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Complex formation equilibria of binary and ternary complexes involving 3,3-bis(1-methylimidazol-2yl)propionic acid and bio-relevant ligands as 1-aminocyclopropane carboxylic acid with reference to plant hormone

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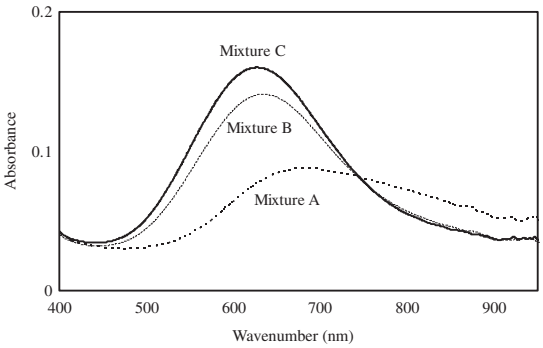
HIGHLIGHTS

- The Cu(II) complex involving bio-relevant ligands was investigated.
- The manuscript is in the domain of SAA as it deals with spectral and potentiometric study.
- The activation of amino acid ester hydrolysis was studied.
- The results is expected to contribute to the chemistry of plant hormone.

GRAPHICAL ABSTRACT



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ABSTRACT

The formation equilibria for the binary complexes of Cu(II) with 1-aminocyclopropane carboxylic acid (ACC) and 3,3-bis(1-methylimidazol-2-yl)propionic acid (BIMP) were investigated. ACC and BIMP form the complexes 110, 120 and 11−1. The ternary complexes of Cu(II) with BIMP and biorelevant ligands as some selected amino acids, peptides and DNA constituents are formed in a stepwise mechanism. The stability constants of the complexes formed were determined and their distribution diagrams were evaluated. The kinetics of hydrolysis of glycine methyl ester in presence of $[Cu(BIMP)]^+$ was investigated by pH-stat technique and the mechanism was discussed.

Introduction

The gaseous plant hormone ethylene, regulates many processes of plant development and defense such as pigmentation, fruit ripening, and senescence [1]. It is directly biosynthesized from 1-aminocyclopropane carboxylic acid (ACC) [2,3], a metabolite of methionine. The last step of ethylene biosynthesis is catalysed by ACC oxidase (ACCO). ACCO is known as the ethylene-forming enzyme. The crystallographic structure of ACCO from *Petunia hybrida* that was recently solved reveals the active site contains a metal ion linked with a side chain of two imidazole and one carboxylate groups, and a member of metal complex involving nitrogenous ligands having imidazole and carboxylate groups as enzyme active sites [4].

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Parallel to the discovery of ACC to be the direct precursor of the plant hormone ethylene, it was shown that 1-aminocyclopropane carboxylic acid and numerous derivatives thereof exhibit herbicidal activity and influence plant growth, caused by intervention in the metabolism [5]. In addition, very small amounts of ACC bring about body weight gain and promotion of protein synthesis in microorganisms and animals [6].

Although, structural characteristics of ACC and 3,3-bis(1-methylimidazol-2-yl)propionic acid complexes were extensively investigated [7], no equilibrium data are available on their equilibria in solution. Work in our laboratories focused on the studies of metal complexes of biological significance [8–12]. It is of considerable interest to study the complex formation equilibria involving 1-aminocyclopropane carboxylic acid, glycine, alanine, glycylglycine and 3,3-bis(1-methylimidazol-2-yl)propionic acid. The latter ligand is considered as a model for ACCO having two imidazole and one carboxylic groups. The results of this investigation will support the biological significance of this class of complexes. In the present study, the complex formation equilibria of binary and ternary complexes of copper(II) invovingACC and a model for ACCO is investigated. The formation constants of the complexes formed in solution are determined and their concentration distribution diagrams will be evaluated. The catalysis of amino acid ester hydrolysis through complex formation with Cu-3,3-bis(1-methylimidazol-2-yl)propionate complex will be investigated.

Experimental

Materials and reagents

1-aminocyclopropane carboxylic acid, glycine and alanine were obtained from Aldrich Chem. Co. The peptides used were glycinamide, glycylglycine, asparagines and glutamine are provided by the Sigma Chem. Co. The DNA constituents investigated are inosine, uracil, uridine, thymine and thymidine and obtained from Aldrich Chem. Co. 3,3-bis(1-methylimidazol-2-yl)propionic acid (BIMP) was prepared as sodium salt as described previously [13]. BIMP and amino acids solutions were prepared in the protonated form with standard HNO_3 solution. $Cu(NO_3)_2{\cdot}4H_2O$ was provided by BDH. The copper content of solutions was determined by complexometric EDTA titrations [14]. Carbonate-free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized H_2O.

Apparatus and measuring techniques

Potentiometric measurements were made using a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland-Herisau). The titroprocessor and electrode were calibrated with standard buffer solutions, prepared according to NBS specification [15]. Electronic spectra were measured using a Shimadzu UV-2101 recording spectrophotometer. All titrations were carried out at 25 ± 0.1 °C, in a double-walled glass cell, through the outer jacket of which water was circulated from a constant temperature bath.

