Potentiometric Study on Complexation of Divalent Transition Metal Ions with Amino Acids and Adenosine 5′-Triphosphate

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Potentiometric equilibrium measurements have been performed at 25 °C and in an $I = 0.10$ M KCl ionic medium for the interaction of nickel(II), copper(II), and zinc(II) with adenosine 5′-triphosphate (ATP) and dicarboxylic amino acids (aa): aspartic acid (Asp) and glutamic acid (Glu). The formation of 1:1 and 1:2 binary and 1:1:1 ternary complexes was inferred from the potentiometric titration curves. It was deduced that adenosine 5′-triphosphate acts as a primary ligand in the ternary complexes involving the dicarboxylic amino acids. The complexation model for systems of adenosine 5′-triphosphate (ATP) and the dicarboxylic amino acids with nickel(II), copper(II), and zinc(II) have been established by the "BEST" software from the potentiometric data. Values of $\Delta \log K$ ($\log \beta_{MAB} - (\log \beta_{MA} + \log \beta_{MB})$) showed that the ternary complexes are less stable than the binary ones, suggesting that no interaction occurred between the ligands in the ternary complexes. The order of the values of the stability constants of all the ternary complexes was M(II)ATP(Asp) > M(II)ATP(Glu), and the same sequence was found in the binary complexes of metal ions with the amino acids. With respect to the metal ions, the stability constants of binary and ternary complexes decrease in the following order: copper(II) > nickel(II) > zinc(II).

Introduction

Nucleotides are important metabolic substances. For example, adenosine-5′-triphosphate (ATP) is used and then resynthesized by some organisms in an amount equal to their mass. ATP is one of the basic components in bioenergetic processes of living organisms, its polyphosphate chain being the center for chemical energy storage and transfer, and is a biologically important ligand that plays a key role in the metabolism of organisms providing transphosphorylation in the presence of metal ions.[1−3] Further, metal−nucleotide complexes may act as a cofactor, substrate, or modifier in promoting enzymatic catalysis of displacement reactions of phosphorus and in maintaining structural integrity and specificity of nucleic acids.[4] Thus, considerable interest has been focused on the study of binary and ternary metal complexes formed with ATP and some secondary ligands.[5−16]

Aspartic acid (Asp) and glutamic acid (Glu) are dicarboxylic amino acids. Containing one amino and two carboxyl groups, Asp and Glu can act as tridentate ligands. Aspartic acid (Asp) is a naturally occurring amino acid. Along with glutamic acid, it acts as a neurotransmitter in the central nervous system. The presence of metal ions in living organisms modifies the character of bioprocesses.[17] The reactions between an amino acid and metal ions are considered as models of the processes which take place at the molecular level in metal/protein systems.[17] Glutamic acid (Glu) occurs in almost all of the protein substances. It is synthesized in a living organism from α-ketoglutaric acid by means of glutamate dehydrogenase. This synthesis is of principal significance for all living cells. Owing to its great importance in many fundamental biochemical processes as well as to its use in treatment of nervous system diseases and states of exhaustion, this acid has become an object of many studies.[18]

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In the literature, there are many studies relating to binary and ternary complexes that include divalent metal ions, aspartic acid, glutamic acid, and various primary ligands.[17−32] However, there are very few studies in the literature relating to ternary complexes of divalent metal ions with ATP and dicarboxylic amino acids. In 1999, Boraei et al. investigated the stability constants of binary and ternary complexes of Co(II), Ni(II), and Cu(II) ions with ATP and dicarboxylic amino acids (aspartic and glutamic acid) at 25 °C and in an $I = 0.10$ M KNO_3 ionic medium.[14] They calculated the acidity constants of the ligands and the stability constants of binary and ternary complexes by using the Irving and Rossotti method. No study exists in the literature on the stability constants of ternary complexes consisting of solutions including zinc(II), ATP, and aspartic acid (or glutamic acid).

In this study, due to their importance in biological systems, protonation constants of ATP, aspartic acid, and glutamic acid and the stability constants of the binary and ternary complexes that these ligands form with nickel(II), copper(II), and zinc(II) have been performed with the potentiometric method at 25 °C and in a 0.1 M KCl ionic medium. The protonation constants of the ligands and the stability constants of the complexes have been calculated with the BEST software.[33] The concentration distribution curves of each complex species in solution were also evaluated by the SPE software.[33] $\Delta \log K$ parameters were calculated which indicate the effect of the bonded primary ligand toward an incoming secondary ligand.

