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Complex Formation Equilibria Between Zinc(II), Nitrilo-tris(Methyl Phosphonic Acid) and Some Bio-relevant Ligands. The Kinetics and Mechanism for Zinc(II) Ion Promoted Hydrolysis of Glycine Methyl Ester

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Abstract Binary and ternary complexes of zinc(II) involving nitrilo-tris(methyl phosphonic acid) (H_6A) and amino acids, peptides (HL), or DNA constituents have been investigated. The stoichiometry and stability constants for the complexes formed are reported. The results show that ternary complexes are formed in a stepwise manner whereby nitrilo-tris(methylphosphonic acid) binds to zinc(II), which is then followed by coordination of an amino acid, peptide or DNA. Zinc(II) was found to form ZnA and $ZnAH_n$ complex species where $n = 3$, 2 or 1. The stabilities of the ternary complexes are compared with the stabilities of their corresponding binary complexes. The concentration distributions of the various complex species have been evaluated. The kinetics of the base hydrolysis of glycine methyl ester in the presence of Zn(II)-NTP complexes was studied in aqueous solution using a pH-stat technique. The pK_a for ionization of the coordinated water molecule is 9.14 as determined from the kinetic results, while direct potentiometric titration of the complex $[Zn(NTP)(H_2O)]$ gave 9.98 (± 0.02). The rate constant for the intramolecular attack of coordinated hydroxide on the ester is $k = (2.65 \times 10^{-4} \pm 0.003)$ $dm^3 \cdot mol^{-1} \cdot s^{-1}$.

Keywords Zinc(II) formation equilibria · Nitrilo-tris(methylphosphonic acid) · Bio-relevant ligands · Kinetics of hydrolysis · Glycine methyl ester

1 Introduction

The study of ternary complexes of transition metal ions with amino acids, peptides or DNA constituents has become the focus of increasing research effort [1–4], which is revealing

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the role of these metal ions at the molecular level. These types of complexes are implicated in the storage and transport of metal ions and of active substances through biological membranes. Consequently, it is worthwhile to study their formation, stability and structure, and the mutual influence of two different ligands bound to the same metal ion. Among the essential trace elements, zinc is second only to iron in terms of the quantity found in the human body and in virtually any other organism. Zinc(II), among other transition metal ions, plays a vital role in biological processes. Zinc deficiency can cause unusual disorders in the development of the body, disorders in the metabolic system and prostate gland, and can result in mental retardation. Studies on model complexes of zinc(II) ions have focused on improving the understanding of the structure-reactivity relationship of the active site in zinc enzymes [5–8].

Natural and synthetic aminopolyphosphonic molecules are very effective ligands, in many cases having high specificity for certain metal ions. This class of compounds and their derivatives has received considerable attention because of their interesting biological activity. They include a variety of herbicides, plant growth regulators, antibodies and inhibitors of metallo-enzymes [9]. As potent metal binders, aminophosphonates might be involved in interactions relevant for the fate of metal ions in the natural environment or in biological systems [10]. Therefore, several studies have aimed at understanding the chelating properties of this class of ligands and determining the stability of the complexes formed [11, 12].

As part of our program on the co-ordination chemistry of transition metals, we have set out to elucidate the formation equilibria of mixed-ligand complexes of Zn(II) involving aminophosphonic acid derivatives (NTP), which are emphasized as they provide information regarding the behavior of this class of ligands in biological systems. The present study is a continuation of published work on binary and ternary complexes of transition metal ions involving amino acids [13, 14], peptides [15, 16] and DNA constituents [17, 18], as well as the base hydrolysis of the mixed-ligand complexes with α-amino acid esters [19, 20]. In the present investigation we describe the equilibria associated with the formation of binary and ternary complexes involving zinc(II), nitrilo-tris(methyl phosphonic acid) and some biorelevant ligands containing different functional groups. Ternary complexes involving amino acid esters can be regarded as mimicking metalloenzyme-substrate complexes. Therefore, it is of interest to study the hydrolysis of α-amino acid esters under complex formation conditions.

2 Experimental

2.1 Materials and Reagents

All of the reagents were of Analar grade. Nitrilo-tris(methyl phosphonic acid) NTP, the tris-phosphonic derivative of nitrilotriacetic acid (NTA), was obtained from Aldrich Chemical Company. The amino acids glycine, alanine, imidazole, threonine, ornithine dihydrochloride, histamine dihydrochloride, histidine monohydrochloride, proline, lysine and cysteine, along with methylamine hydrochloride, ethanolamine and mercaptopropioninc acid, were provided by the Sigma Chemical Company. The peptides glutamine, aspargine, glycylglycine and glycinamide were purchased from Fluka Chemical Company. The DNA constitutents uridine, uracil, thymine, thymidine, inosine and inosine-5′-monophosphate were supplied by BDH-Biochemicals Ltd. $Zn(NO_3)_2$ was provided by BDH. The glycine methyl ester was purchased from Fluka.

The zinc content of solutions was determined by complexometric EDTA titration [21]. Carbonate-free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized H_2O.

