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Kinetics and Mechanism for Hydrolysis of α-Amino Acid Esters in Mixed Ligand Complexes with Zn(II)–Nitrilo-tris(methyl phosphonic Acid)

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Abstract The kinetics of base hydrolysis of the alanine ethyl ester, in addition to glycine, histidine and methionine methyl esters in the presence of the Zn-NTP complex, were studied in aqueous solution by the pH-potentiometric technique, where NTP denotes the nitrilo-tris(methyl phosphonic acid) ligand. The kinetic data fits assumed that hydrolysis proceeds through formation of a M–OH complex, followed by an intramolecular OH^- attack. The effect of an organic solvent on the hydrolysis of coordinated esters was investigated by measuring the rate of hydrolysis in dioxane–water solutions of different compositions at $t = 25.0\,^{\circ}C$ and $I = 0.1$ mol·dm^{-3}. The kinetics of base hydrolysis of the glycine methyl ester was studied at different temperatures. Activation parameters for the base hydrolysis of the complexes were evaluated.

Keywords Zn(II) · Formation equilibria · Nitrilo-tris(methyl phosphonic acid) · Kinetics of hydrolysis · Rate constant · Amino acid ester

1 Introduction

Natural and synthetic aminopolyphosphonic molecules are very effective ligands in many cases with high specificity, for metal ions. This class of compounds and their derivatives have received considerable attention because of their interesting biological activity. They include a variety of herbicides, plant growth regulators, antibodies and inhibitors of metallo-enzymes [1]. For example, N,N′-di(phosphenomethyl)glycine is known as a plant growth regulator [2], whereas N-(phosphenomethyl)glycine is an active ingredient of a popular herbicide [3].

Ternary complexes of α-amino acid esters are known to promote ester hydrolysis, which is an important elucidating feature for bioprocesses, and this topic has been the subject of several studies [4–6]. Also, ternary complexes involving amino acid esters can be regarded

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as mimicking a metallo-enzyme–substrate complex, and they are therefore of considerable interest in the study of the hydrolysis of ester ligands in such complexes.

Newlin et al. [7] have studied the hydrolysis of amino acid esters in mixed ligand complexes of zinc(II) with H_3NTA (nitrilotriacetic acid). Bedell and Nakon [8] investigated the hydrolysis of glycine methyl ester in the presence of [Zn(EGDA)] (EGDA = ethyl glycinate-*N*,*N*-diacetic acid). In view of the above and in conjunction with our research program [9–13] directed to study the kinetics of α-amino acid esters under the effect of complex formation and metal complexes of biological significance, it is desirable to extend these investigations to the mixed complex of Zn(II) with nitrilo-tris(methyl phosphonic acid) (NTP), which provides information regarding the behavior of this class of ligands in biological systems and also helps to solve some of the mechanistic problems associated with these systems.

2 Experimental

2.1 Materials and Reagents

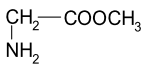
All of the source reagents were of Analar grade. Nitrilo-tris(methyl phosphonic acid) NTP, the tris-phosphonic derivative of nitrilotriacetic acid (NTA), was obtained from Aldrich Chem. Co., and $Zn(NO_3)_2$ was provided by BDH. The glycine methyl ester was purchased from Fluka. The α-amino acid esters provided by Fluka are glycine methyl ester·HCl (GlyOMe), DL-alanine ethyl ester·HCl (DL-AlaOEt), L-histidine methyl ester·2HCl (L-HisOMe), and L-methionine methyl ester·HCl (L-MetOMe). All chemicals were used without further purification. Carbonate-free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized H_2O.

2.2 Kinetic Measurements

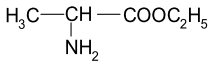
The kinetics of hydrolysis was monitored using a Metrohm 751 Titrino operated with the set mode. The titroprocessor and electrode were calibrated with standard buffer solutions according to NIST specifications [14]. The kinetics of hydrolysis of the complexed ester was investigated by using aqueous solutions (40 cm^3) of a mixture of Zn(II) (6.25×10^{-3} mol·dm^{-3}), NTP (6.87×10^{-3} mol·dm^{-3}), amino acid 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 Zn(II) was used to ensure coordination of all Zn(II). A 20% excess of NTP gave the same rates as with a 10% excess.

