Inorganica Chimica Acta, *157* (1989) 233–238

Mono and Dinuclear M^{2+} Chelates as Catalysts for the Hydrolysis of Organophosphate Triesters

R. G. CLEWLEY, H. ŚLEBOCKA-TILK and R. S. BROWN

Department of Chemistry, University of Alberta, Edmonton, Alta., T6G 2G2, Canada

(Received March 7, 1988; revised August 4, 1988)

Abstract

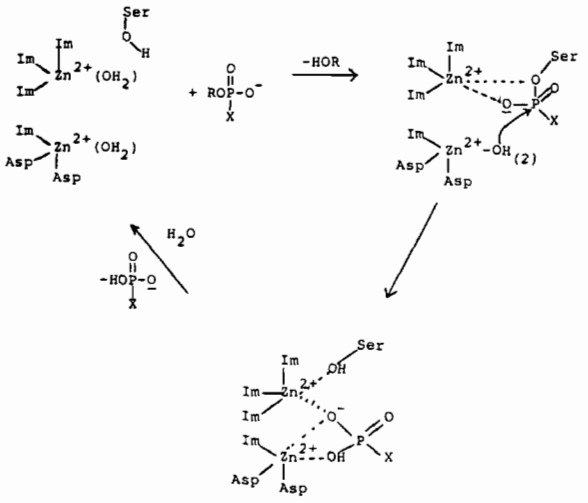
N,*N*,*N*′,*N*′-Tetrakis(1-methylimidazol-2-ylmethyl)-pentane-1,5-diamine (**1**) and bis(1-methylimidazol-2-ylmethyl)ethylamine (**2**) were prepared and their Zn^{2+} and Cu^{2+} complexes studied as catalysts of the hydrolysis of tris-4-nitrophenyl phosphate (**3**) in 33% ethanol–water. A M^{2+}-bound H_2O undergoes ionization in all the $1{:}2M^{2+}$ and $2{:}M^{2+}$ complexes. With $1{:}2Zn^{2+}$ and $2{:}Zn^{2+}$, both the acidic and the basic species promote the hydrolysis of **3**, as do their corresponding Cu^{2+} complexes. There is no evidence of a pre-equilibrium formation of ternary $\mathbf{3}{:}L{:}nM^{2+}$ complexes, so the basic forms of the complexes are acting as bimolecular general bases or nucleophiles towards **3**. The observed order of activity ($\mathbf{2}{:}Cu^{2+} > \mathbf{2}{:}Zn^{2+} > \mathbf{1}{:}2Cu^{2+} > \mathbf{1}{:}2Zn^{2+}$) is a result of the nucleophilicity/basicity of the M^{2+}-bound OH^- decreasing as the pK_a decreases.

Introduction

The hydrolysis of neutral phosphorous esters has received considerable attention due to the possible significance for enzymatic catalysis of phosphoryl transfer and due to the interest in detoxification of acetyl-cholinesterase inhibiting insecticides and chemical warfare agents. Accelerations in the hydrolysis of such esters have principally been effected by the use of cationic micelles [1] and functionalized surfactants [2]; in a few cases the presence of metal ions [3] has been shown to accelerate solvolysis. Our initial work [4] and subsequently that of Breslow and his coworkers [5] has shown that M^{2+} complexes can act as effective nucleophiles in facilitating the destruction of neutral phosphorous esters.

While many of these studies may have biological significance, it must be noted that phosphate triesters are apparently not prominent naturally occurring substances, and enzymes that catalyze phosphoryl transfers in living systems typically act upon anionic substrates [6]. It would therefore be more appropriate to investigate agents that catalyze the hydrolysis of anionic phosphate esters as models for the various phosphoryl transfer enzymes.

