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The different roles of metal ions and water molecules in the recognition and catalyzed hydrolysis of ATP by phenanthroline-containing polyamines

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Abstract

The phenanthroline bridging polyaza ligands L1, L2 and L3 can selectively and strongly bind nucleotides at physiological pH, and hence accelerate the hydrolysis rate of the bound ATP. It is interesting that a phosphoramidate intermediate at 2.88 ppm (should be added 5.63 ppm when compared with other models) was found in the hydrolysis process of L/ATP. By introduction of metal ions (critical Zn^{2+} or hard Mg^{2+}, Ca^{2+}) to the L/ATP system, recognition of the anionic substrates by the protonated ligands was greatly promoted. However, due to the different affinities of metal ions to the receptor and the substrate, ATP hydrolysis in Zn^{2+}/L/ATP system and Mg^{2+}(Ca^{2+})/L/ATP system occurs through different mechanisms. By comparison with the M/ATP ($M=Zn^{2+}$, Mg^{2+}, Ca^{2+}) system, the rates of ATP-hydrolysis in the $Mg^{2+}Ca^{2+}$/L/ATP system and the Zn^{2+}/L/ATP system were enhanced and retarded, respectively. Moreover, the reasons contributing to large rate range of the L/ATP systems and M^{2+}/L/ATP systems were given. The results show that metal ions vertically regulate the recognition and hydrolysis of ATP. On the other hand, water molecule participates in the hydrolysis reactions at different steps with different functions in the L/ATP systems and M(Zn^{2+}, Mg^{2+}, Ca^{2+})L/ATP systems.

Keywords: Metal ions; ATP; Polyamines; Recognition; Catalytic hydrolysis

1. Introduction

In adenosine 5′-triphosphate (ATP), the most important energy-rich substance involved in metabolism, the phosphate groups and the N-site of purine are important binding sites for metal ions [1]. Thus, it is not surprising that interactions [1], stabilities [2] and properties [3] of nucleotide–metal ion complexes in solution have received considerable research interest. In general, metal ions play a structural role or act as one of the cofactors in the promoted hydrolysis of ATP [4]. The biologically high activity of the naturally used Mg(II), Ca(II) or Zn(II) depends on its binding with the bio-substrates [5–8].

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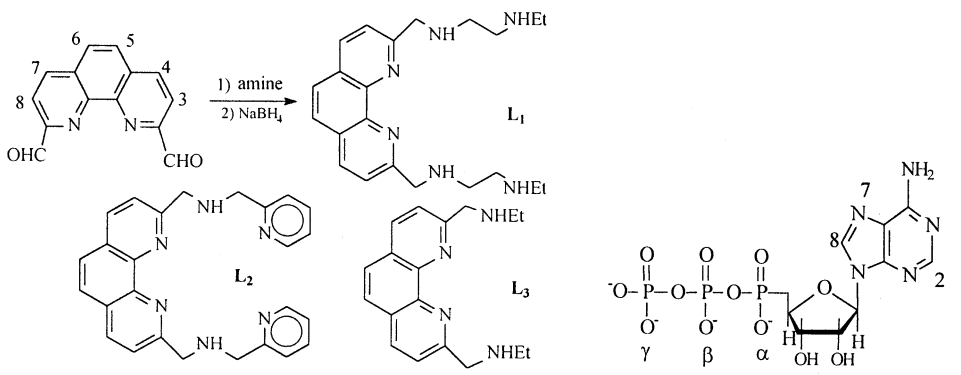


Scheme 1. Synthesis of L and structure of ATP.

However, polyaza ligands have been studied widely because of their importance in coordination chemistry [9], biomimetic studies [10] and supramolecular catalysis [11,12]. It has been reported that polyaza ligands in the protonated forms can selectively bind inorganic phosphates as well as nucleotides, also, they can significantly enhance the hydrolysis rate of the bound anions by combinational factors of entropy effects, electrostatic catalysis, acidic/base catalysis and nucleophilic catalysis [13]. However, successful catalysis by polyammonium ligands relies heavily on the recognition and selectivity of the initially bound substrate and the release of the product formed following chemical transformation.