The temperature of all solutions was maintained at the desired temperature by circulation of thermostatted water through the outer jacket of the cell. The solutions were stirred with a magnetic stirrer under a constant nitrogen flow at an ionic strength of 0.1 mol·dm^{-3} ($NaNO_3$). The ester solution was then added, and the pH brought to the desired value by the automatic addition of 0.05 mol·dm^{-3} NaOH as described previously [9–13]. The data fitting was performed with the OLIS KINFIT set of programs [15] as indicated previously [9–13]. Values of the hydroxide ion concentration were estimated from the pH using $pK_w = 13.997$, and an activity coefficient of 0.772 was calculated from the Davies equation [16]. At the other temperatures studied, the following values of pK_w and γ were employed: at 15.0 °C ($pK_w = 14.34$, $\gamma = 0.776$), at 35.0 °C ($pK_w = 13.68$, $\gamma = 0.768$), and at 45.0 °C ($pK_w = 13.376$, $\gamma = 0.764$).

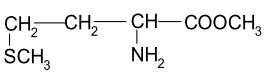
The structural formulae of the investigated amino acid esters are given in Scheme 1.



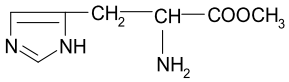
GlyOMe



AlaOEt



MetOMe



HisOMe

Scheme 1 Structural formulae of the investigated ligands

Table 1 Kinetics of hydrolysis of the coordinated glycine methyl ester at different temperatures in aqueous solutions $I = 0.1$ mol·dm^{-3} ($NaNO_3$)

Temperature	pH	[OH$^-$] (mol·dm^{-3})	$10^4 k_{obs.1}$ (s^{-1})	$10^4 k_{OH}$ ($dm^3 \cdot mol^{-1} \cdot s^{-1}$)
Glycine methyl ester				
15 °C	8.80	3.72E−6	0.66 ± 0.3	1.67
	9.00	5.89E−6	0.84 ± 0.1	
	9.20	9.34E−6	1.00 ± 0.2	
	9.40	1.48E−5	1.23 ± 0.4	
	9.60	2.34E−5	1.36 ± 0.3	
25 °C	8.80	0.82E−5	0.99 ± 0.1	2.65
	9.00	1.30E−5	1.29 ± 0.1	
	9.20	2.06E−5	1.55 ± 0.2	
	9.40	3.27E−5	1.93 ± 0.3	
	9.60	5.19E−5	2.11 ± 0.1	
35 °C	8.80	1.72E−5	1.45 ± 0.3	4.64
	9.00	2.72E−5	2.01 ± 0.1	
	9.20	4.31E−5	2.62 ± 0.1	
	9.40	6.83E−5	2.98 ± 0.1	
	9.60	10.8E−5	3.30 ± 0.2	
45 °C	8.80	3.32E−5	1.91 ± 0.1	7.03
	9.00	5.26E−5	2.52 ± 0.1	
	9.20	8.34E−5	3.61 ± 0.2	
	9.40	1.32E−5	4.25 ± 0.1	
	9.60	2.09E−5	4.70 ± 0.2	

3 Results and Discussion

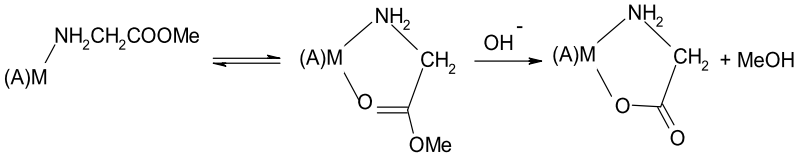
Hydrolysis of the coordinated ester was monitored over the pH range (8.8–9.6) as shown in Tables 1–3. In this range, the rate of hydrolysis of the α-amino acid ester is negligible

Table 2 Kinetics of hydrolysis of the ester group in the complexes Zn(NTP)L[a] at 25 °C and I = 0.1 mol·dm^{-3} ($NaNO_3$)

Ester	pH	10^5 [OH^-] (mol·dm^{-3})	$10^4 k_{obs.}$ (s^{-1})	$10^4 k_{OH}$ (dm^3·mol^{-1}·s^{-1})
DL-AlaOEt	8.80	0.82	0.70 ± 0.1	2.27
	9.00	1.30	0.96 ± 0.2	
	9.20	2.06	1.20 ± 0.1	
	9.40	3.27	1.50 ± 0.2	
	9.60	5.19	1.62 ± 0.3	
L-MetOMe	8.80	0.82	0.94 ± 0.2	2.14
	9.00	1.30	1.16 ± 0.1	
	9.20	2.06	1.37 ± 0.3	
	9.40	3.27	1.63 ± 0.1	
	9.60	5.19	1.82 ± 0.2	
L-HisOMe	8.80	0.82	0.33±0.1	0.95
	9.00	1.30	0.44 ± 0.2	
	9.20	2.06	0.53 ± 0.1	
	9.40	3.27	0.69 ± 0.3	
	9.60	5.19	0.71 ± 0.1	