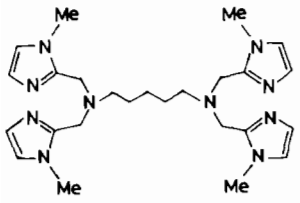
E. coli alkaline phosphatase [7] is a dimeric enzyme that catalyzes the nonspecific hydrolysis of phosphate monoesters. Each active site contains three M^{2+} ions, of which two are Zn^{2+} separated by 3.9 Å and which are required for enzymatic phosphorylation and dephosphorylation. One Zn^{2+} is coordinated to three histidine imidazole groups, and the other to one histidine imidazole and two aspartate carboxylate groups. Also present at the active site is an essential serine. The mechanism of hydrolysis (stylized in Scheme 1), involves nucleophilic displacement by the serine hydroxyl group of a phosphate monoester to form a phosphoryl serine intermediate. This is followed by a second displacement at phosphorous by a zinc bound hydroxide for overall retention of configuration. In addition to activating a coordinated H_2O, the cooperative roles of the Zn^{2+} ions may be to: (i) neutralize the negative charge of the phosphate monoester; (ii) reduce the pK_a of the attacking ser-OH group; (iii) assist by coordination the departure of the leaving group.



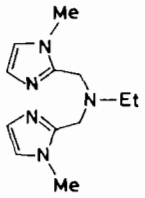
Scheme 1. X = O^-, OH.

0020-1693/89/$3.50

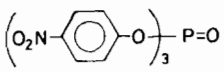
Clearly any reasonable model for the above system must incorporate two M^{2+} ions held in proximity by a suitable ligand. In an effort to address this question, we have prepared ligands **1** and **2** and embarked on a study of the efficacy of their Zn^{2+} and Cu^{2+} complexes in promoting the hydrolysis of a series of mono, bis, and tris phosphate esters. Herein we report the initial results of our studies with a latter species, namely tris-4-nitrophenyl phosphate (**3**).



1



2



3

Experimental

Tris-4-nitrophenyl phosphate (**3**) [8], 2-methylimidazole-1-carboxaldehyde (**4**) [9], 1-hydroxymethyl-2-methylimidazole (**5**) [10] and 1-chloromethyl-2-methylimidazole hydrochloride (**6**) [11] were prepared according to published procedures.

Syntheses of Ligands 2 and 1

Bis(1-methylimidazol-2-ylmethyl)amine (8)

To a solution of 20.0 g of **6** in 250 ml of DMF was added 20 g of NaN_3. The mixture was stirred 2 h, then 200 ml of saturated $NaHCO_3$ solution was added. The solvent was evaporated under reduced pressure and the residue was continuously extracted (Soxhlet) with CH_2Cl_2. Evaporation of the solvent gave 9.0 g of crude 1-azidomethyl-2-methylimidazole (**7**). This was dissolved without further purification in 400 ml of anhydrous THF. To this solution was added 27.0 g of triphenylphosphine. After the initial effervescence had subsided, the solution was refluxed 2 h. Then 8.5 g of **4** in 25 ml of THF was added and the solution was refluxed a further 18 h. After cooling, 200 ml of methanol was added followed by 9.0 g of $NaBH_4$. The mixture was stirred 2 h, then the solvent was evaporated. Water was added to the residue. The mixture was acidified and extracted with $CHCl_3$, then made basic with Na_2CO_3 and again extracted with $CHCl_3$. Concentration of the second extract followed by addition of ether gave 11.3 g of a crystalline product. Recrystallization from benzene gave 8.1 g (60% isolated yield) of white crystals, bis(1-methylimidazol-2-ylmethyl)amine (**8**): melting point (m.p.) 116 °C; ^{1}H NMR ($CDCl_3$) δ 6.97 (s, 2 H), 6.85 (s, 2 H), 3.89 (s, 4 H), 3.65 (s, 6 H), 2.12 (br s, 1 H). *Anal.* Calc. for $C_{10}H_{15}N_5$: C, 58.52; H, 7.36; N, 34.12. Found: C, 58.26; H, 7.37; N, 34.48%.

N,N Bis(1-methylimidazol-2-ylmethyl)acetamide (9)

To a solution of 2.57 g (12.5 mmol) of **8** in 25 ml of alcohol free chloroform was added 5.0 g finely powdered Na_2CO_3. To this mixture was added dropwise 3.0 g (38 mmol) acetyl chloride in 10 ml of chloroform. After stirring for 17 h, 5 ml of water was added. The mixture was stirred 3 h, then the supernatant solution was decanted and dried over Na_2SO_4. Evaporation of the solvent gave 3.19 g of crude product. Recrystallization from $CHCl_3/Et_2O$ gave 2.01 g of white powder, *N,N*-bis(1-methylimidazol-2-ylmethyl)acetamide (**9**); isolated yield 65%: m.p. 55 °C; ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 1 H), 6.89 (s, 1 H), 6.78 (s, 2 H), 6.78 (s, 2 H), 4.72 (s, 2 H), 4.62 (s, 2 H), 3.66 (s, 6 H), 2.33 (s, 3 H).