2.2 Apparatus and Measuring Techniques

Potentiometric measurements were made using a Metrohm 686 titroprocessor equipped with a 665 Dosimat. The electrode and titroprocessor were calibrated with standard buffer solutions prepared according to NBS specifications [22]. The pH meter readings were related to hydrogen ion concentrations by titrating a standard strong acid solution (0.01 mol·dm^{-3}) with standard base (0.10 mol·dm^{-3}) at 25 °C, the ionic strength of which was adjusted to 0.1 mol·dm^{-3} with $NaNO_3$. The pH was plotted against p[H] to yield the relationship pH − p[H] = 0.05. The [OH^-] concentration was calculated using pK_w = 13.997 [23]. All potentiometric titrations were carried out at 25.0 ± 0.05 °C in a double-walled glass cell of 50 mL capacity. The temperature of all solutions was maintained at 25.0 ± 0.05 °C by circulation of thermostatted water through the outer jacket of the double-walled glass cell. The solutions were stirred with a magnetic stirrer, and all titrations were performed at an ionic strength of 0.1 mol·dm^{-3} ($NaNO_3$). The hydrolysis kinetics were monitored by a pH-stat technique using the titroprocessor operated at set mode [20, 24].

3 Equilibrium Measurements

The acid dissociation constants of the ligands were determined potentiometrically by titrating 40 cm^3 of the ligand solution (1.25 × 10^{-3} mol·dm^{-3}) with NaOH at the constant ionic strength of 0.1 mol·dm^{-3} (adjusted with $NaNO_3$). The hydrolysis constant of Zn(II) was determined by titrating 40 cm^3 of Zn(II) solution (1.25 × 10^{-3} mol·dm^{-3}) in 0.1 mol·dm^{-3} $NaNO_3$. The stability constant of the Zn-NTP complex was determined by titrating 40 cm^3 of a solution mixture of Zn(II) (1.25 × 10^{-3} mol·dm^{-3}), NTP (2.5 × 10^{-3} mol·dm^{-3}), and $NaNO_3$ (0.1 mol·dm^{-3}). The stability constants of the ternary complexes were determined using potentiometric data obtained from mixtures of Zn(II) (1.25 × 10^{-3} mol·dm^{-3}), NTP, and the biologically relevant ligand solutions at a concentration ratio 1:1:1. All titrations were performed under a purified N_2 atmosphere, using aqueous 0.05 mol·dm^{-3} NaOH as the titrant.

The stability constants of the ternary Zn(NTP)L complexes, where HL is an amino acid, peptide or DNA, were determined using the data obtained within the pH range corresponding to complete formation of the Zn-NTP complex. Hence, in calculations only complex formation between Zn(NTP) and ligand (HL) were considered and each of these systems could be treated as a binary one.

The equilibrium constants evaluated from the titration data (summarized in Table 1) are defined by Eqs. 1 and 2, where M, L and H denote the [Zn(NTP)]$^{4-}$, ligand and proton, respectively.

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \tag{1}$$

$$\beta = \frac{M_pL_qH_r}{[M]^p[L]^q[H]^r} \tag{2}$$

The calculations were performed using the computer program MINIQUAD-75 [25]. The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models. The selected model was that which gave the best statistical fit and seemed chemically sensible and consistent with the titration data, without giving any systematic drifts in the magnitudes of the various residuals, as described elsewhere [25]. The concentration distribution diagrams were obtained using the program SPECIES (L. Pettit, personal communication) under the experimental conditions used. The results obtained are shown in Table 1.

Table 1 Formation constants of $M_pL_qH_r$ species

System	p	q	r[a]	$\log_{10}\beta$[b]	S[c]
Zn-NTP	0	1	1	11.00(0.01)	5.0E–8
	0	1	2	18.18(0.02)	
	0	1	3	24.28(0.02)	
	0	1	4	28.95(0.03)	
	0	1	5	30.85(0.06)	
	1	0	−1	−7.98(0.08)	5.7E–7
	1	0	−2	−16.08(0.02)	
	1	1	0	12.88(0.01)	1.3E–8
	1	1	1	19.60(0.01)	
	1	1	2	25.01(0.01)	
	1	1	3	29.16(0.01)	

[a] p, q and r are the stoichiometric coefficients corresponding to Zn^{2+}, NTP and H^+

[b] Standard deviations are given in parentheses

[c] Sum of square of residuals (E–x denotes $\times 10^{-x}$)

4 Kinetic Measurements

The kinetics of hydrolysis of the complexed ester was investigated using an aqueous solution (40 cm^3) of a mixture of zinc(II) (6.25×10^{-3} mol·dm^{-3}), NTP (6.87×10^{-3} mol·dm^{-3}), glycine methyl ester (1.25×10^{-3} mol·dm^{-3}), and $NaNO_3$ (0.1 mol·dm^{-3}). In this mixture the [Zn-NTP]:[ester] ratio was adjusted to 5:1 so as to maximize the amount of complexed ester present. A 10% excess of NTP over zinc(II) was used to ensure coordination of all zinc(II), which is itself an excellent catalyst. A 20% excess of NTP gave the same rates as with a 10% excess. In all cases the solutions were equilibrated at the desired temperature under a constant flow of nitrogen. The ester solution was then added, and the pH brought to the desired value by addition of 0.05 mol·dm^{-3} NaOH as described previously [19, 20]. The data fitting was performed with the OLIS KINFIT set of programs [26] as reported previously [19, 20]. Values of the hydroxide ion concentration were estimated from the pH using $pK_w = 13.997$ (± 0.02) and an activity coefficient of 0.772.

