Solution Equilibria and Stabilities of Binary and Ternary Complexes with *N*-(2-Acetamido)iminodiacetic Acid and Ribonucleotides (AMP, ADP, and ATP)

Mohamed M. Khalil

Department of Chemistry, Faculty of Science, Cairo University, Beni-Suef, Egypt

Solution equilibria of binary and ternary complexes involving some transition metal (II) ions with *N*-(2-acetamido)iminodiacetic acid as a primary ligand and the biologically relevant ribonucleotides (adenosine-5′- mono-, -di-, or triphosphate) as secondary ligands were studied using a potentiometric technique. The protonation constants of the ligands were determined and used for determining the stability constants of the complexes formed in aqueous solutions at 25 °C and 0.10 mol dm^{-3} ($NaNO_3$) ionic strength. The formation of 1:1:1 mixed ligand complexes is inferred from the potentiometric titration curves. The order of stability of the binary or ternary complexes in terms of the nature of both the metal ion and the nucleotide is investigated and discussed. Moreover, the complexation behavior of ternary complexes was ascertained using conductivity measurements.

Introduction

The formation of metal ion complexes is among the prominent interactions found in nature,[1,2] and the ribonucleotides adenosine 5′-mono-, 5′-di-, and 5′-triphosphates play a central role in the metabolism of living cells.[3,4] They are important substrates for the enzyme-catalyzed transfers of nucleotidyl or phosphoryl groups—reactions which depend on the presence of metal (II) ions.[3−5] Iminodiacetic acid, having as a peptide group *N*-(2-acetamido)iminodiacetic acid, prepared by Good,[6] is considered as an important zwitterionic amino acid buffer, since it shows significant advantages over conventional buffers, such as (a) maximum buffer capacity at the physiological pH range 6−8.5, (b) no enzyme substrate or enzyme inhibitor properties, and (c) insignificant penetration through a biological membrane.

Stabilities of transition metal (II) ions ternary complexes involving one of the ribonucleotides mentioned above and in the presence of other biologically relevant ligands such as 2,2′-bipyridyl,[7] polybasic oxygen acids,[8,9] and zwitterionic buffers[10] have been evaluated using potentiometric pH-titrations. Solution studies of the ternary complexes involving *N*-(2-acetamido)iminodiacetic acid are scarce.[11,12]

In continuation of our published work oriented toward the study of complexation equilibria and the determination of stability constants of binary and ternary complexes of biological importance,[13−18] the present work concerns a study of the solution equilibria involved in the formation of binary and ternary complexes of some transition metal (II) ions containing *N*-(2-acetamido)iminodiacetic acid and ribonucleotides using potentiometric and conductometric techniques.

Experimental Section

Materials and Solutions. *N*-(2-Acetamido)iminodiacetic acid (H_2ADA) of analytical reagent grade (BDH) was used without further purification. Due to its low solubility in a pure aqueous medium, the monosodium salt was prepared by titration of H_2ADA with a standard sodium hydroxide solution. Chromatographically pure adenosine-5′-mono-, -5′-di-, and -5′-triphosphoric acid disodium salts (AMP, ADP, and ATP) were purchased from Fluka. A fresh sample was weighed, and a solution was prepared for each titration to exclude loss by hydrolysis or photochemical decomposition. The metal salts were provided by BDH as nitrates. All solutions were prepared in bidistilled water. The metal ion solutions were standardized by EDTA using suitable indicators.[19] Sodium hydroxide (titrant, prepared in 0.10 mol dm^{-3} $NaNO_3$ solution) was prepared by dissolving the Analar pellets in bidistilled water, and the solution was standardized potentiometrically with potassium hydrogen phthalate (Merck AG). A nitric acid solution (≈0.04 mol dm^{-3}) was prepared and used after standardization. The acid, base, and $NaNO_3$ were from Merck p.a.

Apparatus. Potentiometric pH titrations were performed on solutions in a double-walled glass vessel at (25 ± 0.1) °C using a Griffin pHJ-300-010 G digital pH meter. The temperature was controlled by circulating water from a constant-temperature bath through the jacket. 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 an 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 assumption usually justified by working in a medium of high ionic strength.[20] The electrode system was calibrated by periodic titrations of HNO_3 (or NaOH) solution (0.1 mol dm^{-3} in $NaNO_3$) with a standard NaOH (or HNO_3) solution. Thus, all constants determined in the present work are concentration constants.