Current interest focuses on the molecular recognition of nucleotides by the polyaza ligands and their metal complexes [14–16], but the effects of different metal ions (hard or soft) as well as water molecules in the process of the catalyzed ATP hydrolysis have not been studied thoroughly. In this paper, the phenanthroline-containing polyaza receptor L1, L2 and L3 have been synthesized in order to achieve strong molecular recognition by stacking with the nucleobase residue of the nucleotides, besides the Coulombic interactions and H-bonding interactions between the ammonium ions of L and the phosphate oxygens of the nucleotides [16]. Furthermore, the activity of L to catalyze the hydrolysis of the bound nucleotides in the absence or presence of metal ions, namely Zn(II) or Mg(II) or Ca^{2+}, has been determined.

2. Materials and methods

Most of the starting materials were obtained commercially and were purified prior to use. The sodium salt of ATP and ADP were purchased from Aldrich Chemical Co. The aqueous stock solutions of the nucleotides were prepared freshly and used within 10 h (Scheme 1).

Synthesis of 2,9-di((2′-(ethylamino)ethyl-eneamino)methyl)-1,10-phenanthroline (**L1**): to a suspension of 2,9-dicarboxaldehydro-1,10-phenanthroline (1.2 g, 5 mmol) [17] in anhydrous EtOH (40 cm^3), a solution of 2-(ethylamino)ethyleneamme (0.97 g, 11 mmol) in 30 cm^3 EtOH was added slowly and then the mixture was stirred under N_2 at 45 °C for 2 h. After the filtration, $NaBH_4$ was added in small quantities and the solution was stirred at RT for 12 h. The solvent was removed, and the white residue remaining was dissolved in water and then was extracted with chloroform. The organic fractions were combined, washed with distilled water and dried over $MgSO_4$. After filtration, the chloroform was removed on a rotary evaporator to give a yellow oil. The product was recrystallized from HCl/EtOH to give white–yellow precipitates 1.28 g, yield: 45.1%. ^{1}H NMR (D_2O): δ 2.86 (*t*, 6H, CH_3CH_2), δ 3.18 (*m*, 4H, CH_2CH_3); δ 3.55–3.69 (*m*, 8H, NCH_2CH_2N); δ 4.80 (*s*, 4H, phen-CH_2); δ 7.82–7.86 (*m*, 4H phen $H_{3,8}$; phen $H_{5,6}$); δ 8.49 (*d*, 2H, phen $H_{4,7}$). IR (KBr pellet): 3395 (ν_{N-H}); 1507 (ν_{CNH}), 865, 1451, 1595 (Ar). Ele-

mental analysis: found H: 6.94%; C: 48.77%; N: 15.72%. Calculated for $C_{22}H_{32}N_6 \cdot 4HCl \cdot H_2O$, H: 7.04%; C: 48.54%; N: 15.44%.

The ligand **L2** [18], 2,9-di(((α-pyridyl)methylamino)methyl)-1,10-phenanthroline and ligand **L3** [19], 2,9-di((ethylamino)methyl)-1,10-phenanthroline, were synthesized using 2,9-dicarboxaldehydro-1,10-phenanthroline [17] and α-aminomethylpyridine and ethylamine, respectively.

For general characterization see Ref. [19]. Potentiometric titrations were carried out according to the reported method [20]. The concentrations of reagents in solution were 5×10^{-4} mol·dm^{-3} and $I=0.1$ mol·dm^{-3} KNO_3. The calculations were made through the curve-fitting computer program (TITFIT), which uses a Newton–Gauss–Marquardt non-linear least-squares program [20].