5 Results and Discussion

5.1 Equilibrium Studies

Nitrilo-tris(methyl phosphonic acid (NTP) differs from nitrilotriacetic acid (NTA) in the stepwise replacement of carboxylic groups by phosphonic groups. NTP has three phosphonic groups and is represented as H_6A. NTP is more basic than NTA due to the electron repelling effect of the dinegative charge on the phosphonic functional groups. The most basic donor for NTP is the tertiary amino group, and the next most basic groups are the phosphonates that protonate in the pH range 4.9–7.2. The remaining phosphonic group is very weakly basic ($\log_{10} K$ is around 2), which can be determined pH-metrically only with rather high uncertainties. Five of the six protons are titrateable and can be measured by the potentiometric technique. The protonation constants listed in Table 1 are in reasonably

good agreement with earlier reports [27, 28]. The acid dissociation constants of amino acids, peptides and DNA constituents have been reported. We re-determined them under the experimental conditions used for determining the stability constants of the mixed-ligand complexes. The results obtained are in good agreement with literature values [29].

Potentiometric data for the Zn-NTP solution mixture were fitted by assuming the formation 110, 111, 112 and 113 species but no 120 species. Formation of the 1:2 complex seems to be generally hindered because NTP acts as a tetradentate ligand. Also, electrostatic repulsion of the negatively charged phosphonate groups precludes coordination of the second ligand where the charge of the 1:2 complex would be −10. In the 1:1 complex $Zn(NTP)^{4-}$, the ligand is tetradentate with nitrogen and phosphate groups acting as donor sites. A comparison between the formation constants of the NTP and NTA complexes with zinc(II) indicates that $Zn(NTP)^{4-}$ ($\log_{10} K = 12.88 \pm 0.01$) is more stable than the corresponding NTA complex ($\log_{10} K = 10.45 \pm 0.01$). Thus, substitution of a carboxylate group by a phosphonate group increases the stability of the complexes due to the higher basicities of the phosphonic functional group.

Ternary complex formation may proceed either through a stepwise or simultaneous mechanism depending on the chelating potentials of NTP and the other ligand (L) [L = amino acid, peptide or DNA]. The formation constant of the 1:1 Zn-NTP complex is higher than 1:1 Zn-ligand (L) complex, see Table 1. It is reasonable to propose that, in presence of both ligands, one molecule of NTP is coordinated to the zinc(II) ion with subsequent coordination of the secondary ligand(L). This assumption was supported by our potentiometric data. A representative set of pH titration curves for the Zn-NTP-glycine system is shown in Fig. 1. The Zn-NTP(1:1) mixture titration curve has a sharp inflection at $a = 6$ (a = number of moles of base added per mole of ligand), corresponding to complete formation of the 1:1 complex. In this respect, the Zn-NTP complex is formed first due to its greater stability compared to the Zn-L complex (Table 1). Beyond $a = 6$, 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 glycine titration data to that of the Zn-NTP titration curve. The mixed ligand system was found to deviate considerably from the resulting composite curve, indicating the formation of a ternary complex. Thus, the formation of ternary complex can be described by the following stepwise equilibria, Eqs. 3 and 4.

The stability constant of the monodentate methylamine complex is slightly higher than that of glycine, indicating that glycine most likely coordinates with Zn(NTP) as a monodentate rather than as a bidentate ligand

$$\mathrm{Zn} + \mathrm{NTP} \rightleftharpoons \mathrm{Zn(NTP)} \tag{3}$$

$$\mathrm{Zn(NTP)} + \mathrm{L} \rightleftharpoons \mathrm{Zn(NTP)L} \tag{4}$$

(Charges are omitted for simplicity)

The observed extra stability of the methylamine complex may be due to the higher basicity of its amino group compared with that of a glycine amino group (as reflected by their pK_a values). This may be considered as further evidence that glycine acts as monodentate ligand. Consequently, ring opening in the Zn(NTP) complex allows for chelation of glycine as a bidentate ligand.

In the case of the ternary complexes of serine and threonine, the potentiometric data could be fitted by assuming that serine or threonine is bound in a glycine-like mode and that the alcohol group of serine is not ionized. The thioether group is not involved in the co-ordination of methionine, because the stability constant of its ternary complex is lower

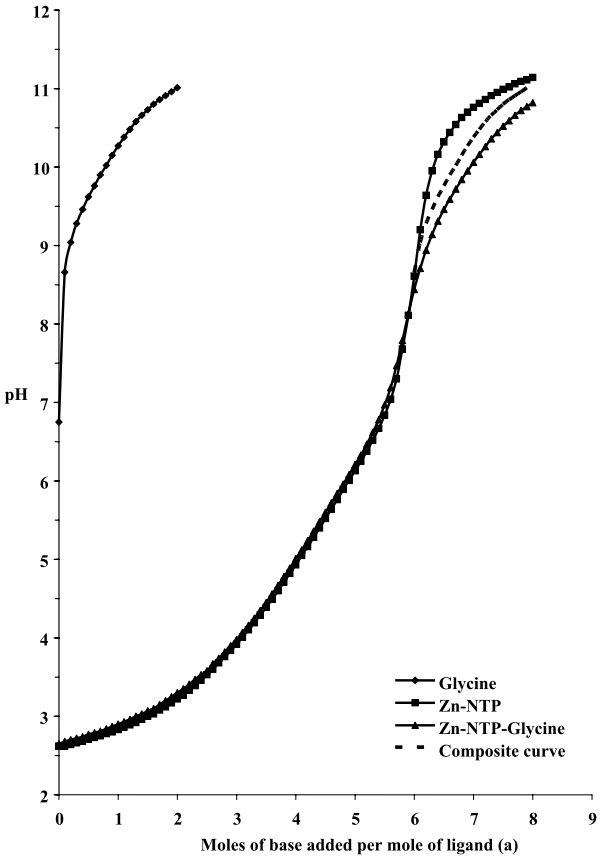

Fig. 1 Potentiometric titration curves of the Zn–NTP–glycine system

than that of mercaptopropanoic acid (S, O donor set) and near to that of glycine (N, O donor set). This indicates that methionine co-ordinates in a glycine-like mode.