Equilibruim studies

The acid-dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 cm^3) solution (2.5×10^{-3} Mol L^{-1}) of constant ionic strength 0.1 Mol L^{-1}, (adjusted with $NaNO_3$). The stability constants of the binary complexes were determined by titrating 40 cm^3 of a solution mixture of Cu^{II} (1.25×10^{-3} Mol L^{-1}), the ligand (2.5×10^{-3} Mol L^{-1}) and $NaNO_3$ (0.1 Mol L^{-1}). The condition for measuring stability constants of the ternary complexes were the same as those adopted for the binary ones, however the solutions contained equivalent amounts of Cu^{II}, 3,3-bis(1-methylimidazol-2-yl)propionate (BIMP) and amino acid, peptide or DNA constituent (1.25×10^{-3} Mol L^{-1}). All titrations were performed in a purified N_2 atmosphere, using aqueous 0.05 Mol L^{-1}NaOH (in 0.1 Mol L^{-1} $NaNO_3$).

The general three component equilibrium can be written as follows (charges are omitted for simplicity):

$$pM + qL + rH \rightleftharpoons (M)_p(L)_q(H)_r \quad (1)$$

for which the formation constants are given by

$$\beta_{pqr} = \frac{[(M)_p(L)_q(H)_r]}{[M]^p[L]^q[H]^r} \quad (2)$$

Calculations were performed using the computer program [16]MINIQUAD-75. The model selected was that which gave the best statistical fit and which was chemically consistent with the titration data without any system bias in residuals [16]. The results obtained are listed in Tables 1–3. The concentration distribution diagrams were obtained using the program SPECIES (L. Pettit, Personal communication) under the experimental conditions described.

Kinetic measurements

The kinetics of the hydrolysis of the complexed ester were monitored by the pH-stat technique [17], by using the titroprocessor operated in the SET mode. The hydrolysis was investigated using an aqueous solution (40 cm^3) containing a mixture of Cu(II) (0.25 mmol), 3,3-bis(1-methylimidazol-2-yl)propionate (0.25 mmol) and amino acid ester (0.05 mmol) and the ionic strength was adjusted to 0.1 Mol L^{-1} with $NaNO_3$. The $[Cu^{2+}]$:[BIMP]:[ester] ratio in the mixture was adjusted to 5:5:1, so as to maximize the amount of complexed ester present. The pH of the mixture was progressively raised to the desired value. The reaction was monitored by the addition of NaOH solution to maintain the given pH. The data fitting was performed with the OLIS KINFIT set of programs [18] as described previously [18].

Spectrophotometric measurements

Spectrophotometric measurements of the binary and ternary complexes were performed by scanning the UV–visible spectra of solution mixtures (*A*–*C*), where (*A*) = 0.02 mmol of Cu(II) + 0.02 mmol of BIMP + 0.06 mmol of NaOH; (*B*) = 0.02 mmol of Cu(II) + 0.02 mmol of BIMP + 0.02 mmol of glycylglycine + 0.08 mmol of NaOH and (*C*) 0.02 mmol of Cu(II) + 0.02 mmol of BIMP + 0.02 mmol of glycylglycine + 0.10 mmol of NaOH. Under these prevailing experimental conditions and after neutralization of the hydrogen ions released, associated with complex formation, it is supposed that the complex formation is nearly complete. In each mixture the volume was brought to 10 cm^3 by addition of deionized water and ionic strength is kept constant at 0.1 Mol L^{-1} $NaNO_3$.

Results and discussion

Complex formation equilibria of 1-aminocyclopropane carboxylic acid

The acid dissociation constants (pK_a) of 1-aminocyclopropane carboxylic acid (HL) in the protonated form were determined by direct potentiometric measurements. The pK_a values were found to be 2.28 ± 0.01 and 8.15 ± 0.01 and corresponding to the carboxylic and protonated amino group. The stability constants of the Cu(II) complex with 1-aminocyclopropane carboxylic acid were

Table 1
Formation constants (log values) for Cu-1-aminocyclopropane carboxylic acidcomplexes at different temperatures and 0.1 mol L^{-1} ionic strength.

T(°C)	p	q	r[a]	Log β[b]	pK_a[c]
15					
	0	1	1	8.33 (0.01)	8.33
	0	1	2	10.63 (0.01)	2.30
	1	1	0	7.82 (0.01)	
	1	2	0	14.69 (0.01)	
	1	1	−1	1.67 (0.01)	
20					
	0	1	1	8.23 (0.01)	8.23
	0	1	2	10.51 (0.01)	2.2 8
	1	1	0	7.79 (0.01)	
	1	2	0	14.60 (0.01)	
	1	1	−1	1.77 (0.01)	
25					
	0	1	1	8.14 (0.01)	8.14
	0	1	2	10.42 (0.01)	2.28
	1	1	0	7.78 (0.01)	
	1	2	0	14.42 (0.01)	
	1	1	−1	1.47423 (0.01)	
30					
	0	1	1	8.07 (0.01)	8.07
	0	1	2	10.32 (0.01)	2.25
	1	1	0	7.25 (0.03)	
	1	2	0	14.25 (0.03)	
	1	1	−1	1.44 (0.06)	
35					
	0	1	1	7.96 (0.01)	7.96
	0	1	2	10.02 (0.01)	2.06
	1	1	0	7.11 (0.03)	
	1	2	0	14.09 (0.03)	
	1	1	−1	1.32 (0.05)	

[a] The *p*, *q* and *r* are the stoichiometric coefficients corresponding to Cu(II), 1-aminocyclopropane carboxylate and H^+, respectively.
[b] Standard deviation is given in parentheses.
[c] Stepwise protonation constants.