^{31}P-NMR spectra were recorded at 25 ± 0.1 °C on a Varian Unity-plus 400 MHz spectrometer. A typical solution (0.5 ml, 20% D_2O/H_2O) containing 0.01 mol·dm^{-3} ATP and 0.01 mol·dm^{-3} L and/or 0.01 mol·dm^{-3} metal ion (Zn^{2+}, Mg^{2+}, Ca^{2+}) was placed in the NMR probe in a 5-mm tube for determination.

Kinetic studies were performed by following the time evolution of the proton-decoupled ^{31}P-NMR spectra (5 min 400 acquisitions) of substrate + catalyst mixtures. Eighty-five percent H_3PO_4 was used as external standard. In a typical experiment, a 0.5-ml solution (20% D_2O/H_2O) in a 5-mm tube containing 0.01 mol·dm^{-3} ATP and 0.01 mol·dm^{-3} L and/or 0.01 mol·dm^{-3} Zn^{2+} (or Mg^{2+} or Ca^{2+}) was placed at the NMR probe and maintained at 70 °C. The hydrolysis of ATP can be monitored accurately ($\pm10\%$) by following the changes in concentration of the various species with time, since most ^{31}P NMR signals of ATP, ADP, AMP, $P_2O_7^{4-}$ and PO_4^{3-} are distinct [13].

3. Results and discussion

The protonation constants of L1, L2 [18] and L3 [19] have been determined by potentiometric titrations. The stepwise protonation constants are listed in Table 1. Although the ligands L1 and L2 consist of hexa-aza donors, they have only four stepwise protonation constants. The two nitrogen atoms of phen (phenanthroline) are not protonated in the pH range studied (2.5–12), due to the lower $pK^{H}_{H\text{-}phen}=4.75$ [21a] vs. 9–11 of secondary nitrogen atoms, as well as the electron-withdrawing effects of the already protonated ammoniums on the electron density of phen. This result can also be verified by the ^{31}P titration of L as a function of pH, which shows that the signals of H-3,8, H-5,6 and H-4,7 of phen were only shifted significantly in strongly acidic solution. Thus, the first two protonation steps of L1 occur at the terminal N1 while the last two steps belong to the benzylic N2, which is linked to the aromatic phen via a methyl group. In contrast to L1, since the baisicity of the pyridyl nitrogen is lower than that of the benzylic N2, the two N2 of L2 are attributed to the first two protonation steps. With regard to L3, the electron-withdrawing effects of the protonated ammonium on phen is weaker, compared with that in the H/L1 system (two protons of L3 vs. four protons of L1 and L2), so that one nitrogen of phen can be protonated and the first two protonation steps involve the nitrogen atoms of the arms. It is observed that the basicity of L1 is larger than that of L3 because of the electron inductive effect of ethyl to the terminal N1 of LI and the linking of the benzylic amino nitrogen of L3 to the aromatic phen via a methyl group. However, due to the additional NH_4^+-π (pyridyl) interactions [22], the protonation constants of ligand L2 are greater than those of both L1 and L3.

All the ligands are good donors for Zn^{2+} ion. The stability constants of complexes formed in 1:1

Table 1
The stepwise protonation constants of L and the stability constants of complexes present in the Zn^{2+}/L system (25 °C, $I=0.1$ mol.dm^{-3}, KNO_3, $C_L=C_{zn}=5\times10^{-4}$ mol.dm^{-3})

	L1	L2[18]	L3[19]
[LH]/[L][H$^+$]	9.95±0.05	10.86±0.09	9.84±0.02
[LH$_2$]/[LH][H$^+$]	9.40±0.04	10.04±0.14	8.50±0.04
[LH$_3$]/[LH$_2$][H$^+$]	6.20±0.02	7.63±0.20	2.09±0.04
[LH$_4$]/[LH$_3$][H$^+$]	5.01±0.03	6.01±0.25	
Logβ	L1	L2[18]	L3[19]
Logβ$_{Zn(L,2H)}$	26.36±0.06	32.83±0.13	
Logβ$_{zn(L,H)}$	20.32±0.04	25.11±0.10	15.23±0.02
Logβ$_{znL}$	13.72±0.05	15.17±0.08	8.89±0.01
Logβ$_{Zn(L,-H)}$	4.45±0.03		0.26±0.03