Conductometric titrations were followed with a SUNTEX conductivity meter SC-170.

Procedure. The following solutions were prepared (total volume 25 cm^3) and titrated potentiometrically against a standard 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}) ADA; (c) solution b + (0.001 mol dm^{-3})

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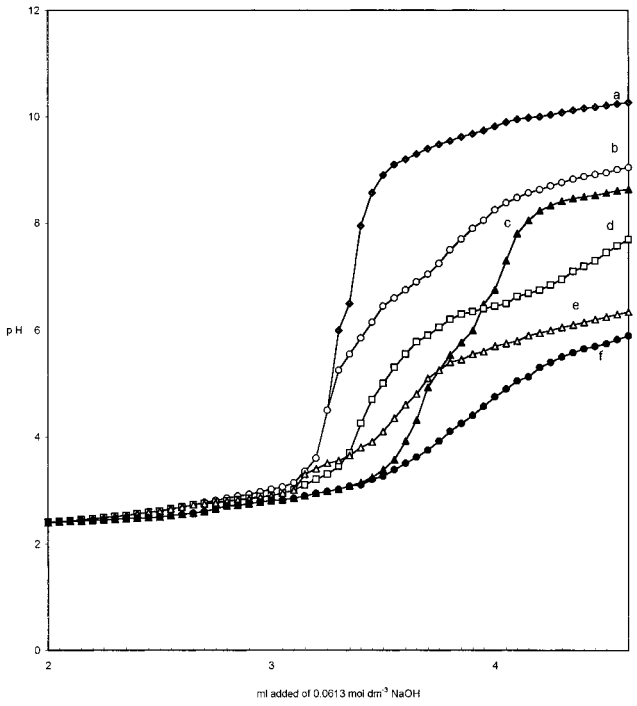

Figure 1. Potentiometric titration curves for the Co^{II}−ADA−ATP system at 25 °C and I = 0.1 mol dm^{-3} $NaNO_3$: (a) 0.0082 mol dm^{-3} HNO_3; (b) solution a + 0.001 mol dm^{-3} ADA; (c) solution b + 0.001 mol dm^{-3} Co(II); (d) solution a + 0.001 mol dm^{-3} ATP; (e) solution d + 0.001 mol dm^{-3} Co(II); (f) solution e + 0.001 mol dm^{-3} ADA.

metal ion; (d) solution a + (0.001 mol dm^{-3}) nucleotide; (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}) ADA + (0.001 mol dm^{-3}) nucleotide.

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[21,22] were used for determination of 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.

The following mixture (g) was titrated conductometrically against a 0.10 mol dm^{-3} NaOH solution: 0.01 mol dm^{-3} metal ion (10 cm^3) + 0.01 mol dm^{-3} ADA (10 cm^3) + 0.01 mol dm^{-3} nucleotide (10 cm^3). The ADA solution was prepared, for conductometric titrations, in an aqueous equimolar HNO_3 solution.

Results and Discussion

Representative potentiometric titration curves obtained according to the sequence described in the experimental part are shown in Figure 1, for the Co^{2+}−ADA−ATP system. The titration curves show a narrow but conspicuous buffer region between pH 6 and 6.5. This buffer region is an indication that the sodium hydroxide solution used was contaminated with carbonate. From the width of the buffer region, the total concentration of carbonate can be estimated to about 1.5 × 10^{-4} mol dm^{-3}.

The protonation constants of the ligands studied have been determined under identical conditions from titration curves a and b for ADA and a and d for the nucleotide, and the results were in a good agreement with the corresponding literature values.[10,23]

The constructed titration curves clearly reveal that the different binary ADA complexes are formed at lower pH (∼2.5). This is attained from the appeared divergence of the 1:1 binary complex titration curve (c) from that of the corresponding free ADA 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 indicates that the ligand ADA is characterized by a high tendency to form stable metal complexes in solution. Concerning the titration curves of M(II)−nucleotide systems, it is evident that these complexes begin to form at a pH of ∼3.5, where the M(II)−nucleotide titration curve (e) diverges from the nucleotide curve (d), denoting the formation of quite stable binary complexes.