Table 2
Formation constants (log values) for Cu-3,3-bis(1-methylimidazol-2-yl)propionate complexes at different temperatures and 0.1 mol L^{-1} ionic strength.

T °C	p	q	r[a]	Log β[b]	pK_a[c]
15					
	0	1	1	7.23 (0.01)	7.23
	0	1	2	11.51 (0.01)	4.28
	0	1	3	13.89 (0.02)	2.38
	1	1	0	9.64 (0.01)	
	1	2	0	17.10 (0.01)	
	1	1	−1	6.34 (0.01)	
20					
	0	1	1	7.19 (0.01)	7.19
	0	1	2	11.43 (0.02)	4.24
	0	1	3	13.71 (0.03)	2.28
	1	1	0	9.70 (0.05)	
	1	2	0	17.20 (0.04)	
	1	1	−1	6.35 (0.06)	
25					
	0	1	1	7.11 (0.01)	7.11
	0	1	2	11.31 (0.02)	4.20
	0	1	3	13.49 (0.03)	2.18
	1	1	0	9.89 (0.03)	
	1	2	0	17.30 (0.05)	
	1	1	−1	6.40 (0.03)	
30					
	0	1	1	7.04 (0.01)	7.04
	0	1	2	11.19 (0.02)	4.15
	0	1	3	13.43 (0.02)	2.24
	1	1	0	10.08 (0.02)	
	1	2	0	17.43 (0.05)	
	1	1	−1	6.43 (0.04)	
35					
	0	1	1	6.97 (0.01)	6.97
	0	1	2	11.09 (0.05)	4.12
	0	1	3	13.32 (0.02)	2.23
	1	1	0	10.42 (0.04)	
	1	2	0	17.78 (0.52)	
	1	1	−1	7.17 (0.03)	

[a] The *p*, *q* and *r* are the stoichiometric coefficients corresponding to Cu(II), 3,3-bis(1-methylimidazol-2-yl)propionate and H^+, respectively.
[b] Standard deviation is given in parentheses.
[c] Stepwise protonation constant.

determined potentiometrically. The data was fitted assuming the formation of 110 $[CuL]^+$, 120 $[CuL_2]$ and 11−1 [CuL(OH)] complexes. This result is in accordance with other amino acids [19]. From the concentration distribution curve, Fig. 1 $[CuL]^+$ species (110) predominates at low pH and has a maximum concentration of 64% at pH 3.8. The $[CuL_2]$ species (120) records the maximum concentration of 84% at pH 7.0, i.e. it is the main species in the physiological pH range. The [CuL(OH)] species (11−1) exists at higher pH range and predominates after pH 11.

- The values obtained for the thermodynamic parameters ΔH^0 and ΔS^0 associated with protonation of 1-amino-1-cyclopropane carboxylic acid and its complex formation with Cu^{II} were calculated from the temperature dependence of the data in Tables 1 and 2. The thermodynamic parameters ΔH^0 and ΔS^0 were obtained by a linear least square fit of lnK*versus* $1/T$ ($\ln K = -\Delta H^0/RT + \Delta S^0/R$) leading to an intercept at $\Delta S^0/R$ and a slope of $-\Delta H^0/R$. The results obtained are summarized in Table 4 and can be interpreted as follows:
- The thermodynamic processes accompanying the protonation and complex formation coreactions are:
  - the neutralization reaction, which is an exothermic process;
  - desolvation of ions, which is an exothermic process;
- The change in the configuration and the arrangements of the hydrogen bonds around the free and protonated ligand.
- The protonation reaction of ACC (reaction 1, Table 4) is exothermic ($\Delta H^0 = -30.45$ kJ mol^{-1}). Reaction (2), ($LH + H^+ \rightleftharpoons LH_2^+$), is less exothermic ($\Delta H^0 = -17.09$ kJ mol^{-1}) as it involves interaction between the neutral species (LH) and the positive hydrogen ion.
- The complex formation reaction (reaction 3) is exothermic, but reaction (4), which involves complexation of the second ACC, is less favoured and is an endothermic reaction. As expained above, the contribution of the large solvation of the left-hand side of reaction (4) contributes more to the endothermic reaction. The large positive entropy change ($\Delta S^0 = 77.40$ J K^{-1} - mol^{-1}) indicates a release of ordered water molecules and the breaking of hydrogen bonds around CuL^+. The net contribution to the free energy change is negative ($\Delta G^0 = 9.08$ kJ mol^{-1}). The formation of the hydrolysed species CuL(OH) (reaction 5) is found to be endothermic with a large change in entropy, indicating a release of ordered water molecules in the bulk of the solution, which contributes more to the total free energy change of the reaction ($\Delta G^0 = -2.26$ kJ mol^{-1})

Complex formation equilibria of 3,3-bis(1-methylimidazol-2-yl)propionic acid

3,3-bis(1-methylimidazol-2-yl)propionic acid protonation constants were determined by direct potentiometric, pH measurements because all protonation reactions were observed to take place within the potentiometrically measurable pH range. Protonated 3,3-bis(1-methylimidazol-2-yl)propionate behaves as triprotonic acid (H_3A^{2+}), where the differential log protonation constants were found to be 2.19, 4.19 and 7.11. The first constant

Table 3
Formation constants of mixed ligand complexes involving Cu(II), 3,3-bis(1-methylimidazol-2-yl)propionate and ligand as amino acid, peptide or DNA constituents of the general formula $(Cu(BIMP))_p(ligand)_qH_r$ at 25 °C and 0.1 M ionic strength.