To 1.98 g (8 mmol) of **9** under argon was added 40 ml of 2.0 M $BH_3 \cdot Me_3S$ in THF. The solution was refluxed 15 h. The excess BH_3 was destroyed with water, and then the solvent was evaporated and 100 ml of 2 M HCl was added. The mixture was refluxed 1 h. On cooling, it was extracted with CH_2Cl_2, made basic with Na_2CO_3, then again extracted with CH_2Cl_2. Evaporation of the solvent from the second extract after drying over Na_2SO_4 gave 1.50 g of a white powder. Recrystallization from CH_2Cl_2/Et_2O gave 1.37 g of white crystals, bis(1-methylimidazol-2-ylmethyl)ethylamine (**2**); isolated yield 73%: m.p. 108 °C; ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 2 H), 6.83 (s, 2 H), 3.70 (s, 4H), 3.50 (s, 6 H), 2.66 (q, J = 6 Hz, 2 H), 1.08 (t, J = 6 Hz, 3 H); exact mass calc. for $C_{12}H_{19}N_5$ *m/e* 233.1641, found 233.1634.

The preparation of **1** was carried out in analogous fashion. *N,N,N',N'*-Tetrakis(1-methylimidazol-2-ylmethyl)pentanediamide (**10**) was prepared by the reactions of 20 mmol of **8** with 10 mmol of glutaryl chloride. The product **10** was obtained in 57% yield: m.p. 193–194 °C ($CHCl_3/Et_2O$); ^{1}H NMR ($CDCl_3$) δ 6.90 (s, 4 H), 6.78 (s, 4 H), 4.73 (s, 4 H), 4.63 (s, 4 H), 3.64 (s, 12 H), 2.70 (t, 4 H), 2.12 (m, 2 H). Reduction of this diamide with 2.0 M $BH_3 \cdot Me_2S$ in THF gave **1** in a 75% yield. The product **1**, had m.p. 124–125 °C ($CHCl_3/Et_2O$); ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 4 H), 6.81 (s, 4 H), 3.64 (s, 8 H), 3.48 (s, 12 H), 2.48 (m, 4 H), 1.25 (m, 6 H). *Anal.*

Calc. for $C_{25}H_{38}N_{10}$: C, 62.76; H, 7.95; N, 29.28. Found: C, 62.77; H, 7.81; N, 29.55%.

Titrations

Buffers employed were MES (pH 5.3–6.1), MOPS (pH 6.5–7.3), HEPES (pH 7.7–8.1), and CHES (pH 8.5–8.9) and were used as supplied. Solubility requirements of **3** necessitated a 33% *w/w* ethanol–water medium. Buffers were 0.01 M; ionic strength was not kept constant since the addition of salts tended to decrease the solubility of the ligands in this medium. pH readings were made with a Radiometer TTT-2 titrator apparatus using a Radiometer GK2321C combination electrode standardized with Fisher Certified Buffers immediately before use. The pH was determined by subtracting 0.09 units from the meter reading according to the method of Bates *et al.* [12]. pK_a values were determined by potentiometric titration using methods and equipment analogous to those described previously [13]. The values reported for the Zn^{2+} complexes are the average of at least two separate determinations and have an error of ±0.10 unit, while those for the Cu^{2+} complexes are single determinations.