Table 1. Proton Ligand Association Constant[a] of *N*-(2-Acetamido)iminodiacetic Acid (ADA) and Stability Constants of Its Binary Complexes at (25 ± 0.1) °C and I = 0.1 mol dm^{-3} $NaNO_3$

metal	Cu(II)	Co(II)	Ni(II)	Zn(II)
log $K^{M}_{M(ADA)}$	7.65 ± 0.08	6.90 ± 0.06	7.15 ± 0.02	7.50 ± 0.03

[a] log K^{H}_{1} = 6.50 ± 0.02.

The formation of a ternary complex is ascertained by comparison of the mixed-ligand titration curve with the composite curve obtained by graphical addition of the nucleotide titration data to that of the (1:l) M(II)−ADA titration curve. The mixed-ligand system was found to deviate considerably from the resultant composite curve, indicating the formation of a ternary complex (cf. Figure 1). Therefore, it is assumed that, in the presence of both ligands, ADA interacts first with the metal ion, followed by interaction of the nucleotide; that is, the ternary complex formation could be considered in stepwise complexation equilibria (eqs 1 and 2).

$$M + ADA \rightleftharpoons M(ADA) \tag{1}$$

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

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

The overall stability constant $\beta^{M}_{M(ADA)(NU)}$ may be represented by eq 4.

$$M + ADA + NU \rightleftharpoons M(ADA)(NU) \tag{4}$$

$$\beta^{M}_{M(ADA)(NU)} = \frac{[M(ADA)(NU)]}{[M][ADA][NU]} = K^{M(ADA)}_{M(ADA)(NU)} K^{M}_{M(ADA)} \tag{5}$$

The mean log K^{H}, log $K^{M}_{M(ADA)}$, log $K^{M}_{M(NU)}$, and log $K^{M(ADA)}_{M(ADA)(NU)}$ values 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 the least-squares method are given in Tables 1−4.

Careful consideration of all presented data reveals that the stability constants of binary and ternary metal (II) complexes with the ligands studied follow the order Co(II) < Ni(II) < Cu(II) > Zn(II), which is in accordance with Irving−William's order.[24] Also, the stability constants of both 1:1 binary complexes of AMP, ADP, or ATP and ternary complexes involving ADA at a molar ratio 1:1:1 were found to lie in the sequence ATP > ADP > AMP. 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−

Table 2. Proton Ligand Association Constants of Adenosine-5′-monophosphate (AMP) and Stability Constants of Its 1:1 Binary Complexes and 1:1:1 Ternary Complexes with *N*-(2-Acetamido)iminodiacetic Acid (ADA) at (25 ± 0.1) °C and I = 0.1 mol dm^{-3} $NaNO_3$

cation	$\log K_1^H$	$\log K_2^H$	$\log K^M_{M(AMP)}$	$\log K^{M(ADA)}_{M(ADA)(AMP)}$	$\log \beta^M_{M(ADA)(AMP)}$	$\Delta \log K$
H	6.20 ± 0.04	3.75 ± 0.06				
Cu			3.19 ± 0.04	3.59 ± 0.06	11.24	0.40
Co			2.57 ± 0.07	2.82 ± 0.09	9.72	0.25
Ni			2.90 ± 0.02	3.20 ± 0.06	10.35	0.30
Zn			2.79 ± 0.06	2.94 ± 0.02	10.44	0.15

Table 3. Proton Ligand Association Constants of Adenosine-5′-Diphosphate (ADP) and Stability Constants of Its 1:1 Binary Complexes and 1:1:1 Ternary Complexes with *N*-(2-Acetamido)iminodiacetic Acid (ADA) at (25 ± 0.1) °C and I = 0.1 mol dm^{-3} $NaNO_3$

cation	$\log K_1^H$	$\log K_2^H$	$\log K^M_{M(ADP)}$	$\log K^{M(ADA)}_{M(ADA)(ADP)}$	$\log \beta^M_{M(ADA)(ADP)}$	$\Delta \log K$
H	6.46 ± 0.03	3.90 ± 0.05				
Cu			5.85 ± 0.06	6.40 ± 0.02	14.05	0.55
Co			4.10 ± 0.08	4.42 ± 0.05	11.32	0.32
Ni			4.40 ± 0.03	4.84 ± 0.07	11.99	0.44
Zn			4.18 ± 0.02	4.38 ± 0.05	11.88	0.20