Experimental Section

Chemicals. Adenosine 5′-triphosphate (ATP) in the form of the disodium salt and the amino acids used were analytical grade (Sigma) products with high purity. All other chemicals employed were of A.R. grade. Metal(II) chloride solutions were prepared by dissolving the metal chlorides (99.9 %) in concentrated HCl. The stock solutions of metal(II) chlorides were standardized

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complexometrically by EDTA titration using a suitable indicator.[34] The excess acid in the metal(II) stock solutions was determined by potentiometric titrations as described previously.[35,36] A carbonate-free KOH solution (0.1 M) was prepared and standardized potentiometrically against the primary standard, potassium hydrogen phthalate.[37,38] A hydrochloric acid solution (0.1 M) was prepared and then standardized by titration against the potassium hydroxide standard. The potassium chloride, which was used as a background electrolyte, was a Merck p.a. reagent. The ionic strength of each solution was adjusted to 0.10 M by the addition of 1 M KCl as the supporting electrolyte. All solutions were prepared with analytical grade water (R = 18 MΩ) using grade A glassware.

Apparatus and Procedure. Potentiometric titrations were carried out using a Schott automatic titrator, model TitroLine Alpha Plus, equipped with a Schott combined pH electrode. The automatic titrator was connected to a computer, and automatic titrations were performed using a suitable computer program to control titrant delivery. The pH-meter was calibrated daily using standard buffer solutions (Merck) with pH values of 4.01 and 8.96 at 25 °C. The potentiometric cell was calibrated before each experiment to measure the hydrogen ion concentration rather than its activity. The K_w values were calculated from several separate series of $[H^+]$ and $[OH^-]$ measurements in 0.1 M KCl.[33,34]

All titrations were carried out in solutions contained in a double-walled glass vessel. The titration cell was thermostatted at 25 °C using a VWR 11405. The titrations were performed in an inert atmosphere where nitrogen gas was bubbled through the titrated solutions before and during the pH measurements.

The ionic strength of the solutions was kept constant (0.10 M), using a KCl solution, and a total volume of 50 mL was used for each titration. The experimental procedure involved the potentiometric titrations of the following solutions:

(a) 5 mL of 0.1 M HCl + 5 mL of 1 M KCl (for cell calibration)

(b) 5 mL of 0.1 M HCl + 0.1 mmol of ligand A or B + 5 mL of 1 M KCl (for the determination of protonation constants of ligands)

(c) 5 mL of 0.1 M HCl + 0.1 mmol of ligand A or B + 10 mL of 0.01 M (0.1 mmol) metal(II) + 5 mL of 1 M KCl (for the determination of stability constants of ML complexes)

(d) 5 mL of 0.1 M HCl + 0.2 mmol of ligand B + 10 mL of 0.01 M (0.1 mmol) metal(II) + 5 mL of 1 M KCl (for the determination of stability constants of ML_2 complexes)

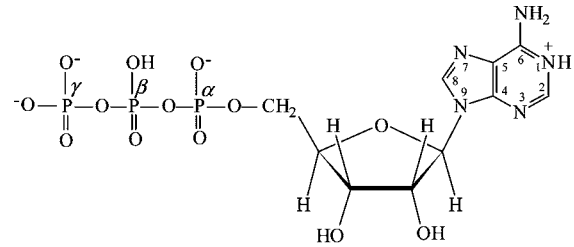
(e) 5 mL of 0.1 M HCl + 0.1 mmol of ligand A + 0.1 mmol of ligand B + 10 mL of 0.01 M (0.1 mmol) metal(II) + 5 mL of 1 M KCl (for the determination of stability constants of MAB ternary complexes)

Potentiometric measurements were carried out by titrating 50 mL of the titrant solution with standard KOH solutions until the formation of scarcely soluble species was noted.

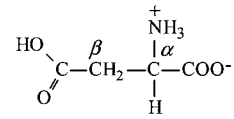
The computations of the protonation constants of the ligands and the stability constants of the binary and ternary complexes of nickel(II), copper(II), and zinc(II) from potentiometric data were carried out by the BEST[33] software. The BEST software was used to minimize the standard deviation of the fit (σ_{fit}) between the observed and calculated pH values for the overall titration data. The potentiometric results were applied by the SPE software, and the distributions of complex species could be drawn for all M(II):ATP:aa systems.

Results and Discussion

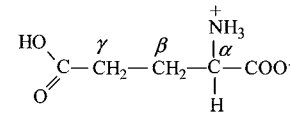
Protonation Constants of the Ligands. The structural formulas of the investigated ligands are given in Figure 1. The



Adenosine 5'-triphosphate disodium salt (ATP, H_2L^{2-})



Aspartic acid (Asp, H_2L)



Glutamic acid (Glu, H_2L)

Figure 1. Structures of the ligands used in this study.