Table 2
Binding strength of protonated L with Nu (Nu = ATP^{4-} and ADP^{3-}) in the absence or presence of Zn(II), $[Zn^{2+}] = [Nu] = [L] = 5\times10^{-4}$ mol·dm^{-3}, at $I = 0.1$ mol·dm^{-3} KNO_3 and at 25 °C

K	LI	L2	K	L3
$[H_2LATP]/[H_2L][ATP]$	6.44 ± 0.06	4.03 ± 0.04	$[HLATP]/[HL][ATP]$	4.26 ± 0.05
$[H_3LATP]/[H_3L][ATP]$	9.64 ± 0.05	5.34 ± 0.09	$[H_2LATP]/[H_2L][ATP]$	5.66 ± 0.04
$[H_4LATP]/[H_3L][HATP]$	9.25 ± 0.08	6.15 ± 0.05	$[H_3LATP]/[H_2L][HATP]$	5.20 ± 0.08
$[H_5LATP]/[H_4L][HATP]$	9.94 ± 0.09	4.15 ± 0.03	$[H_4LATP]/[H_2L][H_2ATP]$	5.08 ± 0.02
$[H_6LATP]/[H_4L][H_2ATP]$	8.02 ± 0.01			
K	LI	L2	K	L3
$[ZnLATPH]/[ZnLH][ATP]$	3.89 ± 0.02	4.67 ± 0.03	$[ZnLATP]/[ZnL][ATP]$	2.61 ± 0.05
$[ZnLATPH2]/[ZnLH_2][ATP]$	6.01 ± 0.02	5.26 ± 0.03	$[ZnLATPH]/[ZnLH][ATP]$	5.65 ± 0.03
$[ZnLATPH3]/[ZnLH_2][HATP]$	5.19 ± 0.04	3.39 ± 0.02	$[ZnLATPH_2]/[ZnLH][HATP]$	4.41 ± 0.02
$[ZnLATPH_4]/[ZnLH_2][H_2ATP]$	4.96 ± 0.03		$[ZnLATPH_3]/[ZnLH][H_2ATP]$	4.27 ± 0.04
$[ZnLATPH_5]/[ZnLH_3][H_3ATP]$	5.13 ± 0.04			
K	LI	L2	K	L3
$[H_2LADP]/[H_2L][ADP]$	5.76 ± 0.04	3.85 ± 0.07	$[HLADP]/[HL][ADP]$	4.05 ± 0.02
$[H_3LADP]/[H_3L][ADP]$	8.25 ± 0.03	4.42 ± 0.06	$[H_3LADP]/[H_2L][ADP]$	5.37 ± 0.03
$[H_4LADP]/[H_3L][HADP]$	7.78 ± 0.04	5.36 ± 0.04	$[H_3LADP]/[H_2L][HADP]$	5.18 ± 0.05
$[H_5LADP]/[H_4L][HADP]$	8.31 ± 0.06	3.08 ± 0.03	$[H_4LADP]/[H_2L][H_2ADP]$	4.55 ± 0.04
$[H_6LADP]/[H_4L][H_2ADP]$	7.54 ± 0.07			
K	LI	L2	K	L3
$[ZnLADPH]/[ZnLH][ADP]$	3.33 ± 0.07	3.98 ± 0.10	$[ZnLADP]/[ZnL][ADP]$	1.87 ± 0.07
$[ZnLADPH_2]/[ZnLH_2][ADP]$	5.21 ± 0.08	4.19 ± 0.05	$[ZnLADPH]/[ZnLH][ADP]$	4.23 ± 0.05
$[ZnLADPH_3]/[ZnLH_2][HADP]$	4.43 ± 0.01	3.01 ± 0.03	$[ZnLADPH_2]/[ZnLH][HADP]$	3.52 ± 0.05
$[ZnLADPH_4]/[ZnLH_2][H_2ADP]$	3.96 ± 0.06		$[ZnLADPH3]/[ZnLH][H_2ADP]$	2.80 ± 0.09
$[ZnLADPH_5]/[ZnLH_3][H_2ADP]$	4.58 ± 0.06			