Kinetics

Stock solutions (0.2 M) of **2**:M^{2+} and **1**:2M^{2+} were prepared by adding 0.50 ml of a 0.4 M solution of the ligand in methanol to 0.50 ml of 0.4 M or 0.8 M zinc or copper nitrate in water. The volume of the resulting solution was assumed to be 1.00 ml; the error of dilution involved is thought to be small (<4%). Known volumes of these solutions were added to 2.00 ml of buffer and then the pH was adjusted to the desired value with HCl of KOH in 33% ethanol–water. Note that because of limited solubility of the organic species and complexes, concentrated buffers could not be employed to regulate the pH, nor could salts be added to control the ionic strength. After thermal equilibration, reaction was initiated by injecting 10 μl of a 0.001 M solution of tris-4-nitrophenyl phosphate in THF. Kinetic data were obtained at 25.0 ± 0.2 °C with a Hewlett-Packard 8451 spectrophotometer by observing the rate of appearance of 4-nitrophenol at 320 nm or 4-nitrophenoxide at 400 nm. Reactions were followed to at least 90% completion and in all cases displayed excellent pseudo-first-order kinetics. pH was monitored immediately following a run; the variation did not exceed 0.03 pH units. Pseudo-first-order rate constants (k_{obs}) were evaluated by fitting the absorbance *versus* time data to a standard exponential model ($A_t = A_\infty + (A_0 - A_\infty)e^{-kt}$) by a non-linear least-squares treatment. The reported values are averages of duplicate or triplicate measurements. From the slopes of the plots of k_{obs} *versus* [L:nM^{2+}] were obtained the values for the second order rate constants (k_{cat}^{obs}) for attack of the complexes on **3**.

Results and Discussion

Quantitative titration experiments on solutions consisting of ligand **2** and 1 equiv. of $M(NO_3)_2$ or ligand **1** with 2 equiv. of $M(NO_3)_2$ together with added H^+ in 33% ethanol–water show that in both cases all added H^+ is accounted for by pH 5.0. This is substantially lower than the values obtained in the absence of Zn^{2+} (final pK_a for **1** and **2**, 7.25 and 6.68 respectively). thus complexes must be fully formed by pH 5.0, with M^{2+} being coordinated to all available ligand nitrogens (or, less likely, being coordinated to some of the available nitrogens with the remainder remaining nonprotonated). In any case, continuing the titration shows release of a proton from some group associated with the complex having a well-defined pK_a: 6.99 and 8.16 for **1**:2Zn^{2+} and **2**:Zn^{2+}, respectively; 7.51 and 8.35 for **1**:2Cu^{2+} and **2**:Cu^{2+}, respectively. This is attributed to the ionization of a metal bound water. With **1**:2M^{2+}, if the titration is continued beyond the point where the ionization of this group is complete, there seems to be additional consumption of OH^-, but no well-defined pK_a is obtained since precipitation occurs above pH 8 at the concentrations required for titration.

^1H NMR spectra of 0.02 M solutions of **1** and **2** in methanol-d_4 were determined as a function of added aliquots of $Zn(NO_3)_2 \cdot 6H_2O$ in D_2O. In the absence of Zn^{2+}, the ^1H resonances for **2** appear as a triplet at 1.10 (J = 7 Hz, CH_2CH_3), a quartet at 2.63 (J = 7 Hz, CH_2CH_3), and singlets at 3.62 ($ImCH_3$), 3.75 ($ImCH_2N$), 6.94 and 7.11 (imidazole 4- and 5-H). After addition of an equimolar amount of Zn^{2+}, the peaks attributable to **2** are replaced by a triplet at 1.13 (J = 7 Hz, CH_2CH_3), a quartet at 3.00 (J = 7 Hz, CH_2CH_3), and singlets at 3.80 ($ImCH_3$), 4.12 ($ImCH_2N$), 7.23 and 7.33 (imidazole 4- and 5-H). Further additions of Zn^{2+} do not alter the appearance of the spectrum; it therefore arises from a 1:1 complex of Zn^{2+}:**2**. The signals are all sharp, indicating that no slow dynamic processes are occurring. The imidazoles are equivalent, and from the magnitude of the downfield shifts for each hydrogen it is clear that Zn^{2+} is bound in a tridentate fashion to the amine and imidazole nitrogens.

The NMR spectrum of **1** in methanol-d_4 consists of multiplets centered at 1.30 ($CH_2(CH_2)_3CH_2$) and 2.48 ($CH_2(CH_2)_3CH_2$), and singlets at 3.61 (CH_3), 3.70 ($ImCH_2N$), 6.96 and 7.12 (imidazole 4- and 5-H). Addition of 2 equiv. of Zn^{2+} results in broad signals centered at 1.45 ($CH_2(CH_2)_3CH_2$), 2.90 ($CH_2(CH_2)_3CH_2$), and sharp singlets at 3.82

(CH_3), 4.15 ($ImCH_2N$), 7.24 and 7.38 (imidazole 4- and 5-H). The appearance of the spectrum is not altered upon further additions of Zn^{2+}. The binding sites for Zn^{2+} in the 2:1 complex are clearly symmetrical with each Zn^{2+} bound to two imidazoles and an amine nitrogen as in Zn^{2+}:**2**.