Table 1. Protonation Constants (log $K \pm \sigma^x)^y$ of ATP, Aspartic Acid, and Glutamic Acid at Ionic Strength I = 0.1 M KCl and 25 °C[a]

ligand	log K_1	log K_2	log K_3
ATP	6.61 ± 0.05	4.13 ± 0.07	
	6.61[11,b]	4.18[11,b]	
	6.614[12,b]	4.006[12,b]	
aspartic acid	9.66 ± 0.01	3.70 ± 0.02	
	9.63[19,c]	3.73[19,c]	
	9.71[27,b]	3.71[27,b]	1.70[27,b]
glutamic acid	9.58 ± 0.03	4.16 ± 0.01	
	9.42[24,d]	4.20[24,d]	
	9.634[27,b]	4.155[27,b]	2.02[27,b]

[a] x: standard deviation. y: ± 95 % confidence interval. [b] 25 °C, 0.1 M KNO_3. [c] 20 °C, 0.1 M KNO_3. [d] 25 °C, 0.1 M $NaNO_3$.

protonation constants of the ligands investigated have been redetermined potentimetrically in an aqueous medium at 25 °C and in I = 0.10 M KCl. The values obtained (Table 1) are in good agreement with the literature. Individual titration curves of ATP and aspartic and glutamic acids are shown as curve I and II in Figure 2 and 3, where m is moles of base added per mole of ligand. Two inflection points were observed in the titration curves of the ligands. In the two buffer zones along the inflection point, the number of titrated protons is two per ligand. For ATP, the first proton is released from the $(N1)H^+$ site followed by the one from the β-phosphate group, $-P(O_2)^-(OH)$.[3,12,13,39] The α-phosphate and two γ-phosphate protons are too acidic to be measured by potentiometry and do not play a role in the complex formation in the pH range studied. In the buffer zone between m = 0.0 and 1.0, aspartic and glutamic acids have indicative dissociation of protons from the β- and γ-carboxyl groups, respectively, followed by dissociation of the $-NH_3^+$ group.[26,27,29]

Binary Complexes of ATP and Aspartic and Glutamic Acid. Potentimetric titrations of the M(II):ATP systems were performed at 25 °C and in 0.1 M KCl ionic medium. Solid Na_2ATP was added to the M(II) solution with a mole ratio of (1:1). Two inflection points were observed at m = 2.0 and 4.0 on the titration curves of the M(II):ATP systems, where m is moles of base added per mole of metal (Figures 2, 3, and 4; curve III). In addition, it has been observed that the curves were

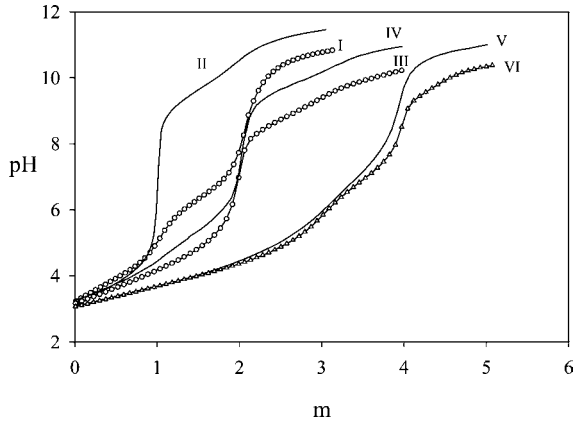


Figure 2. Potentiometric titration curves for the Ni(II):ATP:Asp system at 25 °C and $I = 0.1$ M KCl: I, ATP alone; II, Asp alone; III, Ni(II):ATP (1:1); IV, Ni(II):Asp (1:1); V, Ni(II):Asp (1:2); VI, Ni(II):ATP:Asp (1:1:1).

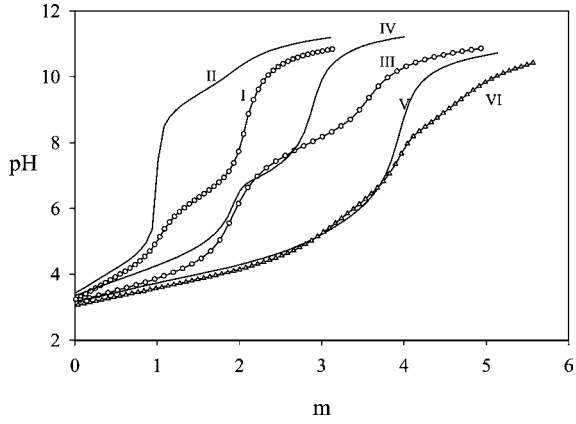


Figure 3. Potentiometric titration curves for the Cu(II):ATP:Glu system at 25 °C and $I = 0.1$ M KCl: I, ATP alone; II, Glu alone; III, Cu(II):ATP (1:1); IV, Cu(II):Glu (1:1); V, Cu(II):Glu (1:2); VI, Cu(II):ATP:Glu (1:1:1).

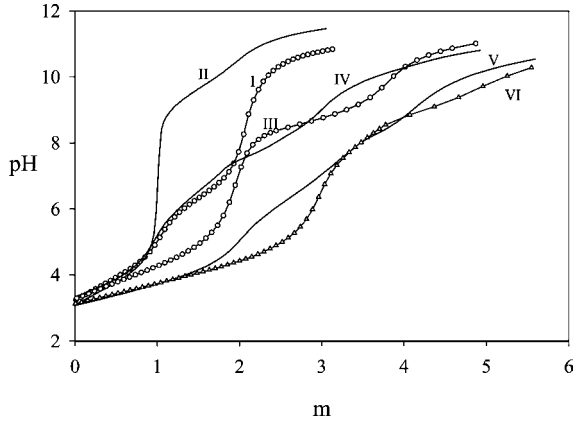


Figure 4. Potentiometric titration curves for the Zn(II):ATP:Asp system at 25 °C and $I = 0.1$ M KCl: I, ATP alone; II, Asp alone; III, Zn(II):ATP (1:1); IV, Zn(II):Asp (1:1); V, Zn(II):Asp (1:2); VI, Zn(II):ATP:Asp (1:1:1).