Zn(II)/L systems are presented in Table 1. There are four species in the Zn^{2+}/L1 system, namely $ZnLH_m$ ($m = 2, 1, 0, -1$). Whereas, Zn^{2+}/L2 and Zn^{2+}/L3 systems lack the complexes $Zn(L2)H_{-1}$ and $Zn(L3)H_2$, respectively. It is as expected that the ligand L1 with more nitrogen atoms forms higher stable complexes with Zn^{2+} than L3 does. Due to the additional interactions occurring between the cations (Zn^{2+}, NH_4^+) and the π-donor (pyridyl) [21b], stability constants of complexes present in the Zn^{2+}/L1 systems are greater than those corresponding in the Zn^{2+}/L1 systems. Due to the different basicity and steric effects of the ligands, their zinc(II) complexes form with different conformations. For example, complexes $Zn(L1)H_2^{4+}$ uses two phen nitrogen atoms and two benzylic N2 to bind Zn^{2+} while the two terminal N1 are protonated. But to $Zn(L2)H_2^{4+}$, the coordination sites involves phen and two pyridyls while the benzylic N2 are protonated. At pH > 7, the coordination bond between Zn(II) and phen becomes loose, and as a consequence, the Zn^{2+}-bound water molecule will deprotonate to give the monohydroxyl complex $ZnLH_{-1}$ (ZnL–OH), which is a good nucleophile in neutral or slightly basic solution.

The stability constants of supramolecular species formed between the various protonated forms of the hexa-aza ligand L and nucleotides were determined using potentiometric equilibrium methods. According to Eqs. (1) and (2), the binding strength of protonated L with nucleotides (ATP, ADP) is listed in Table 2. The binding strength is obtained from the overall stability constants of adduct $LATPH_{(l+n)}^{l+n-4}$ (or $LADPH_{(l+n)}^{l+n-3}$), the protonation constants of LH_m^{m+} and H_nATP^{n-4} (or H_nADP^{n-3}). The protonation constants of H_nATP^{n-4} and H_nADP^{n-3} are cited directly from literature [21b].