Ligand	p	q	r	Log β
Glycine	0	1	1	9.60 (0.01)
	1	1	2	11.93 (0.02)
	1	1	0	7.36 (0.07)
Alanine	0	1	1	9.69 (0.01)
	1	1	2	11.89 (0.02)
	1	1	0	7.41 (0.06)
1-Aminocyclopropane carboxylic acid	0	1	1	8.14 (0.01)
	1	1	2	10.42 (0.01)
	1	1	0	6.51 (0.06)
Glycinamide	0	1	1	8.39 (0.01)
	1	1	0	4.65 (0.03)
	1	1	−1	−3.18 (0.02)
Glycylglycine	0	1	1	8.39 (0.01)
	0	1	2	12.66 (0.01)
	1	1	0	5.67 (0.03)
	1	1	−1	−1.71 (0.04)
Asparagine	0	1	1	8.92 (0.09)
	1	1	0	5.93 (0.14)
	1	1	−1	−3.61 (0.16)
Glutamine	0	1	1	9.21 (0.09)
	1	1	0	6.04 (0.12)
	1	1	−1	−3.41 (0.15)
Inosine	0	1	1	8.43 (0.01)
	1	1	0	4.45 (0.06)
Uracil	0	1	1	9.28 (0.01)
	1	1	0	4.65 (0.04)
	1	2	0	9.15 (0.02)
Uridine	0	1	1	9.01 (0.01)
	1	1	0	4.11 (0.07)
	1	2	0	8.76 (0.02)
Thymine	0	1	1	9.58 (0.01)
	1	1	0	5.08 (0.03)
	1	2	0	9.32 (0.03)
Thymidine	0	1	1	9.5 (0.01)
	1	1	0	5.03 (0.03)
	1	2	0	9.28 (0.03)

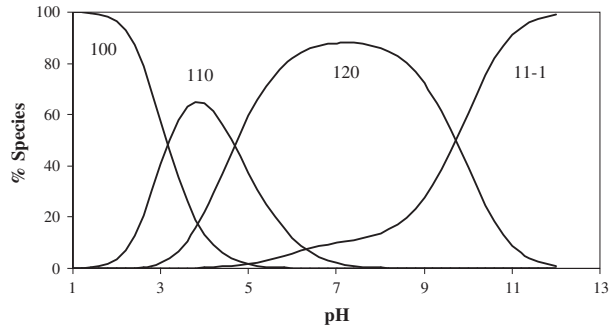


Fig. 1. Concentration distribution diagram of Cu(II)–1-aminocylclopropane carboxylic acid complexes at concentration of 1.25×10^{-3} mol L^{-1} for Cu(II) and 2.5×10^{-3} mol L^{-1} for 1-aminocylclopropane carboxylic acid, $I = 0.1$ mol L^{-1} ($NaNO_3$) and $T = 25$ °C.

is corresponding to the carboxylic group and the second and third constants are corresponding to the protonated imidazole groups.

The model that best fits the potentiometric data of the copper(II)-BIMP complex is found to consist of CuA^+ (110), CuA_2 (120) and CuA(OH) (11−1) species. The validity of the same is proven, where an excellent fit can be observed between the experimental data points from the titration of Cu-3,3-bis(1-methylimidazol-2-yl)propionate complex and the theoretical curve calculated from the values of protonation constants of 3,3-bis(1-methylimidazol-2-yl)propionic acid and formation constant of the corresponding complex. From the concentration distribution curve of Cu^{II} complex, CuA^+ species predominates at low pH and

Table 4
Thermodynamic parameters for the equilibria of Cu(II) complexes with 3,3-bis(1-methylimidazol-2-yl)propionate and 1-aminocyclopropanecarboxylic acid.

Equilibrium[a]	ΔH^0 kJ Mol^{-1}	ΔS^0 JK^{-1} Mol^{-1}
ACC complexes		
(1) $L^- + H + \rightleftharpoons LH$	−30.45	23.35
(2) $LH + H^+ \rightleftharpoons LH_2^+$	−17.09	−6.32
(3) $Cu^{2+} + L^- \rightleftharpoons CuL^+$	−66.38	−33.99
(4) $CuL^+ + L^- \rightleftharpoons CuL_2$	13.98	77.40
(5) $CuL^+ + OH^- \rightleftharpoons CuL(OH)$	30.97	111.53
BIMP complexes		
(6) $A^- + H + \rightleftharpoons AH$	−22.15	26.81
(7) $AH + H^+ \rightleftharpoons AH_2^+$	−14.43	13.88
(8) $AH_2^+ + H^+ \rightleftharpoons AH_3^{2+}$	−11.92	1.41
(9) $Cu^{2+} + A^- \rightleftharpoons CuA^+$	65.60	178.33
(10) $CuA^+ + A^- \rightleftharpoons CuA_2$	−12.32	43.68
(11) $CuA^+ + OH^- \rightleftharpoons CuA(OH)$	−7.05	77.81

[a] Where A and L denote 3,3-bis(1-methylimidazol-2-yl)propionate and 1-aminocyclopropane carboxylate respectively. ΔH^0 and ΔS^0 are estimated by EXCEL from the slope and intercept of the linear plot. The correlation coefficient (r^2) values are 0.93−0.98.

has a maximum concentration of 77.0% at pH 2.5. The CuA_2 species records the maximum concentration of 67% at pH 6.0, i.e. it is the main species in the physiological pH range. The CuAOH species (11−1) exists at higher pH range and predominates above pH 9.0.