The extra amino group in ornithine does not contribute to the stability of the complexes formed because the extra amino group does not participate in complex formation.

The ionized amide residue of the peptide, [–CONH–], behaves as an important ligating group and coordinates to zinc(II) through binding with the ionized amide group [16]. The potentiometric data obtained for the peptides and threonine complexes reveal the formation of ternary Zn(NTP)(L) species rather than the corresponding Zn(NTP)LH_{-1} species, which supports the view that induced ionization of the peptide hydrogen is not favored. This finding is in agreement with the previous investigation carried out on the Zn(II)-diethylenetriamine-peptide system.

The pyrimidines uracil, uridine, thymine and thymidine have only one basic nitrogen N(3). Consequently, the pyrimidines coordinate in the deprotonated form through this site and they do not form protonated complexes. Inosine may become protonated at N(7) with formation of the [N(1)H-N(7)H] monocation. In the present study, only the pK_a of N(1)H was determined because the pK_a of N(7)H is too low to be detected by the potentiometric technique. The potentiometric data of the mixed ligand complexes involving inosine showed the formation of a Zn(NTP)L species, where L is the monoanion of inosine. In the acidic pH range, N(1) remains protonated while the metal ion is attached to N(7). The gradual change from N(7)-binding to N(1)-binding with increasing pH has been frequently documented by

^{1}H-NMR [32] and EPR [33] spectroscopic measurements. Consequently, it is proposed that N(1) serves as a coordination site in the mixed ligand complexes of inosine at higher pH values.

The relative stabilities of the ternary and binary complexes can be quantitatively expressed in a number of different ways. It has been argued that a comparison can best be made in terms of $\Delta \log_{10} K$ [34]. The relative stability of the ternary complexes formed through a stepwise reaction, as compared to those of binary complexes, are expressed in terms of $\Delta \log_{10} K$ as defined by Eq. 5:

$$\Delta \log_{10} K = \log_{10} K^{\mathrm{Zn(NTP)}}_{\mathrm{Zn(NTP)L}} - \log_{10} K^{\mathrm{Zn}}_{\mathrm{ZnL}} \tag{5}$$

One expects to obtain negative values for $\Delta \log_{10} K$ (Table 2) because more coordination positions are available for the bonding of ligand (L) in the binary complex than in the ternary complexes. This indicates that the secondary ligand (L), amino acid, peptide or DNA, forms more stable complexes with zinc(II) ion alone than with the zinc(II)-NTP complex. It is to be noted that imidazole has the least negative $\Delta \log_{10} K$ value. This may be considered as evidence for enhanced stability of a ternary complex involving π-electron back donation from the negatively charged NTP^{6-} ion to the π-system of the imidazole. The $\Delta \log_{10} K$ values of the mixed ligand amino acids complexes are more negative than that for methylamine. This may be described on the basis that the amino acid acts as a bidentate ligand in the binary complex and is a monodentate ligand in the ternary complex formed from the $Zn(NTP)^{4-}$ complex. The negative values obtained for $\Delta \log_{10} K$ are of the order: tridentate (as histidine) > bidentate (as glycine) > monodentate (as imidazole) ligand.

Estimation of the concentration distribution diagrams of the various species in solution provides a useful picture of metal ion binding. To illustrate the main features observed in the species distribution plots for these systems, the speciation diagrams calculated for the Zn-NTP and Zn-NTP-glycine complexes are shown in Figs. 2 and 3, respectively. In Fig. 2, as the pH increases deprotonation of the NTP ligand occurs, the 110 species starts to form at pH ∼5, and reaches a maximum concentration of 99% at pH = 9. In Fig. 3, the mixed ligand species [Zn(NTP)L] (110) starts to form at pH ∼ 7 and, with increasing pH, its concentration reaches a maximum of 35% at pH = 9.5.

5.2 Kinetics of Hydrolysis

The hydrolysis of the coordinated ester was monitored over the pH range 8.8–9.6. In this range, the rate of hydrolysis of MeGly is negligible in the absence of $Zn(NTP)^{4-}$. The volume of base added to change the pH in the pH versus time traces could only be fitted by one exponential as shown in Fig. 4. Various other models were tested without leading to satisfactory fits of the kinetic traces. The results are summarized in Table 3.

The kinetic and equilibrium results can be interpreted in terms of the kinetic scheme.

$$\begin{array}{c} \mathrm{Zn(NTP)(MeGly)(OH_2)} + \mathrm{OH^-} \overset{\mathrm{K}}{\rightleftharpoons} \mathrm{ZN(NTP)(MeGly)(OH)} + \mathrm{H_2O} \\ \downarrow k \\ \mathrm{Zn(NTP)(Gly)} + \mathrm{MeOH} \end{array}$$

Hydrolysis takes place by reaction of the coordinated hydroxide with the nitrogen-bonded amino acid ester. The plots of k_{obs} versus the hydroxide ion concentration are non-linear and show a pronounced curvature Fig. 5 [35]. The kinetic data was fitted by assuming that the