$$LH_l^{l+} + H_nATP^{n-4} = LATPH_{(l+n)}^{l+n-4} \quad (1)$$

$$LH_l^{l+} + H_nADP^{n-3} = LADPH_{(l+n)}^{l+n-3} \quad (2)$$

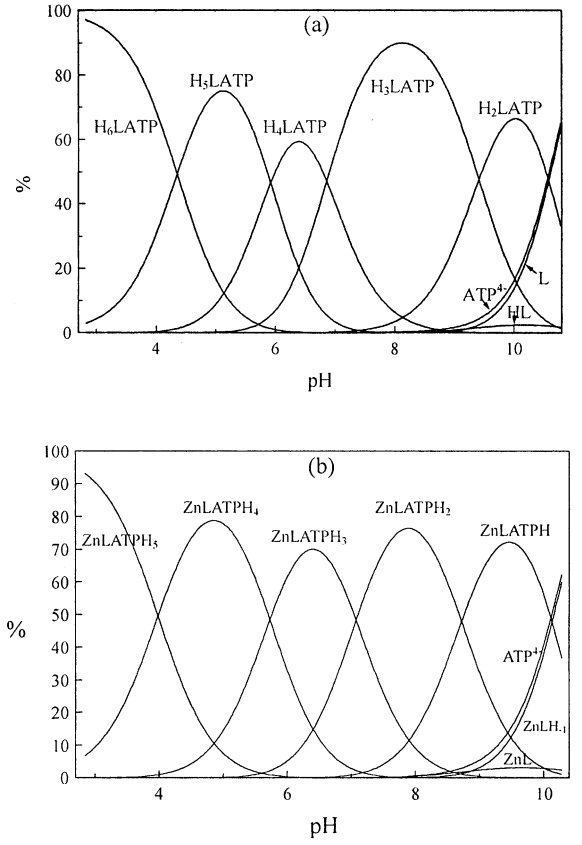


Fig. 1. Distribution diagrams for the species present in (a) L1/ATP; (b) Zn(II)/L1/ATP system. (5×10^{-4} Mol·dm^{-3} Zn^{2+}, 5×10^{-4} mol dm^{-3} L and 5×10^{-4} mol dm^{-3} ATP in water at 25 °C and $I=0.1$ mol dm^{-3} KNO_3.)

As is seen from Fig. 1a, the receptor–substrate adducts mainly form from weakly alkaline to slightly acidic pHs, where the ligand is protonated. An analysis of the binding strength listed in Table 2 indicates that electrostatic interactions and hydrogen bonding interactions [23] play a most important role in the interactions of the protonated ligands with the nucleotides. The binding strength increases with the protonation degree of the receptor, leading to the maximum charge interactions as well as the largest number of H-bonds formed between the polyammonium and the anionic phosphate oxygens. However, neutralization of the substrates ($n>0$) will decrease the binding strength, due to the reduced electrostatic interactions as well as the decreased number of H-bonds. The orders $[H_3L][ATP]>[H_3L]$ [HATP] and $[H_4L][HATP]>[H_4L][H_2ATP]$ of ligand L1 are consistent with the result. However, ^{1}H NMR experiments show that the rigid phen makes significant stacking interactions with the nucleobase residue of ATP in the L/Nu (Nu = ATP and ADP) system. It is observed that all the ^{1}H signals of H-2, H-8 and H-1′ of ATP as well as H-3,8, H-4,7 and H-5,6 of phen are upfield shifted, compared with that of free ATP or L (Table 4). This is very important in the bio-system, since the selectivity of enzymatic reactions of nucleotides largely depends on the interactions of nucleobase residues involving re-stacking interactions [24].

The data listed in Table 1 show that the binding strength of the nucleotide with the receptor is in the order ATP > ADP, which is due to the shorter chain of ADP which has fewer oxygen atoms and then the weaker Coulombic interactions as well as fewer H-bonds and hence, lower binding strength. However, the recognition of the substrates also relies on such properties as structure and composition of the receptors. As shown in Table 1, the trend for polyammonium to interact with ATP is in the order L3 < L1. The ligand L1 has more nitrogen atoms than the corresponding L3 and hence, larger positive charge density in acidic solution, so that they have greater ion-pairing interactions and can form a higher number of H-bonds with the anionic substrate. Furthermore, because the first two protonations belong to the terminal N1 of L1, which has greater freedom than the protonated N2 of L2, the electrostatic interactions and H-bonding interactions occurs easier in the L1/Nu (Nu = ATP^{4-}, ADP^{3-}) systems leading to higher binding strength than the corresponding L2/Nu systems.

However, the pyridyl groups of L2 also stack with the adenosine residue and/or phen to some extent. It has been reported that the tendency for pyridyl group to stack is lower than that of phen or bipy [25,26], so if pyridyls take part in stacking, the chemical shifts of phen and nucleotide will be decreased. This is consistent with the data listed in Table 4. Moreover, the result was also proved

by the calculation using the program MM2 [27], which shows that the phen unit and two pyridyls of L2 simultaneously stack with the nucleoside of nucleotide. In addition, the main effects on the ^{1}H chemical shift of H-5,6 rather than H-3,8 or H-4,7 of phen in the Zn^{2+}/L1/ATP system is probably attributed to the ring–current effect.