located in a lower pH region than the titration curve of ATP alone (Figure 2; curve I). Experimental data have pointed out that, in the $m = 0.0$ to 2.0 buffer zone, the MA^{2-} complex forms between pH 3.0 and 5.0. The equilibria involved in this region can be described by the following equations (β is the overall stability constant of the complex; K is the stepwise stability constant; and L = ATP or aspartic or glutamic acid for the binary complexes).

$$M + L \overset{\beta_{ML}}{\rightleftharpoons} ML \qquad \beta_{ML} = \frac{[ML]}{[M][L]} \tag{1}$$

$$ML + H \overset{K_{MLH}}{\rightleftharpoons} MLH \qquad K_{MLH} = \frac{[MLH]}{[H][ML]} \tag{2}$$

$$ML + H_2O \overset{K_{MLH-1}}{\rightleftharpoons} ML(OH) + H$$

$$K_{MLH\text{-}1} = \frac{[ML(OH)][H]}{[ML]} \tag{3}$$

$$M + L + H \overset{\beta_{MLH}}{\rightleftharpoons} MLH \qquad \beta_{MLH} = \frac{[MLH]}{[M][L][H]} \tag{4}$$

The stability constants of the M(II):ATP complexes were calculated and are listed in Table 2 together with values from the literature. The obtained results appear to be in compliance with the values in the literature. The stability sequence of MA complexes for metal ions was found as Cu(II) > Ni(II) > Zn(II), and the stability sequence of MAH complexes was found as Ni(II) > Zn(II) > Cu(II). Many studies that have been made reported that ATP could bind to metal(II) ions with three donor atoms.[9,13,15] The first two of these atoms are the β- and γ-phosphate oxygen atoms, and the third atom is the N(7) nitrogen atom in adenine. The stability of the MA complex in the Cu(II):ATP system is very high, whereas the stability of the MAH complex is very low. This indicates that the ligand can bind strongly to the Cu(II) ion with its three donor atoms. In the Zn(II):ATP system, the stability of the MA complex is the lowest because the three donor atoms of ATP cannot bind strongly. It has been noted that the N(7) nitrogen atom binds to the metal weakly in Zn(II):ATP complexes.[15]

The potentiometric titrations of (1:1) and (1:2) M(II):aa systems have been carried out under the same experimental conditions. In the (1:1) Ni(II):aa and Cu(II):aa systems, $m = 2.0$ has been observed as the inflection point (Figures 2 and 3, curve IV). It is noticed that a MB complex occurs at the $m = 0.0$ to 2.0 buffer zone and between pH 3.0 and 5.0 in the titration curves. The fact that inflection points have been observed in the Zn(II):aa system, at $m = 1.0$ and $m = 2.0$, points out that the ZnB complex occurs gradually (Figure 4, curve IV). In the potentiometric titrations of (1:2) Ni(II):aa and Cu(II):aa systems, only one inflection point was observed at $m = 4.0$ (Figures 2 and 3, curve V). The potentiometric titration curve of the (1:2) Zn(II):aa system showed three inflection points at $m = 2.0$, $m = 3.0$, and $m = 4.0$ (Figure 4, curve V). The titration of four protons in all systems indicates that the MB_2 complex forms gradually. The gradual reactions and stability constants of the MB, MBH, and MB_2 complexes in the M(II):aa systems have been shown with eqs 1 to 6. The stepwise and overall stability constants of these complexes have been listed in Table 2.

$$ML + L \overset{K_{ML_2}}{\rightleftharpoons} ML_2 \qquad K_{ML_2} = \frac{[ML_2]}{[ML][L]} \tag{5}$$

Table 2. Stepwise and Overall Stability Constants ((log K and log β) $\pm$ $\sigma^x)^y$ of Binary Complexes of Ni(II), Cu(II), and Zn(II) with ATP, Aspartic Acid, and Glutamic Acid at Ionic Strength I = 0.1 M KCl and 25 °C (L = ATP, Glu, or Asp)[a]