The values obtained for the thermodynamic parameters ΔH^0 and ΔS^0 associated with protonation of 3,3-bis(1-methylimidazol-2-yl)propionic acid and its complex formation with Cu^{II} were calculated as described previously and the results were given in Table 3.

The protonation reactions of (BIMP) (reactions 6-8) are exothermic ($\Delta H^0 = -22.15$, −14.43 and −11.92 kJ mol^{-1}), as expected for neutralization reactions. The formation of CuA^+ complex (reaction 9) is endothermic ($\Delta H^0 = 65.8$ kJ mol^{-1}). The large positive entropy change ($\Delta S^0 = 178.33$ JK^{-1} mol^{-1}) indicates a release of ordered water molecules and the breaking of hydrogen bonds. A positive ΔH^0 and large ΔS^0 for the complexation reaction of copper ions and organic phosphates [20] and 6-aminopenicilnic acid [21] were found previously. The formation of CuA_2 and CuA(OH) complexes are exothermic as expected for neutralization reactions.

Ternary complexes of Cu^{II}, 3,3-bis(1-methylimidazol-2-yl)propionic acid and other ligands

Based on thermodynamic aspects the ternary complex formation may proceed either through a stepwise or simultaneous mechanism depending on the chelating potential of 3,3-bis(1-methylimidazol-2-yl)propionic acid and other ligand (HL) [HL = amino acid, peptide or DNA)]. The formation constant value of Cu-3,3-bis(1-methylimidazol-2-yl)propionate is higher than those of 1:1 Cu(II)–ligand (L) complexes, Table 1. It is reasonable to propose that in presence of both ligands, one molecule of 3,3-bis(1-methylimidazol-2-yl)propionate is coordinated to Cu(II) ion, with subsequent coordination of the secondary ligand (L). Similar behavior was found for the systems Zn(II)-NTA-amino acids [22] and Cu(II)–bpy-guanosine-5′-diphosphate [23]. Therefore, it is assumed that in the presence of both ligands, 3,3-bis(1-methylimidazol-2-yl)propionate is coordinated to Cu(II) ion in the region $0 < a < 3$, (a = number of moles of base added per mole of ligand) then followed by coordination of amino acid in the region $3 < a < 4$, i.e the ternary complex formation could be considered in stepwise equilibria

$$Cu + (BIMP) \rightleftharpoons Cu(BIMP) \quad (3)$$

$$Cu(BIMP) + L \rightleftharpoons Cu(BIMP)L \quad (4)$$

The potentiometric data in the region $3 < a < 4$, is fitted considering the formation of the 1:1 complex with amino acids, [Cu(BIMP):amino acid]. The formation constants of the mixed-ligand complexes are given in Table 4.

In the ternary complexes of peptides (HL), the potentiometric titration curves of the mixed ligand system Cu(II)–BIMP-glycylglycine (as a representative of peptides), Fig. 2, coincides with the 1:1 Cu(II)–BIMP curve in the region $0 < a < 3$ (a = number of moles of base added per mole of ligand). Based on thermodynamic aspects, the Cu-BIMP complex is first formed due to its greater stability compared to the Cu(II)–peptide complex (Table 2). Beyond $a = 3$, the formation of a ternary complex was ascertained by comparison of the mixed-ligand titration curve with the composite curve obtained by graphical addition of glycylglycine titration data to that of the Cu-BIMP titration curve. The mixed ligand system was found to deviate considerably from the resulted composite curve in the region $3 < a < 5$, indicating the formation of a ternary complex.

The potentiometric data in this region is fitted considering the formation of the complexes Cu(BIMP)(L) and Cu(BIMP)(LH_{-1}) and their formation constants are given in Table 2. The peptide may form the Cu(BIMP)(L) complex by coordination through the amine and carbonyl groups. On increasing the pH, the coordination sites should switch from the carbonyl oxygen to amide nitrogen. Such changes in coordination centers are now well documented [24,25]. The amide groups undergo deprotonation and the Cu(BIMID)(LH_{-1}) complexes are formed. The pK^H values are calculated by the following equation [26]:

$$pK^H = \log \beta_{110} - \log \beta_{11-1} \quad (5)$$

The pK^H values of the peptide complexes are 7.38, 7.83, 9.54 and 9.45. for glycylglycine, glycinamide, asparagine and glutamine respectively. It is noteworthy that the pK^H for the glycylglycine and glycinamide complexes are lower than that of asparagine complex. This signifies that the more bulky substituent group on the amides may serve to hinder the structural change in going from protonated to deprotonated complexes. The high pK^H value of the glutamine complex is ascribed to the formation of a seven-membered chelate ring, which is more strained and therefore less favoured. Speciation diagram of glycylglycine complex is given in Fig. 3. The mixed ligand species [Cu(BIMP)L] (1 1 0) starts to form at pH = 4 and with increasing pH, its concentration increases reaching the maximum of 64% at pH = 6.5. Further increase of pH is accompanied by a decrease in [Cu(BIMP)L] (1 1 0) complex concentration and an increase of [Cu(BIMP)(LH_{-1})] complex concentration.