Table 4. Proton Ligand Association Constants of Adenosine-5′-Triphosphate (ATP) and Stability Constants of Its 1:1 Binary Complexes and 1:1:1 Ternary Complexes with *N*-(2-Acetamido)iminodiacetic Acid (ADA) at (25 ± 0.1) °C and I = 0.1 mol dm^{-3} $NaNO_3$

cation	$\log K_1^H$	$\log K_2^H$	$\log K^M_{M(ATP)}$	$\log K^{M(ADA)}_{M(ADA)(ATP)}$	$\log \beta^M_{M(ADA)(ATP)}$	$\Delta \log K$
H	6.50 ± 0.07	4.25 ± 0.05				
Cu			4.65 ± 0.02	6.80 ± 0.07	14.45	0.70
Co			5.00 ± 0.02	5.08 ± 0.05	11.98	0.43
Ni			4.90 ± 0.05	5.58 ± 0.09	12.73	0.58
Zn			4.90 ± 0.04	5.26 ± 0.02	12.76	0.36

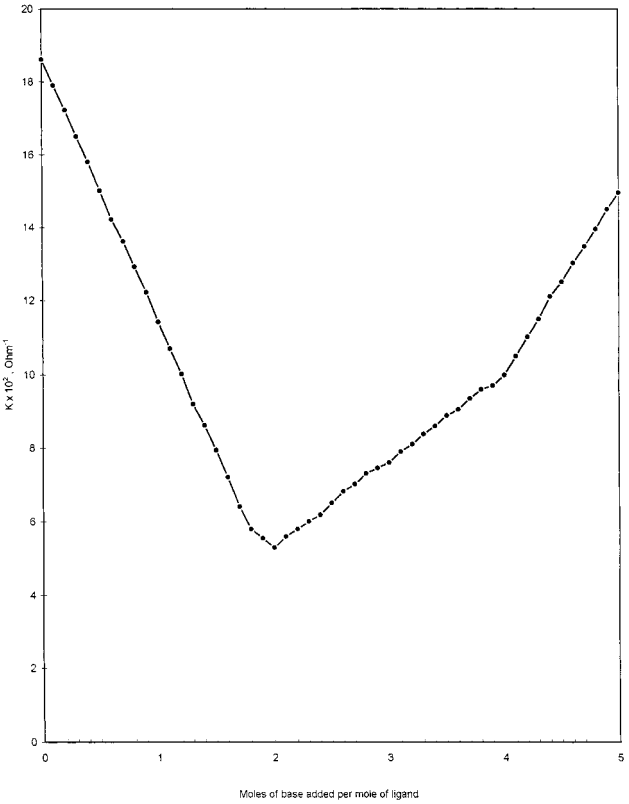

Figure 2. Conductometric titration curve for the Cu^{II}−ADA−ATP system.

phosphate interaction. Therefore, the ternary systems studied 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 ADA. Even insight into the factors which influence the strength are thus becoming available as these systems mimic substrate−metal (II)−buffer interactions.

Let us now consider the ability of metal (II) ions to form ternary complexes with nucleotides. This tendency may be appreciated by examining the specific increments of stability which account for the formation of mixed ligand species relative to the corresponding parent ones. The calculation of these increments is possible using eq 6.[25]

$$\Delta \log K = \log K^{M(ADA)}_{M(ADA)(NU)} - \log K^{M}_{M(NU)} \qquad (6)$$

Thus, M−ADA−NU systems uniformly show positive $\Delta \log K$ values in all cases, which means that the nucleotide ternary complexes are more stable than the binary complexes of ADA. The higher stability constants of mixed ligand complexes compared with those of the binary systems may be ascribed to the interligand interactions or to some cooperativity between the ligands, such as hydrogen bond formation.

The conductometric titration curve for the ternary complex of Cu(II) with ADA and ATP (Figure 2) shows an initial decrease and an inflection at $a = 2$. This probably corresponds to the neutralization of protons originating from the formation of the Cu(II)−ADA complex as well as from the HNO_3 used for the dissolution of ADA. In the $4 \geq a \geq 2$ range, the conductance increases slightly due to the formation of a ternary complex associated with the release of two protons from ATP. Beyond $a = 4$, the conductance increases appreciably due to the presence of an excess of sodium hydroxide.

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