	ligand	log β_{ML}	log K_{MLH}	log β_{MLH}	log K_{MLH-1}	log K_{ML_2}	log β_{ML_2}
Ni(II)	ATP	5.37 ± 0.02 4.85[12,b]	4.35 ± 0.07 4.32[12,b]	9.72 ± 0.07 9.17[12,b]	−3.28 ± 0.01		
	Asp	7.35 ± 0.03 7.09[30,d]	4.08 ± 0.04	11.43 ± 0.04 12.79[30,d]		5.46 ± 0.05	12.81 ± 0.05 12.66[30,d]
	Glu	6.09 ± 0.04 6.06[30,d]	5.67 ± 0.06	11.76 ± 0.06 12.33[30,d]		4.50 ± 0.04	10.59 ± 0.04 10.33[30,d]
Cu(II)	ATP	6.19 ± 0.03 6.27[11,b]	3.22 ± 0.05	9.41 ± 0.05 10.12[11,b]	1.35 ± 0.05 −1.48[11,b]		
	Asp	8.92 ± 0.02 8.76[19,c] 9.04[30,d]	2.98 ± 0.07	11.90 ± 0.07 12.78[19,c] 12.86[30,d]		6.89 ± 0.06	15.81 ± 0.06 15.35[19,c] 15.86[30,d]
	Glu	8.22 ± 0.04 8.43[30,d]	3.85 ± 0.04	12.07 ± 0.03 12.43[30,d]		6.37 ± 0.07	14.59 ± 0.07 15.00[30,d]
Zn(II)	ATP	4.94 ± 0.05 5.16[8,e]	4.14 ± 0.05 4.17[8,e]	9.08 ± 0.04 9.33[8,e]	3.42 ± 0.06		
	Asp	5.87 ± 0.05 5.9253[31,f]	−	−		4.06 ± 0.02 4.242[31,f]	9.93 ± 0.02 10.167[31,f]
	Glu	4.65 ± 0.03 4.682[31,f]	−	−		4.13 ± 0.06 3.425[31,f]	8.78 ± 0.06 8.107[31,f]

[a] x: standard deviation. y: ± 95 % confidence interval. [b] 25 °C, 0.1 M KNO_3. [c] 20 °C, 0.1 M KNO_3. [d] 25 °C, 0.15 M NaCl. [e] 25 °C, 0.1 M $NaNO_3$. [f] 37 °C, 0.1 M $NaClO_4$.

$$M + 2L \overset{\beta_{ML_2}}{\rightleftharpoons} ML_2 \qquad \beta_{ML_2} = \frac{[ML_2]}{[M][L]^2} \tag{6}$$

It has been observed that the stability sequence of the MB and MB_2 complexes formed by aspartic acid or glutamic acid with metal(II) is Cu(II) > Ni(II) > Zn(II). Aspartic acid or glutamic acid has three donor atoms, being two carboxylate oxygen atoms and one amine nitrogen atom. The high level of the stability of the MB and MB_2 complexes formed by Cu(II) with aspartic acid or glutamic acid indicates that the ligand can strongly bind to the three donor atoms. When comparing the potentiometric titration curves of the Zn(II):aa systems with the titration curve of ligand alone, the pH decrease at the m = 0.0 to 1.0 buffer zone is very low, and the two curves almost overlap (Figure 4, curves II and IV). This indicates that aspartic acid or glutamic acid can weakly bind to Zn(II) with the carboxylate oxygens. When comparing the potentiometric titration curves of the M(II):aa systems alone with the ligand titration curve, the pH decrease at the m = 0.0 to 2.0 buffer zone is very high. This indicates that in the M(II) systems the nitrogen atoms of aspartic acid or glutamic acid and metal(II) can bind stronger to each other.

Apart from this, the stability constants of MB and MB_2 complexes formed by aspartic acid are found to be higher than that of glutamic acid. The ligands of aspartic acid or glutamic acid are dicarboxylate amino acids, and glutamic acid has a longer chain of one $-CH_2$ unit when compared with aspartic acid. As a result of the inductive effect caused by this longer chain, the second and third protonation constants of glutamic acid are much higher than those of aspartic acid, which indicates that its bond with protons is stronger. It is expected that ligands that can form strong bonds with protons also form strong bonds with metal ions. However, the stability constants of binary complexes formed by Ni(II), Cu(II), and Zn(II) ions with glutamic acid have been found to be lower than binary complexes formed with aspartic acid. Since aspartic acid and glutamic acid are three dentate ligands, two chelates occur in binary complexes. Aspartic acid forms a five- and a six-membered chelate ring, whereas glutamic acid forms a five- and a seven-membered chelate ring. The formation of the seven-membered chelate ring in binary complexes causes a weaker bond.

Ternary Complexes. Potentiometric titrations of the (1:1:1) M(II):ATP:aa systems were performed in I = 0.1 M KCl ionic medium and at 25 °C. Two inflection points were observed at m = 3.0 and m = 4.0 in the titration curves of the Ni(II):ATP:aa and Zn(II):ATP:aa systems (Figures 2, 4; curve VI). In the Cu(II):ATP:aa system, one inflection point was observed at m = 4.0 (Figure 3; curve VI). Each of the ATP or aspartic acid (or glutamic acid) ligands has two deprotonable protons, and therefore the mixed ligand solutions including ATP and aspartic acid (or glutamic acid) have four deprotonable protons. The potentiometric titration curves of the M(II):ATP:Asp and M(II):ATP:Glu systems have lower pH regions when compared to the curves of the ligands alone and titrate a total of four protons which indicates that the two ligands bind to the metal ions. It is possible to show the formation reaction and the overall stability constant equation of the MAB ternary complex with eq 7.

$$M^{2+} + A^{4-} + B^{2-} \overset{\beta_{MAB}}{\rightleftharpoons} MAB^{4-}$$

$$\beta_{MAB} = \frac{[MAB^{4-}]}{[M^{2+}][A^{4-}][B^{2-}]} \tag{7}$$

The stability constants of the ternary complexes of the MAB type formed by Ni(II), Cu(II), and Zn(II) ions with ATP and Asp (or Glu) have been calculated (log β_{MAB}) and are shown in Table 3.

