Complexation Equilibria and Determination of Stability Constants of Binary and Ternary Complexes with Ribonucleotides (AMP, ADP, and ATP) and Salicylhydroxamic Acid as Ligands

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The binary and mixed ligand complexes of some transition and alkaline earth metal(II) ions with adenosine-5′-mono-, -di-, and triphosphate and salicylhydroxamic acid were studied using potentiometric pH titrations. The Irving and Rossotti technique has been adopted to determine the formation constants corresponding to the various complexation equilibria. The acidity constants of the ligands were measured and used for determining the stability constants of the complexes formed in aqueous solutions under the experimental conditions (t = 25 °C, I = 0.1 mol dm^{-3} $NaNO_3$). The formation of 1:1:1 ternary complexes is inferred from the potentiometric titration curves. The order of stability of the binary or ternary complexes in terms of the nature of the nucleotide is investigated and discussed. The values of Δ log K for ternary systems were evaluated and discussed.

Introduction

It is well-known that metal ion complex formations are among the prominent interactions found in nature (Eichhorn, 1973; Sigel, 1973−1982) and that nucleotides play a central role in the metabolism of living cells (Lippard and Berg, 1994; Fraústo da Silva and Williams, 1991). They serve as substrates for the enzyme-catalyzed transfers of nucleotidyl or phosphoryl groups, which depend on the presence of metal(II) ions (Lippard and Berg, 1994; Fraústo da Silva and Williams, 1991; Sigel and Sigel, 1996). Hydroxamic acids are equally important compounds because they exhibit different biological activities as growth factors, antibiotics, antibiotic antagonists, tumor inhibitors, chelating agents, and cell division factors in biological systems (Kehl and Karger, 1982; Neilands, 1974; Powers and Harper, 1986).

Ternary complexes of transition metal(II) ions with the ribonucleotides adenosine 5′-mono-, 5′-di-, and 5′-triphosphates (AMP, ADP, and ATP) and other secondary ligands of biological importance have been investigated potentiometrically by many workers (Azab et al., 1993, 1994, 1995; Chaudhuri and Sigel, 1977). The stabilities of binary and ternary complexes of metal(II) ions involving hydroxamic acids were recently studied in solution using the same technique (Kurzak and Kroczewska, 1993, 1995; Rao, 1992; Das, 1990; Farkas and Kurzak, 1990).

A detailed knowledge of the complex formation ability of salicylhydroxamic acid (SHAM), a new oral iron chelator, with metal ions, often existing in biological fluids, has confirmed its medical importance. Recently, a study of the solution equilibria involved in the formation of binary and ternary complexes of transition metal(II) ions involving SHAM and other ligands, such as *N*-(2-acetamido)iminodiacetic acid (ADA), iminodiacetic acid (IDA), and nitrilotriacetic acid (NTA), was carried out by us (Khairy et al., 1996).

In continuation of our research program to study the complexation equilibria and determination of stability constants of binary and ternary complexes with ligands exhibiting biochemical effects (Khalil et al., 1985, 1994, 1997; Khalil and Attia, 1999; Khalil and Radalla, 1998), the present work concerns the formation and characterization of binary and mixed-ligand complexes of the type M^{II}−nucleotide−SHAM, to determine the stability constants of the complexes formed, as these systems mimic many biological reactions which may involve ribonucleotide−metal(II) ion−drug interactions.

Experimental Section

Materials and Solutions. Adenosine 5′- monophosphoric acid disodium salt ($Na_2AMP·H_2O$), adenosine 5′-diphosphoric acid disodium salt ($Na_2ADP·2H_2O$), and adenosine 5′-triphosphoric acid disodium salt ($Na_2ATP·3H_2O$) were provided by Fluka and used without further purification. A fresh sample was weighed, and a solution was prepared for each titration to exclude loss by hydrolysis or photochemical decomposition. Salicylhydroxamic acid (SHAM) was purchased from Nasr Pharmaceutical Chemicals Co., Egypt. The metal salts were provided by BDH as nitrates. Stock solutions of the metal salts were prepared in bidistilled water, and the metal concentration was obtained by standard analytical methods (Welcher, 1965). Carbonate-free sodium hydroxide (titrant, prepared in 0.1 mol dm^{-3} $NaNO_3$ solution) was prepared by dissolving the Analar pellets in CO_2-free bidistilled water, and the solution was standardized potentiometrically with KH phthalate (Merck AG). A HNO_3 solution (≈0.04 mol dm^{-3}) was prepared and used after standardization. HNO_3, NaOH, and $NaNO_3$ were from Merck p.a.

