Tribolet, R. *J. Am. Chem. Soc.* **1988**, *110*, 6857.
(47) Sigel, H. *Eur. J. Biochem.* **1987**, *165*, 65.

Received for review October 22, 1992. Revised June 29, 1993. Accepted July 12, 1993.*

* Abstract published in *Advance ACS Abstracts*, September 1, 1993.