In all systems, the mixed ligand interactions result in only the MAB complex as shown in Table 3. The log β_{MAB} values indicating the stability of the MAB ternary complexes do not show which ligand (ATP, Asp, or Glu) binds more strongly with the metal(II) ion. For this reason, to identify which of the ligands are primary or secondary, the log K^{MA}_{MAB} and log K^{MB}_{MAB} constants are calculated with the following equations.

$$\log K^{MA}_{MAB} = \log \beta^{M}_{MAB} - \log K^{M}_{MA} \quad \text{and} \quad \log K^{MB}_{MBA} = \log \beta^{M}_{MBA} - \log K^{M}_{MB} \tag{8}$$

Table 3. Stepwise and Overall Stability Constants ((log K and log β) $\pm$ $\sigma^x)^y$ of Ternary Complexes of Ni(II), Cu(II), and Zn(II) at Ionic Strength I = 0.1 M KCl and 25 °C[a]

metal	ligand A	ligand B	log β_{MAB}	log K^{MA}_{MAB}	log K^{MB}_{MAB}	Δ log K
Ni(II)	ATP	Asp	10.85 ± 0.05	5.48 ± 0.05 8.95[14,b]	3.50 ± 0.05	−1.87
		Glu	9.86 ± 0.02	4.49 ± 0.02 7.90[14,b]	3.77 ± 0.04	−1.60
Cu(II)	ATP	Asp	13.14 ± 0.03	6.95 ± 0.03 9.43[14,b]	4.22 ± 0.03	−1.97
		Glu	12.65 ± 0.07	6.46 ± 0.07 8.89[14,b]	4.43 ± 0.07	−1.76
Zn(II)	ATP	Asp	9.40 ± 0.05	4.46 ± 0.05	3.53 ± 0.05	−1.41
		Glu	8.58 ± 0.07	3.64 ± 0.07	3.93 ± 0.07	−1.01

[a] x: standard deviation. y: ± 95 % confidence interval. [b] 25 °C, 0.1 M KNO_3

The log K^{MA}_{MAB} and log K^{MB}_{MAB} are listed in Table 3. The fact that the log K^{MA}_{MAB} constants were higher in all systems indicates that the ATP ligand is the primary ligand.

The stabilities of the binary and ternary complexes can also be compared in another way. For example, the Δ log K parameter expresses the effect of the bonded primary ligand toward an incoming secondary ligand. The Δ log K parameters have been calculated with the equation below and are listed in Table 3.

$$\Delta \log K = \log K^{MA}_{MAB} - \log K^{M}_{MB} = \log K^{MB}_{MAB} - \log K^{M}_{MA} \quad (9)$$

This reaction represents the following overall equilibrium

$$MA + MB \rightarrow MAB + M \text{ and hence}$$

$$\Delta \log K = \log \beta_{MAB} - (\log \beta_{MA} + \log \beta_{MB}) \quad (10)$$

The Δ log K values were found to be negative for all systems. This result shows that the stability of the ternary complexes is lower than that of the binary complexes.

Boraei et al. found that the stability constants of MAB ternary complexes in Cu(II):ATP:aa and Ni(II):ATP:aa (aa: Asp and Glu) systems were higher than the binary complexes and that the Δ log K parameters were positive.[14] They reported in their article that the dicarboxylic amino acids binded more strongly to negatively loaded M(II):ATP complexes. However, in our study, the stability constants of MAB type ternary complexes for the Cu(II) and Ni(II) ions were found to be lower than the binary complexes. Aspartic and glutamic acids form stable binary complexes with metal(II) ions. However, when these amino acids bind to M(II):ATP complexes as secondary ligands and form ternary complexes, there appears to be steric and electrostatic effects. ATP is a voluminous ligand, and it is more difficult for an amino acid to bind to a M(II):ATP complex than to bind to a M(II) ion. Apart from this, the MA complex formed by the metal(II) with ATP that is shown with formula H_2L^{2-} is negatively loaded. It is difficult for an anionic secondary ligand as aspartic (or glutamic) acid to bind to a negatively loaded MA complex due to electrostatic effects. Due to these two effects, the stabilities of the ternary complexes are lower than the binary complexes.