The binding strength of $ZnLH_m^{m+2}$ with H_nATP^{n-4} or H_nADP^{n-3} according to Eqs. (3) and (4) are listed in Table 2. The binding strength is obtained from the result of stability constants of complexes $ZnLATPH_{(m+n)}^{m+n-2}$ (or $ZnLADPH_{(m+n)}^{m+n-1}$), stability constants of complexes $ZnLH_m^{m+2}$ and protonation constants of H_nATP^{n-4} (or H_nADP^{n-3}) [21b]]. The recognition of the substrates was significantly promoted after Zn(II) was added into the L/nucleotide system. In the first approach it should be assumed that the additional stabilization is related to the presence of non-covalent intramolecular interactions [1,2,9–16].

$$ZnLH_m^{m+2} + H_nATP^{n-4} = ZnLATPH_{(m+n)}^{m+n-2} \quad (3)$$

$$ZnLH_m^{m+2} + H_nADP^{n-3} = ZnLADPH_{(m+n)}^{m+n-1} \quad (4)$$

As is seen from Fig. 1b, there are five ternary complexes, namely $ZnLATPH_x$ ($x=1$–5), in the Zn(II)/L1/ATP system. Complex Zn(L1)ATPH is a major species at pH > 9 while $Zn(L1)ATPH_2$ forms in the 5–10 pH range and binds 80% of the metal at pH ca. 8 (Fig. 1b). Taking into account the protonation degree of the nucleotide and the metal complexes, it should be assumed that the complex Zn(L1)ATPH might be the mixture of [Zn(L1)H][ATP] and [Zn(L1)][HATP] due to the fact that $pK^H_{HATP}=6.50$ [21b] and $pK^H_{Zn(L1,H)}=6.6$ (Table 1). Since $pK^H_{Zn(L2,H)}=10$ and $pK^H_{Zn(L3,H)}=6.4$ (Table 1), the complex Zn(L2)ATPH must be formed by [Zn(L2)H] and [ATP] whereas complex Zn(L3)ATPH is the mixture of [Zn(L3)H][ATP] and [Zn(L3)][HATP]. At the same time, the benzylic nitrogens of L1 or pyridyl nitrogens of L2 and/or the nitrogen atom(s) of phen [Zn(II)-phen bond becomes weaker at high pH] are bound to Zn(II). Because of $pK^H_{H2ATP}=4.20$ [21b] and $pK^H_{Zn(L1,2H)}=6.3$ and $pK^H_{Zn(L2,2H)}=7.6$, the second proton in complex $ZnLATPH_2$ should be located at the terminal nitrogen atom of L1 and the benzylic N2 of L2, respectively. Complex $Zn(L1)ATPH_3$, which exists in the 4–9 pH range and binds 70% of the metal at pH ca. 6 (Fig. 1b), should be adducted by $[Zn(L1)H_2]$ and [HATP] for the same reason mentioned above. Although the species $[ZnLH_3]$ (we supposed its $pK_a=3$, L = L1, L2) was not observed in the potentiometric titrations due to the pH range studied (2.5–12), we do believe the existence of it in the acidic solution. Thus, both $[ZnLH_3][HATP]$ and $[ZnLH_2][H_2ATP]$ are reasonable for species $ZnLATPH_4$ (L = L1, L2). Finally, in acidic solution complex $ZnLATPH_5$ prefers $[ZnLH_3][H_2ATP]$ rather than $[ZnLH_2][H_3ATP]$) since $pK^H_{H3ATP}=7$.