Before discussing the results of spectrophotometric measurements, it should be pointed out that the spectrum of the aquated copper(II) ion consists of a broad, weak band with a maximum wavelength at 817 nm, being attributed to the $^2B_{1g} \leftarrow {}^2A_{1g}$ transition [27,28]. The spectral bands of the binary and ternary copper(II) complexes are quite different from that of the aquated copper(II) ion in the position of the maximum wavelength. The spectrum of the $[Cu(BIMP)]^+$ complex (mixture A) shows an absorption maximum, at 680 nm (Fig. 4). On the other hand the spectrum obtained from the ternary copper(II) complex with BIMP and glycylglycine (1 1 0) (mixture B) exhibits a band at 635 nm. The spectrum obtained for [Cu(BIMP)–glycylglycinate;H_{-1}] complex (1 1 −1) (mixture C) exhibits a band at 626 nm. The progressive shift toward shorter wavelength in the absorption spectrum may be taken as an evidence, supporting the potentiometric measurement, for the induced ionization of amide hydrogen upon complex formation [24,25].

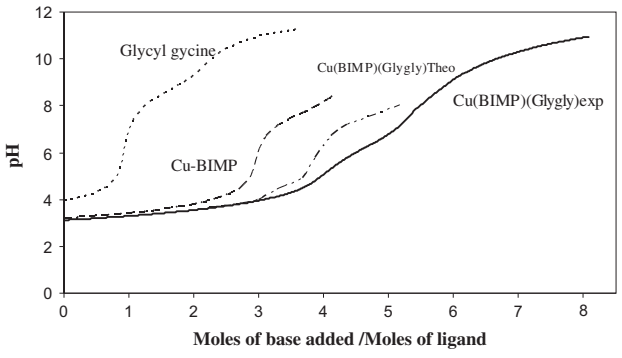


Fig. 2. Potentiometric titration curves of Cu(BIMP)–glycylglycine system at concentration of 1.25×10^{-3} mol L^{-1} for Cu(II), BIMP and glycylglycine, $I = 0.1$ mol L^{-1} ($NaNO_3$) and $T = 25$ °C.

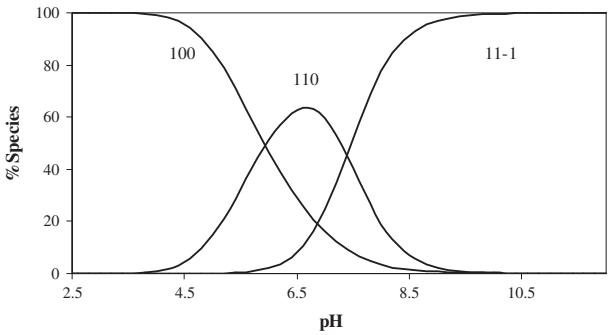


Fig. 3. Concentration distribution diagram of Cu(BIMP)–glycylglycine complex at concentration of 1.25×10^{-3} mol L^{-1} for Cu(II), BIMP and glycylglycine, $I = 0.1$ mol L^{-1} ($NaNO_3$) and $T = 25$ °C.