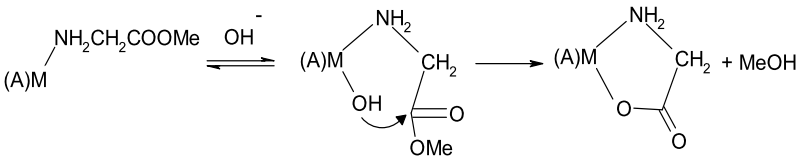
[a]L = DL-AlaOEt, L-HisOMe, and L-MetOMe

in the absence of $Zn(NTP)^{4-}$. Metal ion promoted hydrolysis of amino acid esters has been studied by a number of research groups [17–20]. The first-order kinetic dependence on OH^- concentration may be accounted for by three mechanisms. One involves an initial, rapidly established, equilibrium in which the carbonyl oxygen of the ester group coordinates to the metal ion, followed by a rate-determining OH^- attack (Eq. 1):



(1)

The second mechanism involves rapid equilibrium formation of a M–OH complex, followed by an intramolecular OH^- attack (Eq. 2):



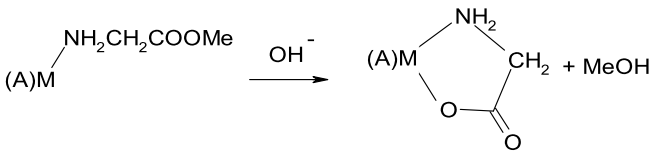
(2)

The third mechanism involves only OH^- attack on the uncoordinated carbonyl carbon of the ester group (Eq. 3):

Table 3 Kinetics of hydrolysis of the coordinated glycine methyl ester in dioxane–water solutions of different compositions at 25 °C and $I = 0.1$ mol·dm^{-3} ($NaNO_3$)[a]

Dioxane (%)	pH	10^6 [OH$^-$] (mol·dm^{-3})	$10^5 k_{obs.1}$ (s^{-1})	$10^4 k_{OH}$ (dm^3·mol^{-1}·s^{-1})
Glycine methyl ester				
12.50	8.80	3.31	5.70 ± 0.3	2.32
	9.00	5.24	8.20 ± 0.1	
	9.20	8.32	10.50 ± 0.2	
	9.40	13.1	13.50 ± 0.4	
	9.60	20.9	15.00 ± 0.4	
25.0	8.80	1.20	4.50 ± 0.1	2.62
	9.00	1.90	7.00 ± 0.1	
	9.20	3.02	9.70 ± 0.2	
	9.40	4.78	11.80 ± 0.2	
	9.60	7.58	13.80 ± 0.3	
37.50	8.80	0.46	3.50 ± 0.3	3.04
	9.00	0.74	5.50 ± 0.1	
	9.20	1.17	8.20 ± 0.1	
	9.40	1.86	10.20 ± 0.1	
	9.60	2.95	12.70 ± 0.1	
50.0	8.80	0.22	2.60 ± 0.1	4.56
	9.00	0.34	4.20 ± 0.1	
	9.20	0.55	6.32 ± 0.2	
	9.40	0.87	8.71 ± 0.1	
	9.60	1.38	12.0 ± 0.1	

[a]The pK_w values are 14.28, 14.72, 15.13 and 15.46 for 12.5%, 25%, 37.5% and 50.0% dioxane in water, respectively. These data were taken from Ref. [9]



(3)

The volume of base added to keep the pH constant versus time traces, could only be fitted by one exponential as shown in Figs. 1 and 2. Various other models were tested without leading to satisfactory fits of the kinetic traces. The plot of k_{obs} versus the hydroxide ion concentration is not linear and shows a pronounced curvature, see Fig. 3 [21]. which is consistent with the second mechanism (Eq. 2).