In this study, the stability sequence of MAB ternary complexes is Cu(II) > Ni(II) > Zn(II), in terms of metal ions. This behavior may be attributed to the nature of the interaction of the metal ions Ni(II), Cu(II), and Zn(II) during their binding to the ATP. In aqueous solutions, macro-chelate formation with N(1) and N(7) nitrogen atoms is not possible due to steric reasons in the mixed ligand complexes of some metal ions.[16] It has been stated that in a Zn(II):ATP complex the N(7) nitrogen atom of the ATP binds weakly to the Zn(II) ion and that during the formation of a mixed ligand complex the N(7) nitrogen−metal bond is broken.[15]

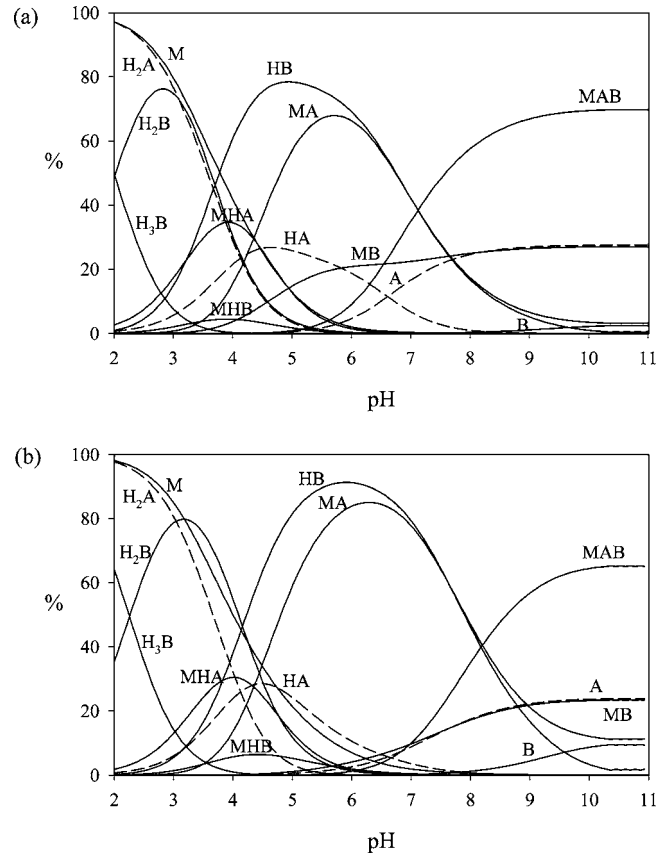


Figure 5. Distribution of species as a function of pH for the systems (a) Ni(II):ATP:Asp in the ratio 1:1:1 and (b) Ni(II):ATP:Glu in the ratio 1:1:1, at 25 °C and 0.1 M KCl (M, Ni(II); A, ATP; B, Asp or Glu).

The stability constants of MAB ternary complexes that form in the M(II):ATP:Asp and M(II):ATP:Glu systems have been found suitable with nature of the secondary ligand as was the case for the binary systems. The stability constants of MAB ternary complexes that formed in the M(II):ATP:Asp systems have been found to be higher that those of glutamic acid.

Distribution Diagrams. The concentration distribution of complex species that occur in all mixed-ligand systems could be obtained by the SPE software. The distribution of the species for the mixed-ligand systems of Ni(II), Cu(II), and Zn(II) is given in Figures 5, 6, and 7. The MA binary complex has a maximum concentration in the acidic region for all systems. The maximum concentration of MAB is in the basic region.

The concentration distribution curves are drawn for two different ternary systems of Ni(II) as can be seen in Figure 5a and 5b. Only the MA complex predominates at pH ca. 5.5 for each of the two systems. At basic pH values, pH 9 and 10, the concentrations of the MAB complex for the Ni(II):ATP:Asp and Ni(II):ATP:Glu systems are ca. 70 % and ca. 64 %, respectively. The concentration of the MB complex is ca. 20 % at these pH values.

In Figure 6a, the concentrations of MA and MB complex species are much the same (ca. 50 %) in the acidic region for the Cu(II):ATP:Asp system. In the ternary system including Glu