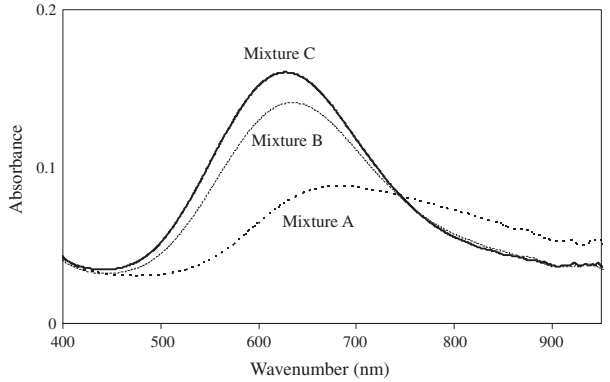
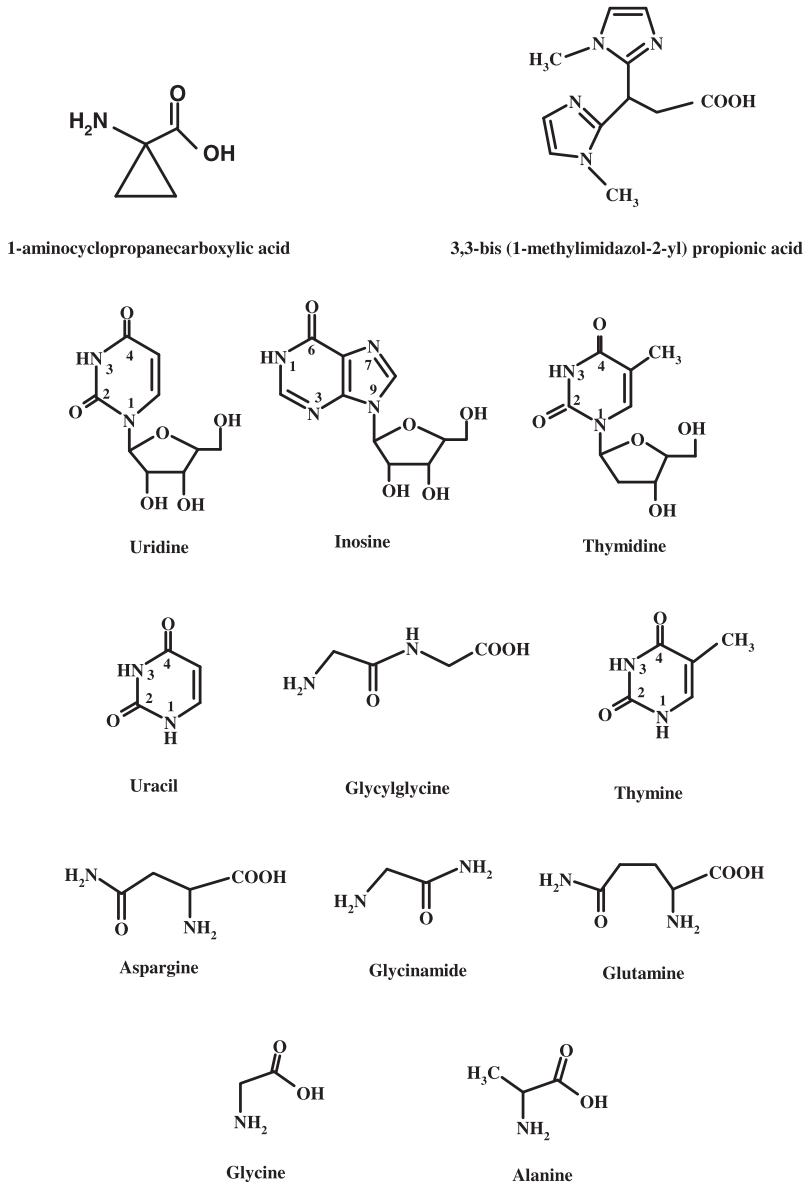


Fig. 4. The electronic spectra of Cu(BIMP)–glycylglycine complexes, Composition of solution mixtures A, B and C were given in the Experimental part.

The potentiometric titration data of Cu(BIMP)–DNA complexes shows that DNA forms complexes by stepwise mechanism in the same way of amino acids and peptides complexes. The potentiometric data of the DNA complexes were fitted with the model composed of the 110 and 120 species. The pyrimidinic species (uridine, uracil, thymine and thymidine) have a dissociable proton at N_3–C_4O group [29,30]. The acid dissociation constants obtained from this study were compared with that of the N_1 proton of inosine. The purinic derivative (inosine) is slightly more acidic than the pyrimidinic species (uridine, uracil, thymine and thymidine). This can be related to the existence of the anion form of purinic derivatives in a higher number of resonance forms due to the presence of two condensed rings in this ligand (inosine) as shown in Scheme 1. Based on the existing data, uracil, uridine, thymine and thymidine coordinate in the deprotonated form as monoanion, through N_3 and they do not form protonated complexes. The thymine and thymidine complexes are more stable than those of



Scheme 1. Structural formulae of the investigated ligands.

uracil and uridine, most probably due to the higher basicity of the N_3 site of thymine and thymidine resulting from the inductive effect of the extra electron-donating methyl group. As a result of the high pK_a values of pyrimidines ($pK_a \approx 9$) and the fact that they are monodentates, the complexes are formed only above pH 6, supporting the view that the negatively charged nitrogen donors of pyrimidine bases are important binding sites in the neutral and slightly basic pH range

Hydrolysis of coordinated amino acid ester

Metal ion catalyzed hydrolyses of amino acid esters have been suggested to proceed by several mechanisms [31]. All require initial coordination of the ester and NH_2 groups by the metal ion. The ester may then get attacked at the carbonyl carbon atom by OH^-, OH_2, or MOH (a metal hydroxo complex). The coordinated amino acid ester hydrolysis may be represented by:

$$[Cu(BIMP)(H_2O)_2]^+ + NH_2CH_2CO_2CH_3 \underset{}{\overset{k_f}{\rightleftharpoons}} [Cu(BIMP)(NH_2CH_2CO_2CH_3)]^+ + 2H_2O \quad (6)$$

$$\downarrow \; OH,\ k_{OH}$$

$$[Cu(BIMP)(NH_2CH_2CO_2)] + CH_3OH \qquad (6)$$

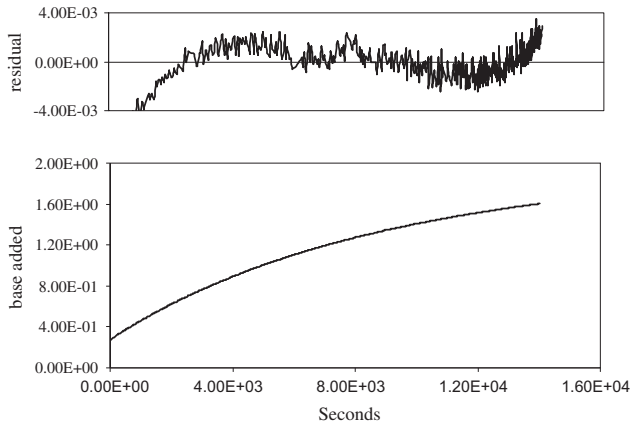


Fig. 5. Typical plot of volume of base added *versus* time in seconds fitted with a single exponential function for the hydrolysis of $[Cu(BIMP)(GlyOMe)]^+$ at 25 °C and 0.1 M ionic strength.