Hydrolysis takes place by reaction of a coordinated hydroxide with the nitrogen-bonded amino acid ester. Kinetic data was fitted assuming that the hydrolysis involves equilibrium formation of the hydroxo complex followed by an intramolecular attack. The same mechanism was proposed for the hydrolysis of an amino acid ester in the mixed-ligand complex

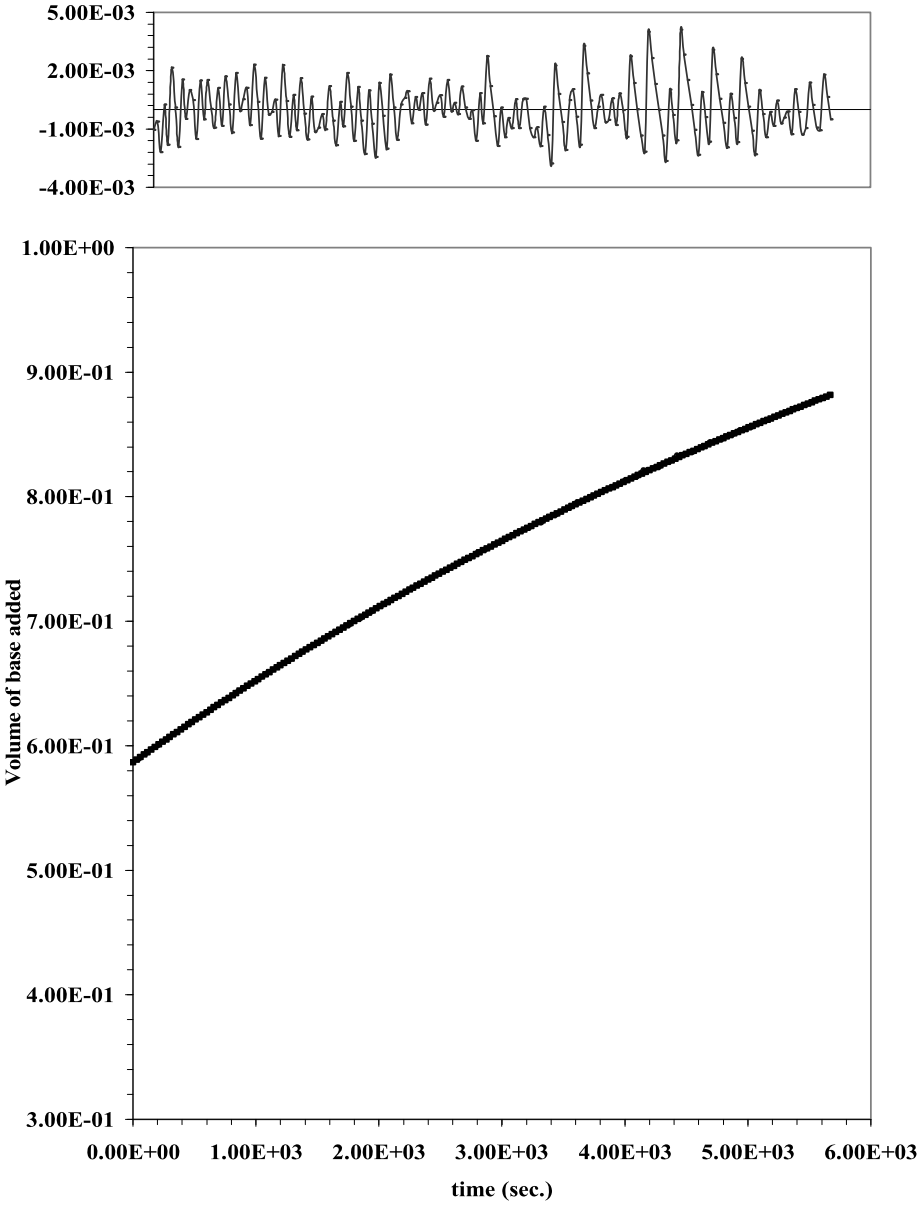


Fig. 1 Typical volume of base added versus time trace for the hydrolysis of coordinated AlaOEt fitted with one exponential function. The top of the figure shows the volume of base difference between the measured and calculated kinetic traces. Conditions: $I = 0.1$ mol·dm^{-3} ($NaNO_3$), pH = 9.0, and $t = 25$ °C

of Cu(II) with dipyridylamine [21]. The rate expression can therefore be represented by Eq. 4 [21]:

$$k_{\text{obs}} = kK\left[\text{OH}^-\right]/\left(1 + K\left[\text{OH}^-\right]\right) \tag{4}$$