Apparatus. Potentiometric pH measurements were performed on solutions in a double-walled glass vessel at 25 °C using a Griffin pH J-300-010 G digital pH meter. The temperature was controlled by circulating water through the jacket, from a constant-temperature bath. The cell was equipped with a magnetic stirrer and a tightly fitting rubber stopper, through which an Amel 882 delivery dispenser, readable to 1 μL, and electrode system were inserted. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities. It is to be assumed that the activity coefficient is constant, an

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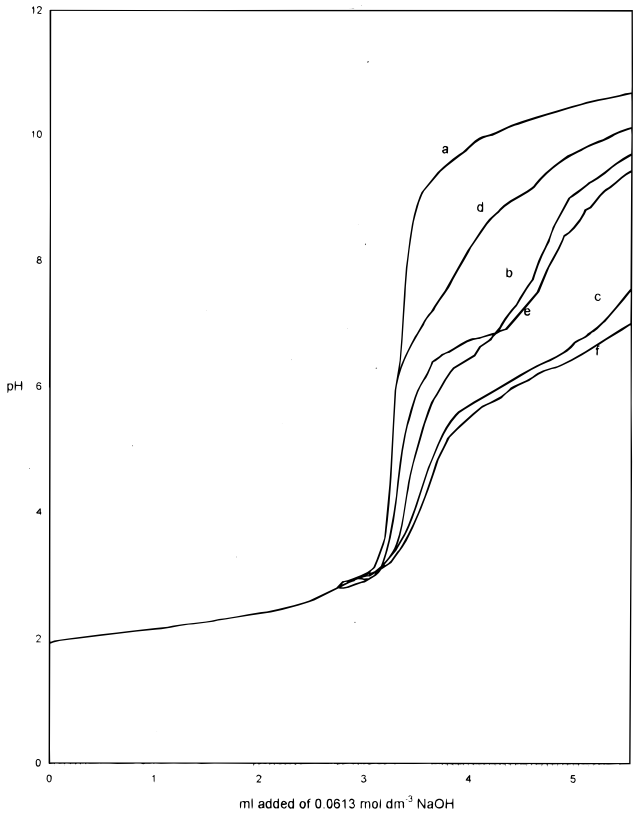

Figure 1. Potentiometric titration curves for the Co(II)−ATP−SHAM system at 25 °C and I = 0.10 mol dm^{-3} $NaNO_3$.

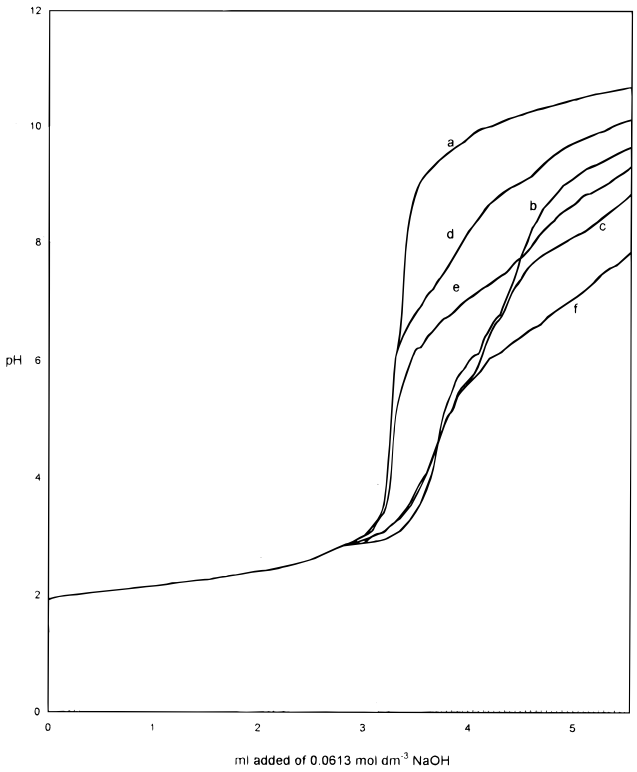

Figure 2. Potentiometric titration curves for the Zn(II)−ADP−SHAM system at 25 °C and I = 0.10 mol dm^{-3} $NaNO_3$.

assumption usually justified by working in a medium of high ionic strength (Ringbom, 1963). The electrode system was calibrated by periodic titrations of HNO_3 (or NaOH) solution (0.1 mol dm^{-3} in $NaNO_3$) with standard NaOH (or HNO_3) solution. The resulting titration data were used to calculate the standard electrode potential $E°$ and the dissociation constant of water. These values were then used in the calculation of hydrogen ion concentration from potential readings. Thus, all constants determined in this work are concentration constants.