Kinetic Studies

The Zn^{2+} and Cu^{2+} complexes of **1** and **2** all produce accelerations in the rate of hydrolysis of tris-4-nitrophenyl phosphate (**3**). Preliminary experiments showed that each of the ligands and M^{2+} alone also catalyze the hydrolysis of **3**, but at the concentrations and pH values employed the complexes are essentially completely formed, as indicated by the titration results, so the accelerations can be attributed to catalysis afforded by the complexes. That complexation is complete under the kinetic conditions is also verified by the fact that the plots of pseudo-first-order rate constants, k_{obs}, *versus* [L:$n$$Zn^{2+}$] are linear and show no curvature between 0.2–4 mM for **1**:2Zn^{2+} and **2**:M^{2+}. The linearity of the k_{obs} *versus* [L:$n$$M^{2+}$] plots also indicates that there is no significant pre-equilibrium formation of any **3**:L:$n$$M^{2+}$ ternary complexes. Due to the formation of precipitates the L:$n$$Zn^{2+}$ complexes could only be studied at pH values <8 (**1**:2M^{2+}) and <9 (**2**:M^{2+}); the observed second-order rate constants (k_{cat}^{obs}) are given in Tables 1 and 2.

The k_{cat}^{obs}/pH profiles for the Zn^{2+} and Cu^{2+} complexes attacking **3** are shown in Figs. 1 and 2 respectively. Both series of **1**:2M^{2+} and **2**:M^{2+} show similar profiles: activity increases with increasing pH at intermediate pH values, but levels off at low and high pH. This is consistent with both the acidic and basic forms of the complexes being active as in eqn. (1).

$$\text{Products} \xleftarrow[k']{3} \text{L:}n\text{M}^{2+}(\text{H}_2\text{O})_x \overset{K_a}{\rightleftharpoons} \text{L:}n\text{M}^{2+}(\text{H}_2\text{O})_y(\text{OH}^-) \xrightarrow[k'']{3} \text{Products} \quad (1)$$

Nonlinear least-squares fitting of the data to eqn. (2)

$$k_{cat}^{obs} = \frac{k'[H^+] + k''K_a}{[H^+] + K_a} \quad (2)$$

gives the various parameters tabulated in Table 3.

The calculated dependences are shown as solid lines in Figs. 1 and 2. With the exception of **1**:2Cu^{2+}, the calculated lines fit the data well. In the latter case, the fit is rather poor and could not be realized until the K_a value was fixed at the titration value. Even so, an appreciable error exists in the calculated values for **1**:2Cu^{2+} given in Table 3. The reasons for this are at present unclear, but may be attributable to relatively poor solubility and undetectable clustering of the complex at higher pH value.

TABLE 1. Catalysis of the Hydrolysis of **3** by L:$n$$Zn^{2+}$ [a]

pH	k_{cat}^{obs} (M^{-1} s^{-1})[b]	
	1:2Zn^{2+}	**2**:Zn^{2+}
5.30	5.10×10^{-2}	$(2.86 \pm 0.45) \times 10^{-2}$
5.50		
5.70	5.74×10^{-2}	
5.90		
6.10	9.71×10^{-2}	4.94×10^{-2}
6.50	1.58×10^{-1}	8.47×10^{-2}
6.90	2.38×10^{-1}	1.71×10^{-1}
7.30	3.30×10^{-1}	3.76×10^{-1}
7.70	3.61×10^{-1}	9.09×10^{-1}
8.10		1.45
8.50		2.29
8.90		3.11

[a]Followed by observing rate of appearance of 4-nitrophenol or 4-nitrophenoxide; T = 25.0 °C, 33% ethanol/H_2O. [b]k_{cat}^{obs} refers to the second order rate constant for attack of L:$n$$Zn^{2+}$ on **3** determined from slopes of k_{obs} *vs.* [L:$n$$Zn^{2+}$] plots.