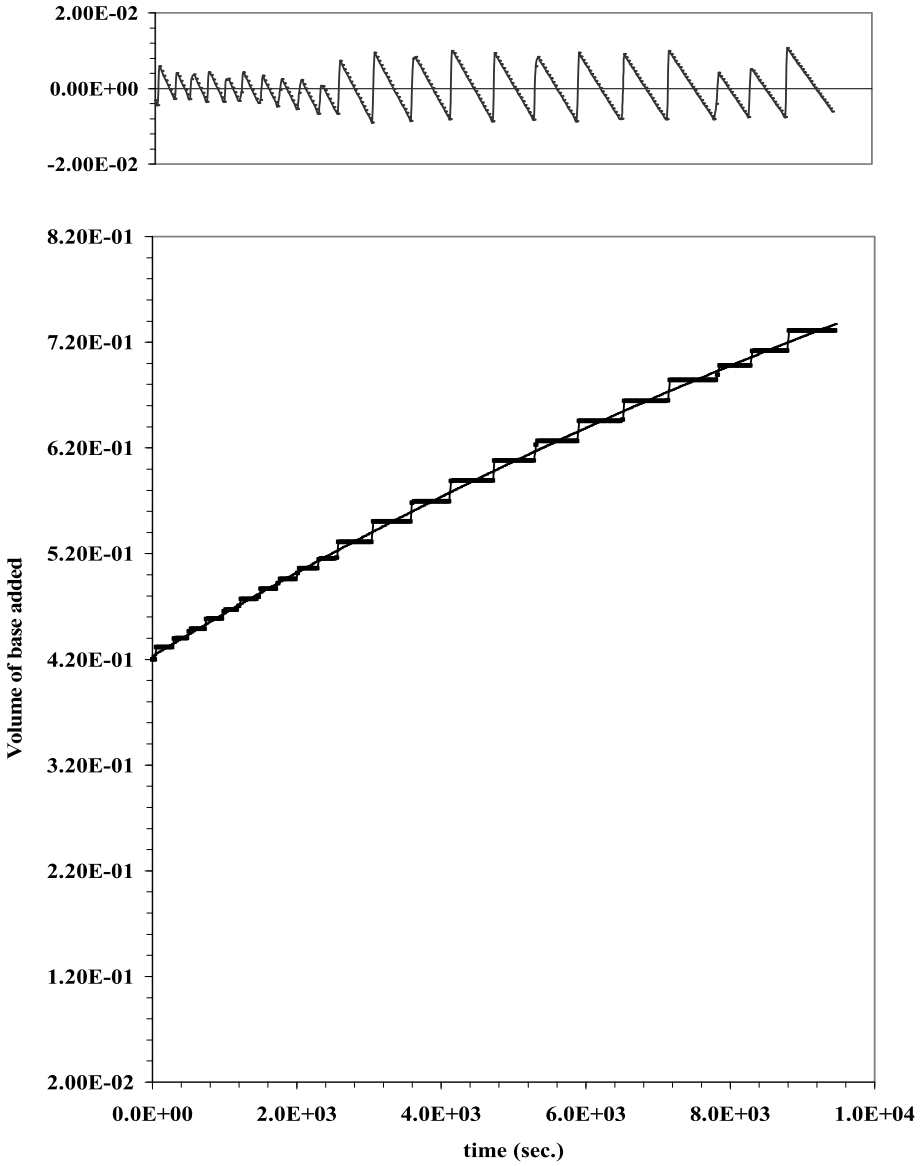


Fig. 2 Typical volume of base added versus time trace for the hydrolysis of coordinated HisOMe fitted with one exponential function. The top of the figure shows the volume of base difference between the measured and calculated kinetic traces. Conditions: $I = 0.1$ mol·dm^{-3} ($NaNO_3$), pH = 9.2, and $t = 25\,^{\circ}C$

which can be rewritten as Eq. 5

$$1/k_{obs} = 1/kK\left[OH^-\right] + 1/k \tag{5}$$

A plot of $1/k_{obs}$ versus 1/[OH^-] should be linear with a slope of $1/kK$ and an intercept of $1/k$, Fig. 4. Here K is the formation constant of the hydroxo complex and k is the rate con-