Procedure. The following solutions were prepared (total volume 25 cm^3) and titrated potentiometrically against standard carbonate-free NaOH (0.0613 mol dm^{-3}) solution:
(a) HNO_3 (0.0082 mol dm^{-3}) + $NaNO_3$ (0.10 mol dm^{-3})
(b) solution a + (0.001 mol dm^{-3}) nucleotide
(c) solution b + (0.001 mol dm^{-3}) metal ion
(d) solution a + (0.001 mol dm^{-3}) SHAM
(e) solution d + (0.001 mol dm^{-3}) metal ion
(f) solution a + (0.001 mol dm^{-3}) metal ion + (0.001 mol dm^{-3}) nucleotide + (0.001 mol dm^{-3}) SHAM

Each of the above solutions was thermostated at 25 °C with an accuracy of ±0.1 °C, where the solutions were left to stand for about 15 min before titration. The equations of Irving and Rossotti (1953, 1954) were used to determine the protonation constants of the ligands and the formation constants of the metal binary and ternary complexes. Multiple titrations have been performed for each system.

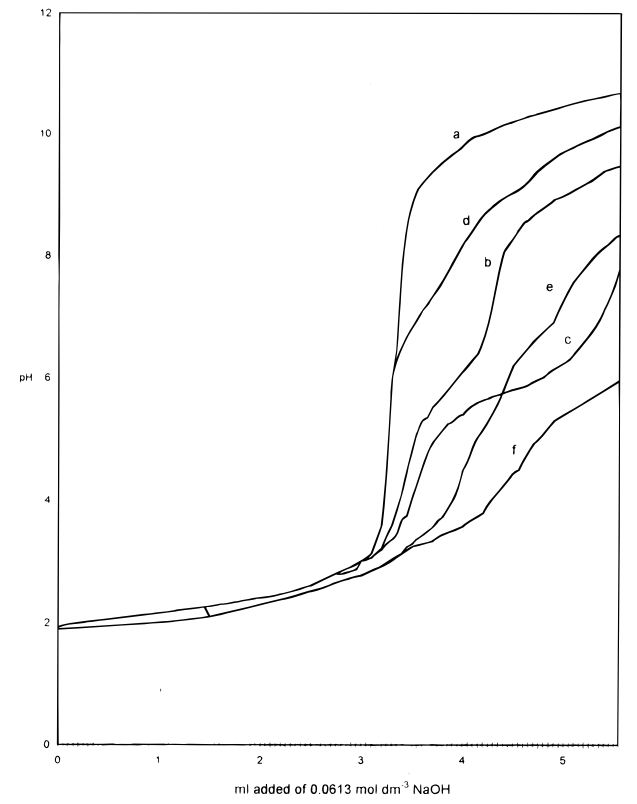

Figure 3. Potentiometric titration curves for the Cu(II)−AMP−SHAM system at 25 °C and I = 0.10 mol dm^{-3} $NaNO_3$.

Results and Discussion

Figures 1−3 display representative sets of experimental titration curves obtained according to the sequence mentioned in the Experimental Section, for Co^{2+}−ATP−SHAM, Zn^{2+}−ADP−SHAM, and Cu^{2+}−AMP−SHAM, respectively. The first and second proton association constants of the nucleotides studied (AMP, ADP, or ATP) and SHAM have been determined under identical conditions from titration curves a and b for nucleotides and a and d for SHAM.

In an excellent review, Izatt et al. (1971) have surveyed the literature dealing with proton and metal ion interaction with ribonucleic acid, deoxyribonucleic acid, and their constituent bases, nucleosides, and nucleotides. It is con-

Table 1. Acidity Constants[a] of Salicylhydroxamic Acid and Stability Constants of 1:1 Binary Complexes at 25 °C and I = 0.1 mol dm^{-3} $NaNO_3$

	Cu(II)	Co(II)	Ni(II)	Zn(II)	Mn(II)	Cd(II)	Mg(II)	Ca(II)	Sr(II)	Ba(II)
$\log K^{M}_{M(SHAM)}$	13.10 ± 0.05	6.62 ± 0.04	6.07 ± 0.07	5.92 ± 0.08	4.60 ± 0.05	4.90 ± 0.05	3.39 ± 0.05	3.24 ± 0.04	3.12 ± 0.02	3.20 ± 0.04

[a] $\log K^{H}_{1} = 9.68 \pm 0.03$ and $\log K^{H}_{2} = 7.34 \pm 0.06$.