Table 2 Formation constants of $M_pL_qH_r$ species

System	p	q	r[a]	$\log_{10}\beta$[b]	S[c]	$\log_{10} K^{Zn}_{ZnL}$	$\Delta\log_{10} K$
Zn(NTP)–OH	1	0	−1	−9.98(0.02)	7.5E–8	–	–
	1	0	−2	−22.45(0.06)			
Glycine	0	1	1	9.60(0.01)	1.5E–7	4.96	−1.84
	0	1	2	11.92(0.03)			
	1	1	0	3.12(0.01)	4.8E–8		
	1	1	−1	−7.84(0.02)			
Alanine	0	1	1	9.69(0.01)	9.2E–8	4.58	−1.68
	0	1	2	11.88(0.02)			
	1	1	0	2.95(0.03)	7.3E–8		
	1	1	−1	−7.02(0.01)			
Proline	0	1	1	10.52(0.01)	7.9E–9	5.27	−1.15
	0	1	2	12.03(0.02)			
	1	1	0	4.12(0.03)	2.2E–7		
	1	1	−1	−7.16(0.04)			
Threonine	0	1	1	9.06(0.01)	7.9E–9	4.63	−1.16
	0	1	2	11.03(0.02)			
	1	1	0	3.48(0.009)	2.2E–8		
	1	1	−1	−6.38(0.007)			
Serine	0	1	1	9.14(0.01)	1.7E–8	4.65	−1.72
	0	1	2	11.40(0.01)			
	1	1	0	3.30(0.01)	5.2E–8		
	1	1	−1	−6.21(0.006)			
Methionine	0	1	1	9.10(0.01)	1.9E–9	4.38	−1.26
	0	1	2	11.08(0.02)			
	1	1	0	3.25(0.01)	1.6E–8		
	1	1	−1	−6.36(0.006)			
Imidazole	0	1	1	7.04(0.01)	2.6E–9	2.55	0.04
	1	1	0	2.59(0.07)	1.4E–7		
	1	1	−1	−4.72(0.01)			
Methylamine	0	1	1	10.55(0.004	8.9E–9	3.59	0.73
	1	1	0	4.32(0.03)	2.7E–7		
	1	1	−1	−6.95(0.06)			
Histidine	0	1	1	9.53(0.01)	1.6E–7	6.51	−2.47
	0	1	2	15.81(0.03)			
	0	1	3	17.81(0.06)			
	1	1	0	4.04(0.02)	1.5E–7		
	1	1	−1	−6.77(0.03)			
Histamine	0	1	1	9.82(0.01)	2.4E–8	5.22	−1.30
	0	1	2	15.97(0.01)			
	1	1	0	3.92(0.08)	2.7E–7		
	1	1	−1	−6.46(0.06)			

Table 2 (*Continued*)

System	p	q	r[a]	$\log_{10}\beta$[b]	S[c]	$\log_{10} K^{Zn}_{ZnL}$	$\Delta \log_{10} K$
Lysine	0	1	1	10.52(0.01)	1.6E–7	4.11	0.38
	0	1	2	19.65(0.03)			
	0	1	3	21.91(0.04)			
	1	1	0	4.49(0.004)	6.3E–9		
	1	1	1	14.25(0.007)			
	1	1	−1	−6.65(0.008)			
Ornithine	0	1	1	10.57(0.01)	1.6E–7	3.75	0.39
	0	1	2	19.43(0.03)			
	0	1	3	21.38(0.02)			
	1	1	0	4.14(0.01)	3.0E–8		
	1	1	1	13.70(0.01)			
	1	1	−1	−7.04(0.01)			
Mercapto-propionic acid	0	1	1	9.88(0.01)	5.9E–8	6.32	−2.17
	0	1	2	13.77(0.01)			
	1	1	0	4.25(0.06)	4.5E–7		
	1	1	−1	−7.12(0.08)			
Cysteine	0	1	1	10.63(0.01)	4.7E–8	9.11	−3.16
	0	1	2	19.18(0.02)			
	0	1	3	21.62(0.05)			
	1	1	0	5.95(0.04)	2.8E–7		
	1	1	1	14.90(0.04)			
Ethanolamine	0	1	1	9.62(0.01)	4.6E–8	2.41	0.86
	1	1	0	3.27(0.009)	1.5E–8		
	1	1	−1	−7.05(0.008)			
Glycinamide	0	1	1	7.88(0.01)	4.6E–8	3.28	−0.8
	1	1	0	2.48(0.06)	1.6E–7		
	1	1	−1	−6.03(0.02)			
Glycylglycine	0	1	1	7.97(0.01)	4.6E–8	3.38	−0.98
	1	1	0	2.40(0.05)	7.0E–8		
	1	1	−1	−6.11(0.01)			
Glutamine	0	1	1	9.09(0.01)	4.5E–8	4.12	
	1	1	0	3.23(0.01)	2.0E–8		
	1	1	−1	−6.60(0.008)			
Aspargine	0	1	1	8.55(0.01)	5.9E–8	4.52	−1.96
	1	1	0	2.56(0.02)	3.1E–8		
	1	1	−1	−6.77(0.01)			
Uracil	0	1	1	9.28(0.01)	1.1E–7	3.98	–
	1	1	0	3.39(0.008)	1.4E–8		
	1	1	−1	−6.39(0.005)			

Table 2 (*Continued*)