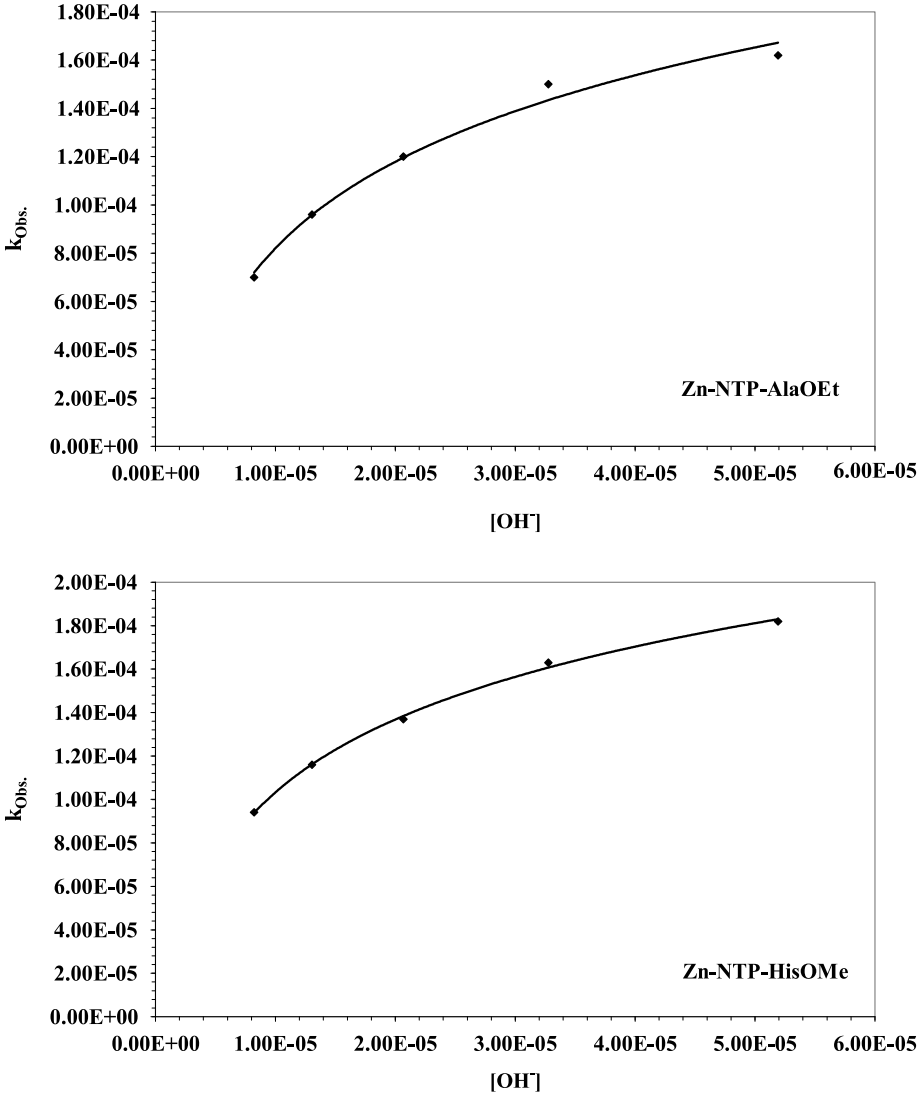


Fig. 3 Plots of k_{obs} versus [OH^-] for the hydrolysis of coordinated AlaOEt and HisOMe at 25 °C

stant for intramolecular hydrolysis by coordinated hydroxide in the nitrogen-bonded amino acid ester complex.

Activation parameters ($\Delta S^{\neq}$ and $\Delta H^{\neq}$) for the hydrolysis of the coordinated glycine methyl ester, as a representative example of α-amino acid esters, were determined from the temperature dependence of the data in Table 1 using an Eyring plot of $\ln(k_{OH}/T)$ versus $1/T$ as displayed in Fig. 5. The slope of the plot is $\Delta H^{\neq}/R$ and the intercept is related to $\Delta S^{\neq}$ by Eq. 6 (where K, h and R are the Boltzmann, Plank and gas constants, respectively):