Table 2. Acidity Constants of Adenosine-5′-monophosphate (AMP) and Stability Constants of 1:1 Binary Complexes and 1:1:1 Ternary Complexes with Salicylhydroxamic Acid at 25 °C and I = 0.1 mol dm^{-3} $NaNO_3$[a]

cation	$\log K^{H}_{1}$	$\log K^{H}_{2}$	$\log K^{M}_{M(AMP)}$	$\log K^{M(AMP)}_{M(AMP)(SHAM)}$	$\log \beta^{M}_{M(AMP)(SHAM)}$	$\Delta \log K$
H	6.20 ± 0.04	3.75 ± 0.06				
Cu			3.19 ± 0.04			11.11
Co			2.57 ± 0.07	2.64 ± 0.06	5.21	−3.98
Ni			2.90 ± 0.02	2.98 ± 0.04	5.88	−3.09
Zn			2.79 ± 0.06	2.86 ± 0.07	5.65	−3.06
Mn			2.40 ± 0.06	2.48 ± 0.08	4.88	−2.12
Cd			2.65 ± 0.08	2.73 ± 0.08	5.18	−2.17
Mg			1.97[b]			
Ca			1.85[b]			
Sr			1.79[b]			
Ba			1.73[b]			

[a] $\log K^{Cu(SHAM)}_{Cu(SHAM)(AMP)} = 14.30 \pm 0.05$ and $\log \beta^{Cu}_{Cu(SHAM)(AMP)} = 27.40$. [b] Sillén and Martell, 1971.

Table 3. Acidity Constants of Adenosine-5′-diphosphate (ADP) and Stability Constants of 1:1 Binary Complexes and 1:1:1 Ternary Complexes with Salicylhydroxamic Acid at 25 °C and I = 0.1 mol dm^{-3} $NaNO_3$[a]

cation	$\log K^{H}_{1}$	$\log K^{H}_{2}$	$\log K^{M}_{M(ADP)}$	$\log K^{M(ADP)}_{M(ADP)(SHAM)}$	$\log \beta^{M}_{M(ADP)(SHAM)}$	$\Delta \log K$
H	6.46 ± 0.03	3.90 ± 0.05				
Cu			5.85 ± 0.06			9.25
Co			4.10 ± 0.08	4.22 ± 0.02	8.32	−2.40
Ni			4.40 ± 0.03	4.50 ± 0.02	8.90	−1.57
Zn			4.18 ± 0.02	4.35 ± 0.03	8.53	−1.57
Mn			4.00 ± 0.04	4.10 ± 0.05	8.10	−0.50
Cd			3.90 ± 0.05	4.02 ± 0.07	7.92	−0.88
Mg			3.24 ± 0.05	3.43 ± 0.08	6.67	0.04
Ca			2.86 ± 0.07	3.06 ± 0.05	5.92	−0.18
Sr			2.54 ± 0.08	3.17 ± 0.04	5.71	0.05
Ba			2.36 ± 0.08	3.26 ± 0.07	5.62	0.06

[a] $\log K^{Cu(SHAM)}_{Cu(SHAM)(ADP)} = 15.10 \pm 0.03$ and $\log \beta^{Cu}_{Cu(SHAM)(ADP)} = 28.20$.

Table 4. Acidity Constants of Adenosine-5′-triphosphate (ATP) and Stability Constants of 1:1 Binary Complexes and 1:1:1 Ternary Complexes with Salicylhydroxamic Acid at 25 °C and I = 0.1 mol dm^{-3} $NaNO_3$[a]

cation	$\log K^{H}_{1}$	$\log K^{H}_{2}$	$\log K^{M}_{M(ATP)}$	$\log K^{M(ATP)}_{M(ATP)(SHAM)}$	$\log \beta^{M}_{M(ATP)(SHAM)}$	$\Delta \log K$
H	6.50 ± 0.07	4.25 ± 0.05				
Cu			6.10 ± 0.02			9.40
Co			4.65 ± 0.02	6.89 ± 0.04	11.54	0.27
Ni			5.00 ± 0.05	6.39 ± 0.06	11.39	0.32
Zn			4.90 ± 0.04	6.19 ± 0.07	11.09	0.27
Mn			4.80 ± 0.07	4.89 ± 0.08	9.69	0.29
Cd			4.55 ± 0.08	5.10 ± 0.05	9.65	0.20
Mg			4.30 ± 0.06	3.49 ± 0.07	7.79	0.10
Ca			4.05 ± 0.05	3.35 ± 0.07	7.40	0.11
Sr			3.92 ± 0.05	3.20 ± 0.04	7.12	0.08
Ba			3.70 ± 0.05	3.30 ± 0.05	7.00	0.10