TABLE 2. Catalysis of the Hydrolysis of **3** by L:$n$$Cu^{2+}$ [a]

pH	k_{cat}^{obs} (M^{-1} s^{-1})[b]	
	1:2Cu^{2+}	**2**:Cu^{2+}
6.10	0.276 ± 0.045	
6.20	0.28 ± 0.04	
6.30		0.129 ± 0.027
		0.125 ± 0.007
6.50	0.37 ± 0.02	0.161 ± 0.009
6.60	0.34 ± 0.07	
6.80	0.37 ± 0.05	
6.90		0.28 ± 0.02
		0.298 ± 0.015
		0.30 ± 0.04
7.10	0.516 ± 0.034	
7.30	0.98 ± 0.03	0.334 ± 0.026
7.40	1.04 ± 0.01	
7.50		0.694 ± 0.06
7.55	1.276 ± 0.035	
7.70	1.48 ± 0.08	
7.75		0.94 ± 0.07
7.90		1.13 ± 0.06
8.30		2.246 ± 0.065
8.50		3.215 ± 0.125
8.80		4.215 ± 0.205

[a]Followed by observing rate of appearance of 4-nitrophenoxide; T = 25.0 °C; 33% ethanol/H_2O. [b]Same caption as for Table 1 but change L:$n$$Zn^{2+}$ to L:$n$$Cu^{2+}$.

From the shapes of the k_{cat}^{obs} *versus* pH profile, a common mechanism for all species can be proposed. The plateau at low pH is attributable to the complexes acting as Lewis acids facilitating the attack of

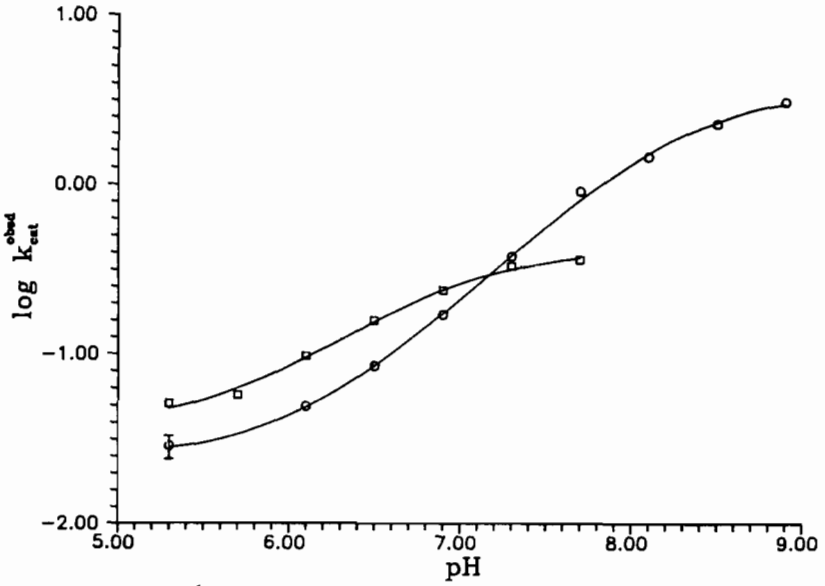


Fig. 1. Plot of log k_{cat}^{obs} *vs.* pH for the hydrolysis of 3 catalyzed by 1:2Zn^{2+} (□) and 2:Zn^{2+} (○) in 33% EtOH–H_2O at 25 °C.

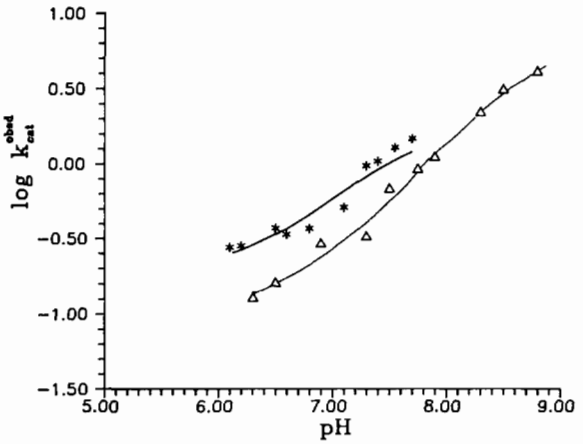


Fig. 2. Plot of log k_{cat}^{obs} *vs.* pH for the hydrolysis of 3 catalyzed by 1:2Cu^{2+} (*) and 2:Cu^{2+} (△) in 33% EtOH–H_2O at 25 °C.