$$\Delta S^{\neq} = \left[\text{intercept} - \ln(K/h)\right]R \tag{6}$$

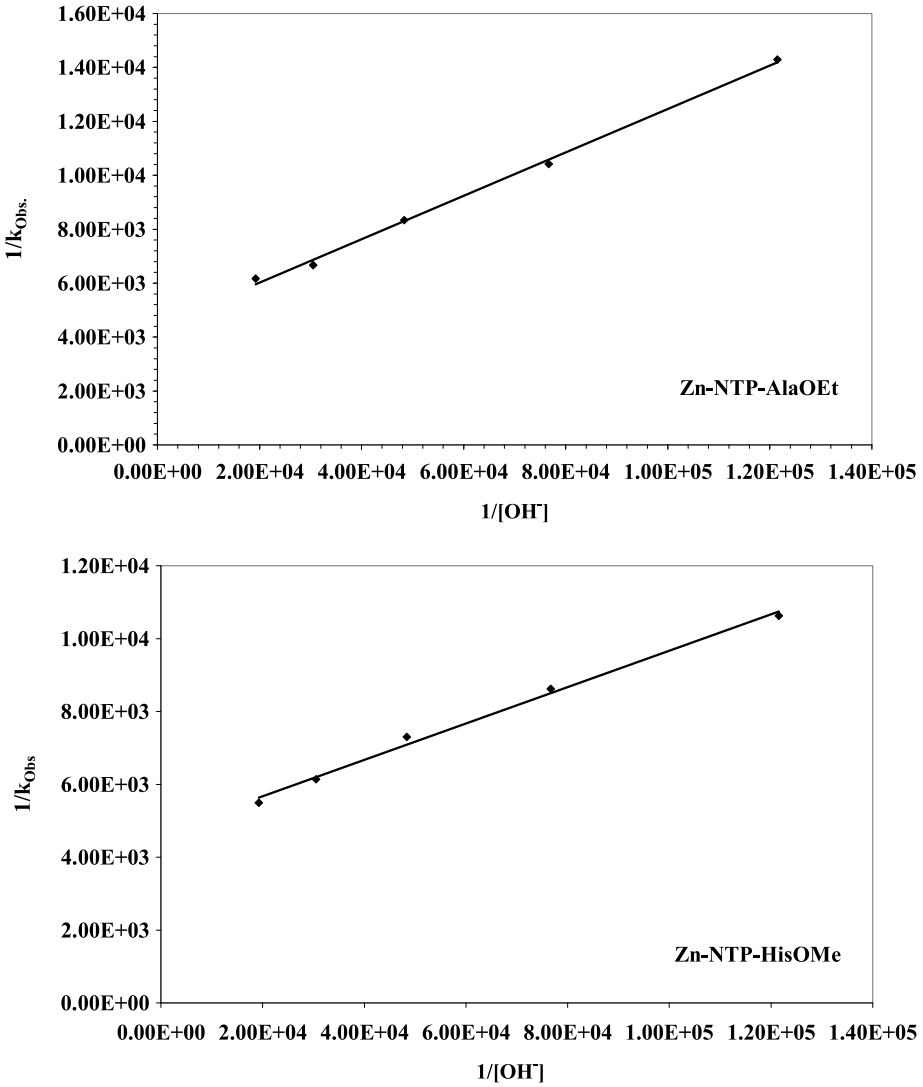


Fig. 4 Double reciprocal plots for the Zn–NTP promoted hydrolysis of AlaOEt and HisOMe at 25 °C and $I = 0.1$ mol·dm^{-3}

The activation parameters were determined for the hydrolysis of $[Zn(NTP)(GlyOMe)]^{4-}$ at $I = 0.1$ mol·dm^{-3}. The temperature dependence of the rate constants k_{OH} is summarized in Table 1.

The values obtained for base hydrolysis of GlyOMe incorporated in the [Zn–NTP] complex can be compared with those of the free ester [22]. For base hydrolysis of the free glycine methyl ester, the activation parameters are found to be $\Delta H^{\neq} = 39.7$ kJ·mol^{-1} and $\Delta S^{\neq} = -117$ J·K^{-1}·mol^{-1}. The values for coordinated GlyOMe are $\Delta H^{\neq} = 34.5$ kJ·mol^{-1} and $\Delta S^{\neq} = -197$ J·K^{-1}·mol^{-1}. It can thus be concluded that the enhanced rate for base hydrolysis is due to contributions from a decreased value of $\Delta H^{\neq}$. The entropy of activation ($\Delta S^{\neq}$) is related to the charge of the complex. Therefore, positively charged complexes