[a] $\log K^{Cu(SHAM)}_{Cu(SHAM)(ATP)} = 15.50 \pm 0.07$ and $\log \beta^{Cu}_{Cu(SHAM)(ATP)} = 28.60$.

cluded that the N_1H^+ group is the site of proton ionization ($pK_a \approx 4$) in adenine and adenosine and presumably in the ribonucleotide derivatives AMP, ADP, and ATP as well. The second proton ionization constant for the three nucleotides studied was attributed to the phosphate groups.

Salicylhydroxamic acid (SHAM) contains two hydroxyl groups, so that the potentiometric titration curve (d) shows two buffer regions. The first one extends up to the neutralization of the hydroxamic proton, and the second region is most likely associated with the neutralization of the phenolic hydroxyl group of SHAM. The stepwise protonation constants amount to $\log K^{H} = 7.34$ for the hydroxamic hydroxyl group and $\log K^{H} = 9.68$ for the phenolic group. To verify the nature of the donor groups, it is helpful to invoke the protonation constant of the benzohydroxamic acid ($\log K^{H} = 8.60$) analogue, where the protonation center is the hydroxamic OH group. The protonation constant of the hydroxamic hydroxyl group of benzohydroxamic acid is presumably comparable with that of SHAM, amounting to 7.34. The difference between the pK^{H}'s of SHAM and the selected model compound, namely benzohydroxamic acid, is apparently due to different structural parameters.

Examination of the different titration curves obtained for M(II)−nucleotide solutions reveals that the complexation equilibria involved occur in a stepwise manner. Generally, M(II)−nucleotide binary complexes begin to form at pH values lower than that of free nucleotide. This

is attained from the divergence of each of the 1:1 binary M(II)−nucleotide titration curves c from that of the corresponding free nucleotide solution, curve b. The complex solutions of such binary systems do not show any precipitation due to hydrolysis up to higher pH's, where nearly complete complex formation takes place. This behavior strongly suggests that the nucleotides are characterized by a high tendency to form stable metal complexes in solution. With respect to the titration curves of the different M(II)−SHAM complexes, it is evident that these complexes begin to form at pH 2.5−6.0.

The existence of a ternary complex is proved by comparison of the mixed ligand titration curve (f) with the composite curve, obtained by graphical addition of the secondary ligand titration data to that of the 1:1 M(II)−primary ligand titration curve. Therefore, it is assumed that, in the presence of both ligands, nucleotide is ligated to the metal ion followed by ligation of SHAM; that is, the ternary complex formation could be considered in stepwise complexation equilibria (eqs 1 and 2).

$$\mathrm{M} + \mathrm{NU} \rightleftharpoons \mathrm{M(NU)} \tag{1}$$

$$\mathrm{M(NU)} + \mathrm{SHAM} \rightleftharpoons \mathrm{M(NU)(SHAM)} \tag{2}$$

$$K^{\mathrm{M(NU)}}_{\mathrm{M(NU)(SHAM)}} = \frac{[\mathrm{M(NU)(SHAM)}]}{[\mathrm{M(NU)}][\mathrm{SHAM}]} \tag{3}$$

It is observed, from the titration curves representing Cu(II)−nucleotide−SHAM systems, that the complexation equilibria are as follows:

$$\mathrm{Cu} + \mathrm{SHAM} \rightleftharpoons \mathrm{Cu(SHAM)} \tag{4}$$

$$\mathrm{Cu(SHAM)} + \mathrm{NU} \rightleftharpoons \mathrm{Cu(SHAM)(NU)} \tag{5}$$

$$K^{\mathrm{Cu(SHAM)}}_{\mathrm{Cu(SHAM)(NU)}} = \frac{[\mathrm{Cu(SHAM)(NU)}]}{[\mathrm{Cu(SHAM)}][\mathrm{NU}]} \tag{6}$$

This behavior reveals that, in the presence of the two ligands, SHAM interacts first with Cu(II), forming a highly stable binary complex (log $K^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)(SHAM)}} = 13.10$), and then interacts with nucleotide, forming a ternary Cu(II) complex.