H_2O on 3, or a kinetic equivalent such as assistance of the departure of the leaving group. In this region, the Cu^{2+} complexes are about 3–4 fold more efficacious than the Zn^{2+} complexes. Also, the bimetallic complexes are roughly 2-fold more active than their monometallic counterparts suggesting a simple statistical enhancement of catalysis. Hence no evidence for a cooperativity of interaction in the bimetallic species is evident.

In the high pH region, all complexes show evidence of plateauing indicative of a metal-bound hydroxide being the active form. This rules out a Lewis acid role for the metal enhancing the attack of external OH^- on a transiently coordinated phosphate. The data for all complexes suggests that there is a Brønsted relationship between the pK_a of the complex and log k'' (β = 0.78, r = 0.972, 4 data) which includes both Zn^{2+} and Cu^{2+} complexes.

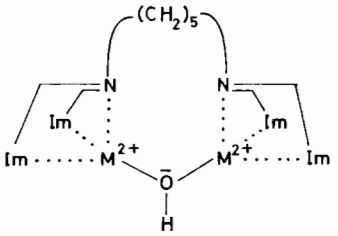
TABLE 3. Computed Rate Constants k' and k'' for the Neutral and Basic Forms Respectively of the Complexes Attacking 3 and Thermodynamic pK_a Values[a]

Constant	Complex			
	1:2Zn^{2+}	2:Zn^{2+}	1:2Cu^{2+} [b]	2:Cu^{2+}
k' (M^{-1} s^{-1})	3.75×10^{-2}	2.39×10^{-2}	1.6×10^{-1}	9.8×10^{-2}
k'' (M^{-1} s^{-1})	4.19×10^{-1}	3.73	2.0	6.67
pK_a[c]	6.85	8.27	7.51[d]	8.41

[a]Computed by non-linear least-squares fitting of k_{cat}^{obs}/pH data in Tables 1 and 2 to eqn. (1); T = 25.0 °C, 33% ethanol/H_2O. Uncertainty ±5% of quoted number unless otherwise specified. [b]Uncertainty in k' ±0.7; k'' ±0.4. [c]From titration pK_a values for 1:2Zn^{2+}, 6.99; 2:Zn^{2+}, 8.16; 2:Cu^{2+}, 8.35. [d]Titration value used in fitting of data for 1:2Cu^{2+} to eqn. (1).

The fact that the bimetallic complexes fit on the same line as the monometallic ones indicates that the mode of activity is the same and that the second M^{2+} does not interact cooperatively to promote the reaction.

The data in Table 2 suggest some reasons for the failure of these bimetallic complexes to be better catalysts than their monometallic counterparts. The pK_a for the $1{:}2M^{2+}$ species in both the Zn^{2+} and Cu^{2+} complex is ~1 unit lower than in the corresponding $2{:}M^{2+}$ complex. This is a likely indication that the $1{:}2M^{2+}$-OH^- complexes generated have the coordinated hydroxide stabilized by affiliation with both metal centers perhaps by bridging as in **11**. The net consequence of such stabilization is to lower both the nucleophilicity and basicity and hence led to lower activity. This leads to the conclusion that perhaps one can make effective bimetallic catalysts if the ligand system is designed in such a way as to prevent the two M^{2+}-centers from being bridged by a single OH^-.



11

Finally, it should be pointed out that in the hydrolyses of **3** only 1 equiv of 4-nitrophenol (or 4-nitrophenoxide) was released. No following hydrolysis of the product bis-4-nitrophenyl phosphate was ever observed so that if catalysis of the diester hydrolysis is occurring, it must be far less efficient than that of the triester. No reason is offered for this. However, with the mono-ester 4-nitrophenyl phosphate in 50% aqueous acetonitrile, $1{:}2Zn^{2+}$ inhibits the hydrolysis [14], clearly indicating that coordination of 4-nitrophenyl phosphate to $1{:}2Zn^{2+}$ is occurring and preventing attack by external nucleophiles.

Supplementary Material

Tables S1-S4 giving pseudo-first-order rate constants for the catalyzed hydrolysis of **3** are available from the authors on request.