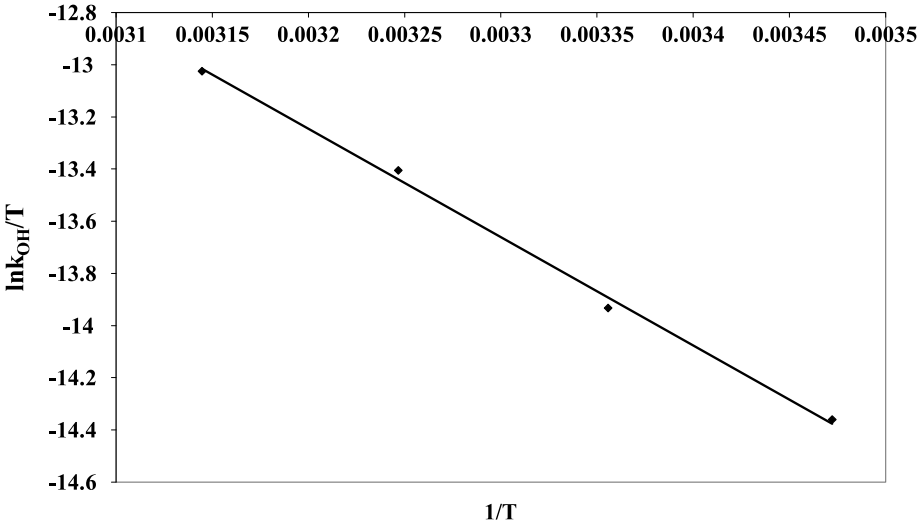


Fig. 5 Plot of $\ln k_{OH}/T$ versus $1/T$ for the hydrolysis of the coordinated glycine methyl ester

reacting with OH^- are expected to have smaller entropies of activation due to charge neutralization in the transition state, leading to desolvation. Neutral and negatively charged complexes will have more negative entropies because of the developing charge in the transition state.

Least-squares analysis gives $k = 2.65 \times 10^{-4}$ mol^{-1}·dm^3·s^{-1}, 2.27×10^{-4} mol^{-1}·dm^3·s^{-1}, 2.14×10^{-4} mol^{-1}·dm^3·s^{-1} and 9.5×10^{-5} mol^{-1}·dm^3·s^{-1} for GlyOMe, AlaOEt, MetOMe and HisOMe, respectively, at 25.0 °C and $I = 0.1$ mol·dm^{-3} $NaNO_3$ as given in Table 2.

Traditionally, water has been considered to be the solvent that best represents biological conditions. Although this is a general assumption, a lower polarity has been detected in some biochemical microenvironments, such as the active sites of enzymes and side chains in proteins where the dielectric constant values are 30–50 [23–27]. It has been suggested that these properties approximately correspond to those (or can be simulated by those) existing in water/dioxane mixtures. Consequently, investigation of amino acid ester hydrolysis in water–dioxane mixture is of biological significance.

In order to examine the effect of organic solvents on hydrolysis of the esters, the rate constants for hydrolysis of coordinated esters were determined in various dioxane–water solutions of different compositions. The rate constant values (k_{OH}), given in Table 3, increase with increasing amount of dioxane. This may be explained on the premise that, as the dioxane content increases, the dielectric constant of the solution decreases. This favors the interaction of a negatively charged OH^- ion with the electropositive carbon atom of the ester. Consequently, hydrolysis proceeds faster.

The kinetics of hydrolysis of α-glycine methyl ester with Cu–NTP [11] is more rapid than with Zn–NTP, due to the fact that direct attack on the carbonyl ester in the case of the Cu–NTP–glycine methyl ester is more effective than the intramolecular OH^- attack on the Zn–NTP–glycine methyl ester.

4 Conclusions

The Zn^{II}–NTP chelate promoted hydrolysis of amino acid esters may be accounted for by the proposed intra-molecular mechanism. This trend is supported by Hay and Chen's study [21]. The Cu^{II}–NTP chelate-promoted hydrolysis was accounted for by an external OH^- attack on the carbonyl ester. This may be explained on the premise that it is unlikely that an OH^- ion would add to form a stable five coordinated derivative, because Cu^{II} is known to prefer four coordination [4]. Hydrolysis of α-amino acid esters is significantly catalyzed and the enhanced rate for base hydrolysis is due to contributions from a decreased value of $\Delta H^{\neq}$.

Hydrolysis of the glycine methyl ester was investigated in dioxane–water solutions of different composition to simulate conditions that resemble those under biological conditions with comparable dielectric constants. The solvent effect on the hydrolysis of esters shows that, as the dielectric constant of the medium decreases (by increasing the dioxane content), hydrolysis of the ester is favored. This is interesting from the biological point of view since the solutions in biochemical microenvironments have dielectric constant values of 30–50, whereas the dielectric constant of water is 76.

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