System	p	q	r[a]	$\log_{10}\beta$[b]	S[c]	$\log_{10} K^{Zn}_{ZnL}$	$\Delta \log_{10} K$
Uridine	0	1	1	9.01(0.01)	1.1E–7	–	–
	1	1	0	2.89(0.01)	1.0E–8		
	1	1	−1	−6.72(0.005)			
Thymidine	0	1	1	9.50(0.01)	8.7E–8	–	–
	1	1	0	2.79(0.01)	2.7E–8		
	1	1	−1	−7.67(0.01)			
Thymine	0	1	1	9.58(0.006)	8.1E–8	–	–
	1	1	0	3.86(0.02)	1.5E–7		
	1	1	−1	−6.26(0.02)			
Inosine	0	1	1	8.43(0.01)	5E–9	–	–
	1	1	0	1.94(0.05)	1.3E–8		
	1	1	−1	−6.67(0.006)			
5′-IMP	0	1	1	9.21(0.01)	2.4E–8	–	–
	0	1	2	15.21(0.01)			
	1	1	0	3.38(0.01)	4.9E–8		
	1	1	−1	−6.34(0.01)			

[a] p, q and r are the stoichiometric coefficients corresponding to Zn(NTP); amino acids, peptides or DNA units; and H^+

[b] Standard deviations are given in parentheses

[c] Sum of squares of residuals (E–x denotes $\times 10^{-x}$)

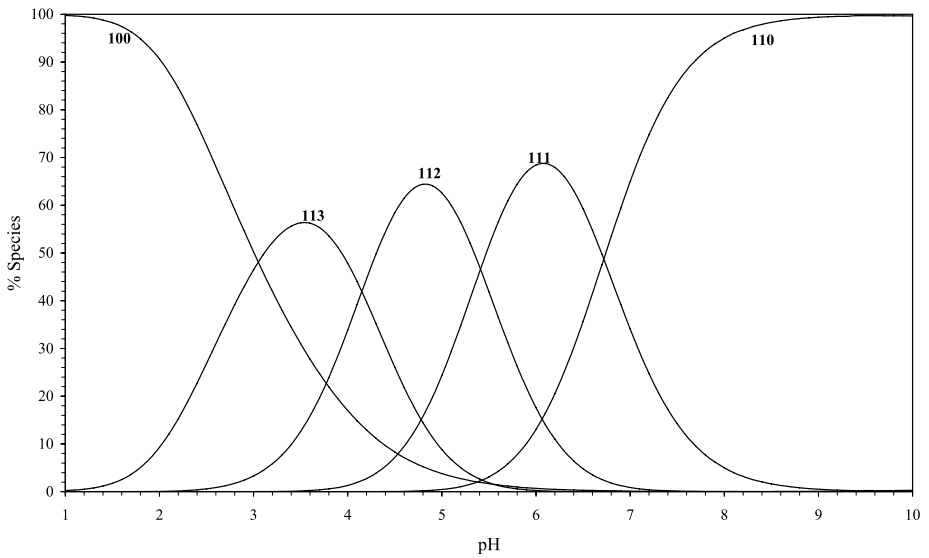

Fig. 2 Concentration distribution of various species as a function of the pH in the Zn–NTP–system

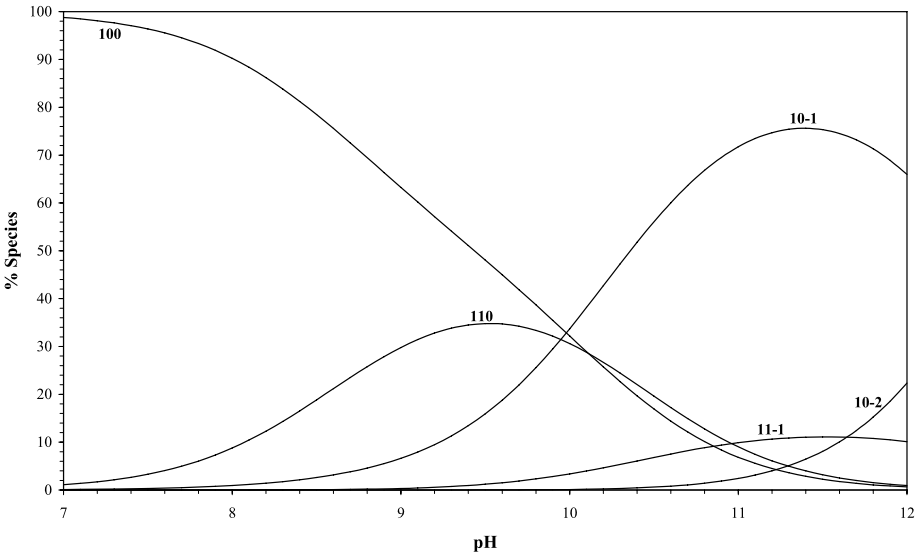

Fig. 3 Concentration distribution of various species as a function of pH in the Zn–NTP–glycine system where (100) denotes $Zn(NTP)^{4-}$, (110) denotes $Zn(NTP)(glycinate)^{5-}$, (11-1) denotes $Zn(NTP)(glycinate)(OH)^{6-}$, (10-1) denotes $Zn(NTP)(OH)^{5-}$, and (10-2) denotes $Zn(NTP)(OH)_2^{6-}$

hydrolysis involves equilibrium formation of the hydroxo complex Zn(NTP)(MeGly)(OH), followed by an intramolecular attack. The rate expression can therefore be represented by Eq. 6 [19],

$$k_{obs} = kK[OH^-]/(1 + K[OH^-]) \tag{6}$$

which can be rewritten as Eq. 7

$$1/k_{obs} = 1/kK[OH^-] + 1/k \tag{7}$$

A plot of $1/k_{obs}$ versus $1/[OH^-]$ should be linear with a slope of $1/kK$ and intercept of $1/k$. Such a plot is found to be linear, Fig. 6. Least-squares analysis gives $k = (2.65 \pm 0.003) \times 10^{-4}$ dm^3·mol^{-1}·s^{-1} and $K = (7.24 \pm 0.02) \times 10^4$ dm^3·mol^{-1} at 25 °C and ($I = 0.1$ mol·dm^{-3}). The rate constant $k = (2.65 \pm 0.003) \times 10^{-4}$ dm^3·mol^{-1}·s^{-1} is the rate constant for intramolecular hydrolysis by coordinated hydroxide in the nitrogen bonded methyl glycinate complex. In this system it has been possible to determine the involvement of a coordinated hydroxide ion in the Zn(II)-catalyzed hydrolysis of the amino acid ester.