Acknowledgements

We gratefully acknowledge the financial support of the United States Army Medical Research and Development Command (Contract No. DAMD17-85C-5173) and the University of Alberta.

References

1 (a) C. A. Bunton and L. Robinson, *J. Org. Chem., 34* (1968) 773; (b) C. A. Bunton, J. Frankson and L. S. Romsted, *J. Phys. Chem., 84* (1980) 2607; (c) U. Tonellato, in K. L. Mittal (ed.), *Solution Chemistry of Surfactants*, Plenum, New York, 1979, and refs. therein; (d) J. M. Brown, C. A. Bunton, S. Diaz and Y. Ihara, *J. Org. Chem., 45* (1980) 4169; (e) C. A. Bunton, Y. S. Hong, L. S. Romsted and C. Quau, *J. Am. Chem. Soc., 103* (1981) 5785; (f) C. A. Bunton, M. M. Mhala, J. R. Moffatt, D. Mannarres and G. Savelli, *J. Org. Chem., 49* (1984) 426; (g) F. M. Menger, L. H. Gan, E. Johnson and D. H. Durst, *J. Am. Chem. Soc., 109* (1987) 2800.

2 (a) Y. Okahata, H. Ihara and T. Kunitake, *Bull. Chem. Soc. Jpn., 54* (1981) 2072; (b) J. Epstein, J. J. Kaminski, N. Bodor, R. Enever, J. Sowa and T. Higuchi, *J. Org. Chem., 43* (1978) 2816; (c) R. A. Moss and H. Ihara, *J. Org. Chem., 48* (1983) 588; (d) R. A. Moss, H. Ihara and G. O. Bizzigotti, *J. Am. Chem. Soc., 104* (1982) 7476; (e) C. A. Bunton and L. G. Ionescu, *J. Am. Chem. Soc., 95* (1973) 2912; (f) C. A. Bunton, S. E. Nelson and C. Quan, *J. Org. Chem., 47* (1982) 1157; (g) C. A. Bunton and H. Ihara, *J. Org. Chem., 82* (1977) 2865; (h) U. Tonelatto, *J. Chem. Soc., Perkin Trans. 2*, (1977) 821; (i) J. M. Brown, C. A. Bunton and S. Diaz, *J. Chem. Soc., Chem. Commun.*, (1974) 971; (j) C. A. Bunton, L. Robinson and M. Stam, *J. Am. Chem. Soc., 92* (1970) 7393; (k) F. M. Menger and L. G. Whitesell, *J. Am. Chem. Soc., 107* (1985) 707.

3 (a) W. S. Wadsworth, Jr., *J. Org. Chem., 46* (1981) 4080; (b) W. G. Wadsworth and W. S. Wadsworth, Jr., *J. Am. Chem. Soc., 105* (1983) 1631; (c) P. Hendry and A. M. Sargeson, *J. Chem. Soc., Chem. Commun.*, (1984) 164; (d) G. H. Posner, J. W. Ellis and J. Ponton, *J. Fluorine Chem., 19* (1981) 191.

4 R. S. Brown and M. Zamkanei, *Inorg. Chim. Acta, 108* (1985) 201.

5 S. H. Gellman, R. Petter and R. Breslow, *J. Am. Chem. Soc., 108* (1986) 2388.

6 C. Walsh, in *Enzymatic Reaction Mechanisms*, W. H. Freeman, San Francisco, 1979, pp. 179–209.

7 (a) J. E. Coleman and P. Gettins, *Adv. Enzymol., 55* (1983) 381, and refs. therein; (b) H. W. Wyckoff, M. Handschumacher, K. Murthy and J. M. Sowadski, *Adv. Enzymol., 55* (1983) 453.

8 K. Kasse, *Houbenweyl, 12(2)* (1964) 324.

9 P. E. Iverson and H. Lund, *Acta Chem. Scand., 20* (1966) 2649.

10 V. Somayaji, K. I. Skorey and R. S. Brown, *J. Org. Chem., 51* (1986) 4866.

11 P. C. Jocelyn, *J. Chem. Soc.*, (1957) 3305.

12 R. G. Bates, M. Paabo and R. A. Robinson, *J. Phys. Chem., 67* (1963) 1833.

13 R. S. Brown and J. Huguet, *Can. J. Chem., 58* (1980) 889.

14 V. Somayaji, unpublished observation.