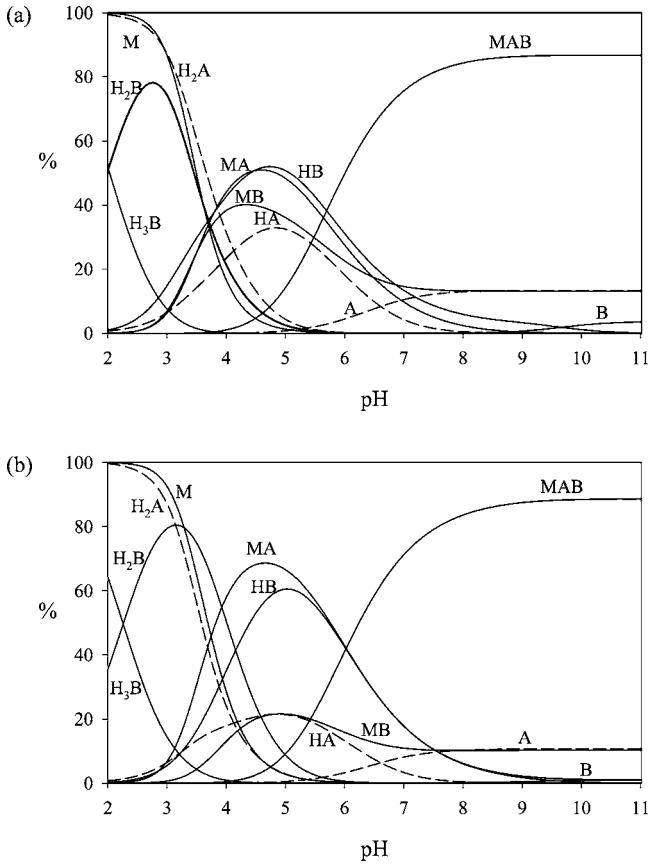


Figure 6. Distribution of species as a function of pH for the systems (a) Cu(II):ATP:Asp in the ratio 1:1:1 and (b) Cu(II):ATP:Glu in the ratio 1:1:1, at 25 °C and 0.1 M KCl (M, Cu(II); A, ATP; B, Asp or Glu).

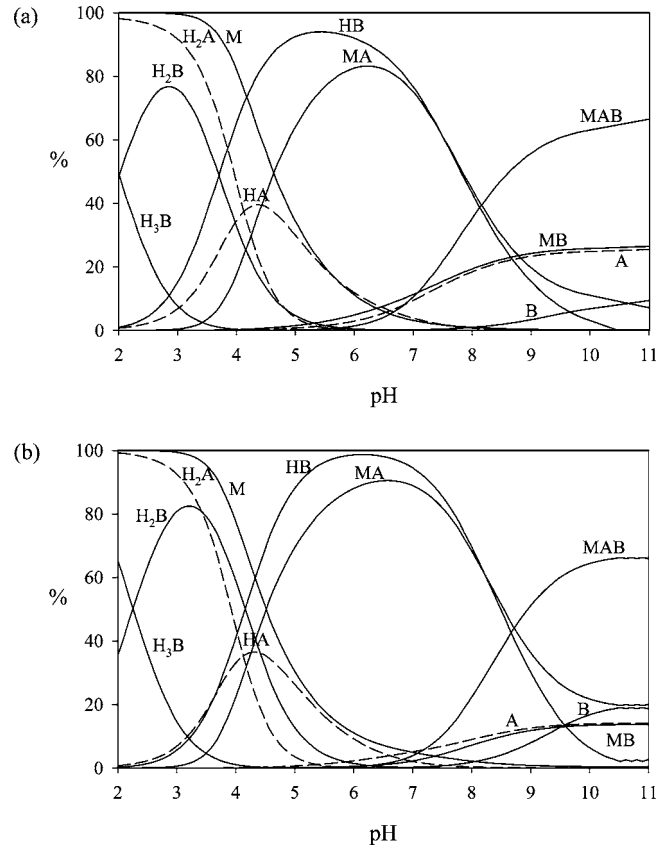


Figure 7. Distribution of species as a function of pH for the systems (a) Zn(II):ATP:Asp in the ratio 1:1:1 and (b) Zn(II):ATP:Glu in the ratio 1:1:1, at 25 °C and 0.1 M KCl (M, Zn(II); A, ATP; B, Asp or Glu).

(Figure 6b), the concentration of MA is ca. 70 % and MB is ca. 20 % at pH ca. 5.0. In the ternary systems of Cu(II), while the MA concentration decreases, the MAB complex starts to form at pH ca. 4.0 and reaches a maximum concentration of ca. 90 % at pH ca. 8.0.

Figure 7a shows that the concentration of the MA complex is high (ca. 90 %) at ca. pH 6.5 in the ternary system of Zn(II). MAB and MB complexes start to form after pH 6.0, and the maximum concentration of MAB is 65 % at ca. pH 10.

In the distribution diagrams of all systems, the MA complex appears at a lower pH region and in a higher rate which indicates that the ATP ligand binds first to the M(II) ion. It is sterically a little bit more difficult for aspartic and glutamic acid to bind to the MA complex that appears after the ATP has been bound. Therefore, in our study, the log K^{MA}_{MAB} values were found to be lower than the log K^{M}_{MB} values.

Conclusion

In the present study, potentiometric equilibrium measurements have been performed at 25 °C and ionic strength $I = 0.1$ M KCl for interaction of biologically important ligands aspartic acid and glutamic acid and Ni(II), Cu(II), and Zn(II) with ATP in a 1:1:1 ratio.

In all mixed ligand systems, it has been found that ternary complexes are only formed in the MAB type and that ATP is the primary ligand, whereas aspartic or glutamic acid is the secondary ligand. In terms of metal ions, the stability sequence of MAB ternary complexes is Cu(II) > Ni(II) > Zn(II), just as is the case in the binary complexes. This sequence has been found to be in agreement with the sequence found by Irving and Williams.[40] Apart from this, the stability of the ternary complexes formed by aspartic acid with M(II) and ATP is higher than those formed by glutamic acid.

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