K is the formation constant for formation of the hydroxo complex ($\log_{10} K = 4.86$). The pK_a for the ionization of the aqua complex can readily be calculated as:

$$pK_a = pK_W - \log_{10} K = 9.14 \tag{8}$$

Potentiometric titration of $[Zn(NTP)(OH)_2]^{4-}$ with NaOH gives a well defined end point after the addition of one equivalent of sodium hydroxide, which can be attributed to the ionization of one of the coordinated water molecules. The value of pK_a determined from the titration results is 9.98 (±0.02) at 25 °C and $I = 0.1$ mol·dm^{-3}, and is almost in agreement with the kinetically determined pK_a value.

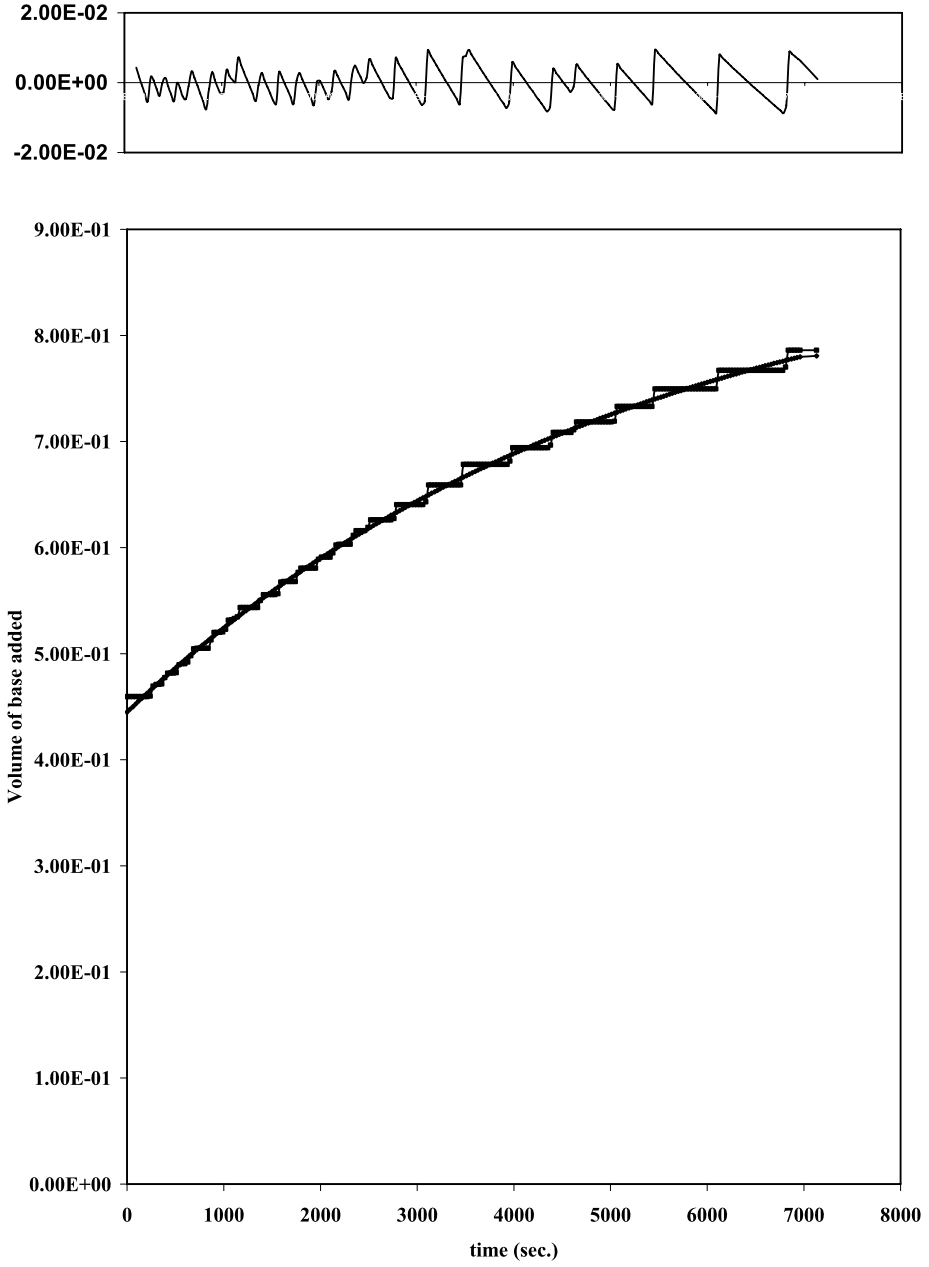

Fig. 4 Typical volume of added base versus time trace for the hydrolysis of Zn–NTP–GlyOMe ester fitted with one exponential function. The *top* of the figure shows the volume of base difference between the measured and calculated kinetic traces at $I = 0.1$ mol·dm^{-3}, pH = 9.4 and $t = 25$ °C