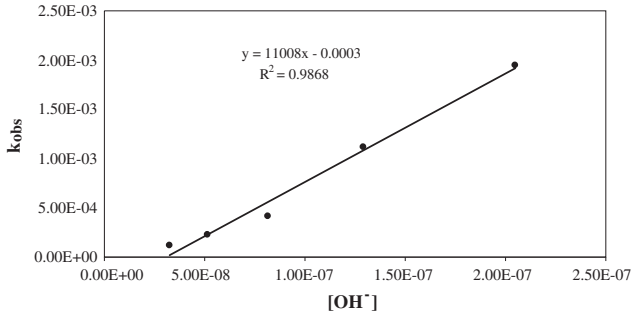


Fig. 6. Kinetic data for the hydrolysis of Cu(BIMP)–glycine methyl ester at 25 °C and 0.1 M ionic strength.

Table 5
Kinetics of hydrolysis of $[Cu(BIMP)(glycine\ methyl\ ester)]^+$ at 25 °C and 0.1 M ionic strength.

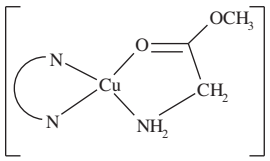
Ester	pH	$[OH^-]$	$k_{obs}(s^{-1})$
Glycine methyl ester	6.4	3.25E-8	1.15E-4
	6.6	5.15E-8	2.28E-4
	6.8	8.17E-8	4.2E-4
	7.0	1.29E-7	1.12E-3
	7.2	2.05E-7	1.95E-3

In the solution mixture of Cu(II), BIMP and glycine methyl ester in concentration ratio 5:5:1, and based on the high stability constant of Cu(II)–BIMP complex (log K = 9.89), it is proposed that reaction (6) proceeds to completion. The kinetic data, *viz.* the volume of base added to keep the pH constant *versus* time, could be fitted by a single exponential function as shown in Fig. 5. Various other kinetic models were tested without leading to satisfying fits of the data. A plot of k_{obs} *versus* hydroxide ion concentration is linear as shown in Fig. 6 and follows the rate expression given in

$$K_{obs} = k_o + k_{OH}[OH^-] \quad (7)$$

The k_o term is suggested to arise from the spontaneous hydrolysis reaction with water.

The observed rate constant (k_{obs}) at different pH values are given in Table 5. The linear dependence of the rate on the OH^- concentration is consistent with the direct attack of the OH^- ion on the ester group [32], Fig. 6. k_{OH} value is 1.1×10^4 dm^3 mol^{-1} s^{-1}. The catalytic ratio value $C = k_{OH}/k_{OH}^{ester}$, where k_{OH}^{ester} represents the rate constant for the hydrolysis of the free glycine methyl ester and its value is 1.28 dm^3 mol^{-1} s^{-1} as taken from Lit [17]. For the coordinated glycine methyl ester, the catalytic ratio amounts to 8.6 E+3. A catalytic ratio of this magnitude is fully consistent with the formation of the mixed ligand complex of structure I, where Cu(II) is coordinated with BIMP by two imidazole nitrogen atoms (N–N) and there is a direct interaction between Cu(II) and the carbonyl group of the ester.



I

Comparative values of k_{OH} at 25 °C for the base hydrolysis of the glycine methyl ester incorporated in $[Cu(bpy)L]^{2+}$ is 4.88×10^4 dm^3 mol^{-1} s^{-1} [33], where bpy = 2,2′-bipyridine. The k_{OH} value for $[Cu(BIMP)L]^+$ (1.1×10^4 dm^3 mol^{-1} s^{-1}) is lower than that of $[Cu(bpy)L]^{2+}$. This may be explained on the premise that the coordinated mononegatively charged BIMP leads to a decrease of electrophilicity of the Cu(II) centre. The electrophilicity is one of the factors determining the donor-acceptor interaction between the ester and Cu(II) ion, the complex which binds the ester more tightly, would withdraw the most electron density from the ester making it more susceptible to OH^- attack. This will lead to increase of the respective catalysis ratio.

Conclusion

The gaseous plant hormone, ethylene, is biosynthesised from 1-aminocyclopropane carboxylic (ACC) acid using ACC oxidase as catalyst. 3,3-bis(1-methylimidazol-2-yl)propionate (BIMP) is considered as a model of ACCoxidaxe. ACC and BIMP react with Cu(II) ion forming 1:1, 1:2 and the monohydroxo-species of the 1:1 complex. The thermodynamic functions (ΔH^0 and ΔS^0) are evaluated and discussed. The ternary complex of Cu(II) involving ACC and BIMP is supporting the biological activity of this class of ligands in production of plant hormone (ethylene). The combination of stability constants of Cu(II) with BIMP and ACC and biorelevant ligands as amino acids, peptides and DNA constituents will allow the equilibrium distribution of the complexes formed in biological fluids. $Cu(BIMP)^+$ complex is catalysing the hydrolysis of glycine methyl ester and the mechanism was discussed.

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