The mean values of the acidity constants of the ligands studied and the formation constants of their binary and ternary complexes are determined from the corresponding experimental formation curves using the average value and straight line methods. The values obtained along with the estimated error using least-squares refinement are given in Tables 1−4.

By following the Irving−Rossotti technique (1953, 1954), the protonation constants of salicylhydroxamic acid (SHAM) and the stability constants of its normal 1:1 binary complexes with some transition and alkaline earth metal(II) ions were determined (Table 1) for comparison with those of ternary systems. In the binary systems, the stabilities decrease in the order Cu(II) ≫ Co(II) > Ni(II) > Zn(II) > Cd(II) > Mn(II) > Mg(II) > Ca(II) > Ba(II) > Sr(II).

The acidity constants of the nucleotides studied (AMP, ADP, and ATP) and the stability constants of their M(II) complexes were determined from the titration curves, and the results were in good agreement with those reported in the literature (Sillén and Martell, 1971).

The low stabilities of the 1:1 alkaline earth metal ion complexes with AMP made the determination of stability constants, under the experimental conditions, very inaccurate.

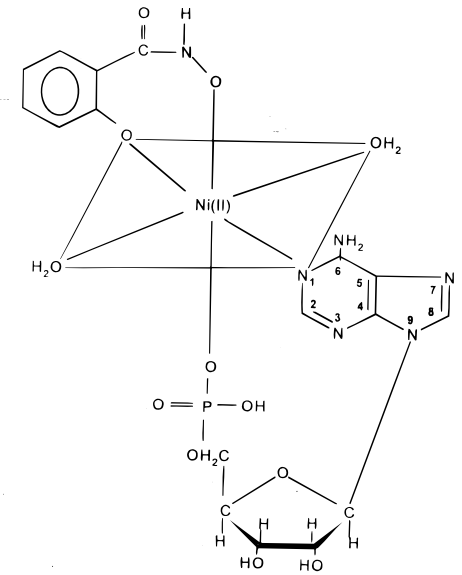

Figure 4. Tentative structure of the Ni(II)−AMP−SHAM ternary complex.

Careful consideration of all the data in Tables 2−4 reveals that the stability constants of both 1:1 binary complexes of the nucleotides studied and 1:1:1 ternary complexes involving SHAM increase in the order AMP < ADP < ATP. This behavior indicates that the phosphate moieties of the ribonucleotides are favored rather than the base as the primary metal(II) binding site. Thus, the metal(II) bound to the base moiety may promote intramolecular base−phosphate interaction. Therefore, the ternary systems investigated may be considered as relatively simple models from which information may be gained concerning the properties of ribonucleotides and their base moieties regarding the strength of their interactions with the biologically relevant salicylhydroxamic acid (SHAM). Even insight into the factors which influence the strength are thus becoming available as these systems mimic substrate−metal(II)−drug interactions.

The relative stability of the mixed-ligand complexes, as compared to that of the corresponding binary systems involving secondary ligands, can be quantitatively expressed (Martin and Prados, 1974) in terms of Δ log K. Tables 2−4 demonstrate the difference in stabilities of the binary and ternary complexes in terms of Δ log K, as defined by eq 7.

$$\Delta \log K = \log K^{\mathrm{M(NU)}}_{\mathrm{M(NU)(SHAM)}} - \log K^{\mathrm{M}}_{\mathrm{M(SHAM)}}$$

$$= \log K^{\mathrm{Cu(SHAM)}}_{\mathrm{Cu(SHAM)(NU)}} - \log K^{\mathrm{Cu}}_{\mathrm{Cu(NU)}} \tag{7}$$

The second form of eq 7 is used in the case of Cu(II) ternary systems, where SHAM acts as a primary ligand and the nucleotide as a secondary ligand, as mentioned previously in this work.

It can be observed (Table 2) that the values of Δ log K for AMP systems are negative, in accordance with statistical, steric, and electrostatic factors (Sigel, 1973) which result in a lower stability constant for the ternary AMP complexes as compared with those for the binary SHAM systems. The Δ log K values for ATP systems are found to be invariably positive (Table 4). This means that the ATP ternary systems are more stable than the binary complexes of SHAM. The higher stability constants of mixed ligand complexes compared with those of binary systems can be

ascribed to interligand interactions or to some cooperativity between the ligands, possibly hydrogen bond formation.

On the basis of the foregoing discussion, Figure 4 shows a representative structure of the Ni(II)−AMP−SHAM ternary complex formed in solution under the experimental conditions.

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