Table 3 The Zn–NTP promoted hydrolysis of glycine methyl ester at 25 °C and $I = 0.1$ mol·dm^{-3} ($NaNO_3$)

Ester	pH	10^6 [OH$^-$]	$10^4 k_{obs}$
GlyOMe	8.8	0.823	0.99 ± 0.1
	9.0	1.304	1.29 ± 0.1
	9.2	2.067	1.55 ± 0.2
	9.4	3.276	1.93 ± 0.3
	9.6	5.192	2.03 ± 0.1

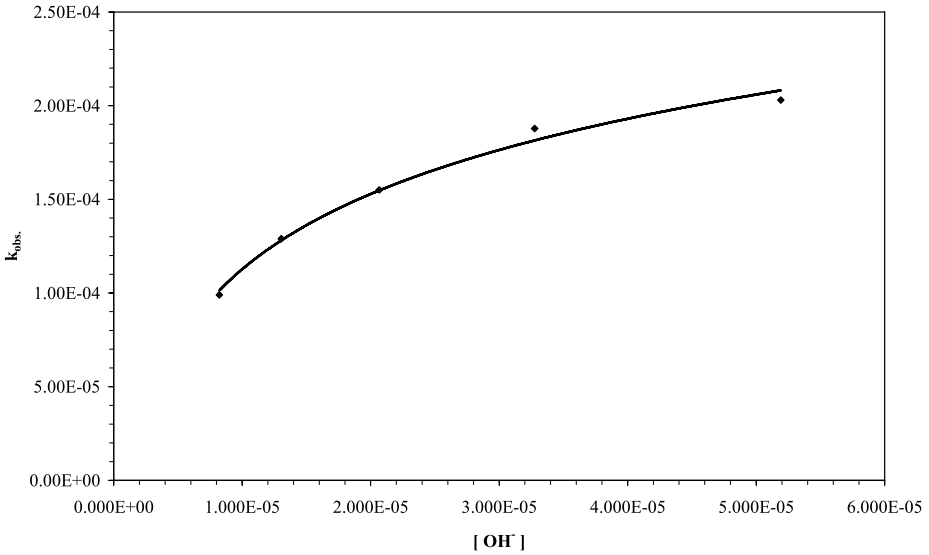

Fig. 5 Plot of k_{obs} versus the hydroxide ion concentration for the Zn–NTP promoted hydrolysis of GlyOMe at 25 °C and $I = 0.1$ mol·dm^{-3}

6 Conclusions

The present investigation describes the formation equilibria of Zn(II) complexes involving tris(methyl phosphonic acid) (NTP) and some ligands of biological significance. In combination with the stability constants of such Zn(II) complexes with amino acids, peptides and DNA constituents, it will be possible to calculate the equilibrium distribution diagrams of the metal species in biological fluids where all types of ligands are present simultaneously. This would form a clear basis for understanding the mode of action of such metal species under physiological conditions. From the above results it may be concluded that ternary complex formation proceeds through a stepwise mechanism. Amino acids form highly stable complexes, whereas the extra amino group in ornithine does not contribute to the stability of the complexes formed because the extra amino group is not competing with the carboxylic group in complex formation. The negative values obtained for $\Delta \log_{10} K$ fall in the order: tridentate (as histidine) > bidentate (as glycine) > monodentate (as imidazole) ligand.

The Zn(II) chelate promoted hydrolysis of an amino acid ester may be accounted by the proposed intramolecular mechanism. This trend is supported by the Hay and Yau-Quan studies [35]. The pK_a for ionization of the aqua complex from potentiometric titration is in agreement with the kinetically determined pK value.

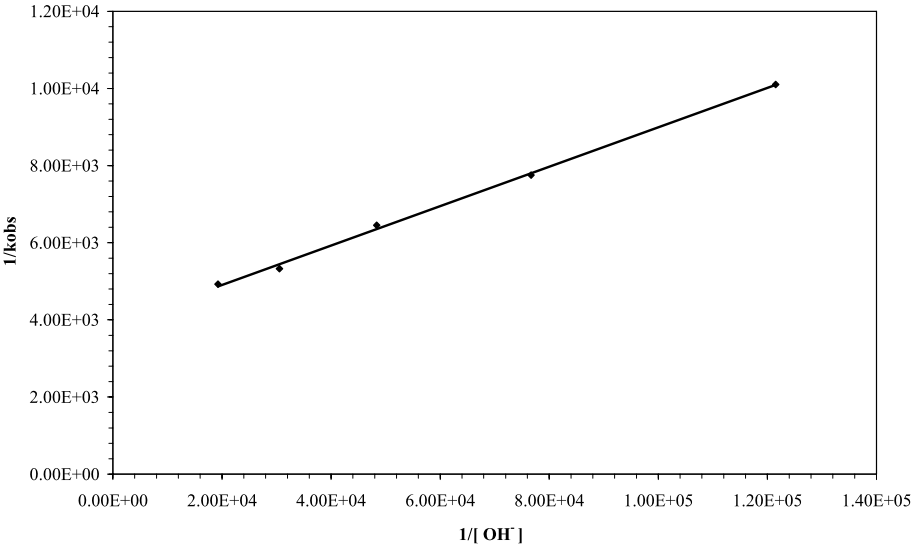

Fig. 6 Double reciprocal plot for the Zn–NTP promoted hydrolysis of GlyOMe at 25 °C and $I = 0.1\ \text{mol}\cdot\text{dm}^{-3}$

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