Table 3
^{31}p NMR chemical shifts for ATP (0.01 mol·dm^{-3}) in the presence of L (0.01 mol·dm^{-3}) and/or Zn^{2+}, Mg^{2+}, Ca^{2+} (0.01 mol·dm^{-3}) at pH 7.6 and at 25 °C[a]

L	M^{2+}	Nucleotides	α	β	γ
–	Zn^{2+}	ATP	0.33	2.79	0.92
–	Mg^{2+}	ATP	0.29	2.66	0.74
–	Ca^{2+}	ATP	0.40	2.56	0.87
L1	–	ATP	−0.02	0.40	1.12
L2	–	ATP	0.02	0.35	1.25
L3	–	ATP	0.02	0.39	1.05
L1	Zn^{2+}	ATP	−0.29	0.70	0.48
L2	Zn^{2+}	ATP	−0.11	1.39	0.64
L1	Mg^{2+}	ATP	0.36	2.77	0.90
L2	Mg^{2+}	ATP	0.35	2.77	0.85
L1	Ca^{2+}	ATP	0.36	2.74	0.79
L2	Ca^{2+}	ATP	0.36	2.73	0.84

[a] The chemical shifts of ATP are $P_\alpha = -15.54$ ppm, $P_\beta = -26.44$ ppm and $P_\gamma = -11.92$ ppm, respectively.

The interactions of Mg^{2+}(Ca^{2+}/L/ATP system were determined by ^{31}P NMR and ^{1}H NMR spectra. The hard metal ions are more favorable for the phosphate oxygen of the substrate rather than the nitrogen atoms of the receptor in the ternary systems. Similar to the Zn(II)/L/ATP system, there are coordination bonds, ion pair, hydrogen bonds (^{31}P NMR, Table 3), bridged π–π stacking interactions between the phen of L and the nucleobase residue of the nucleotide (^{1}H NMR, Table 4) in the $Mg^{2+}Ca^{2+}$/L/ATP systems (see Scheme 3b). Also, cation (Mg^{2+}, Ca^{2+}, NH_4^+)-π-donor (phen, nucleoside and/or pyridyl) [22,28], hydrophobic (phen, nucleoside, etc.) and

Table 4
^{1}H NMR chemical shifts for ATP (0.01 mol·dm^{-3}) in the presence of L (0.01 mol·dm^{-3}) and/or Zn^{2+}, Mg^{2+}, Ca^{2+} (0.01 mol·dm^{-3}) at pH 7.6 and at 25 °C[a]

System	H-2	H-8	H-1′	Phen H_{38}	Phen H_{47}	Phen H_{56}
Zn^{2+}/ATP	−0.17	−0.14	−0.13			
Mg^{2+}/ATP	−0.09	−0.11	−0.04			
Ca^{2+}/L/ATP	−0.14	−0.17	−0.13			
L1/ATP	−0.67	−0.79	−0.80	−0.38	−0.45	−0.40
L2/ATP	−0.35	−0.41	−0.40	−0.37	−0.35	−0.38
L2/ADP	−0.44	−0.50	−0.72	−0.26	−0.21	−1.14
L3/ATP	−0.52	−0.60	−0.49	−0.29	−0.32	−0.30
Zn^{2+}/L1/ATP	−0.37	−0.73	−0.70	−0.23	−0.67	−0.25
Zn^{2+}/L2/ATP	−0.25	−0.29	−0.23	−0.20	−0.19	−0.21
Mg^{2+}/L1/ATP	−0.63	−0.81	−0.76	−0.35	−0.34	−0.37
Mg^{2+}/L2/ATP	−0.09	−0.35	−0.10	−0.25	−0.11	−0.69
Ca^{2+}/L1/ATP	−0.65	−0.82	−0.79	−0.36	−0.33	−0.37
Ca^{2+}/L2/ATP	−0.12	−0.37	−0.11	−0.27	−0.12	−0.66

[a] The chemical shifts for H-2, H-8 and H-1′ of ATP are 8.25, 8.58 and 6.16 ppm, respectively.

even van der Waals interactions are possibly involved in the supramolecular interactions.

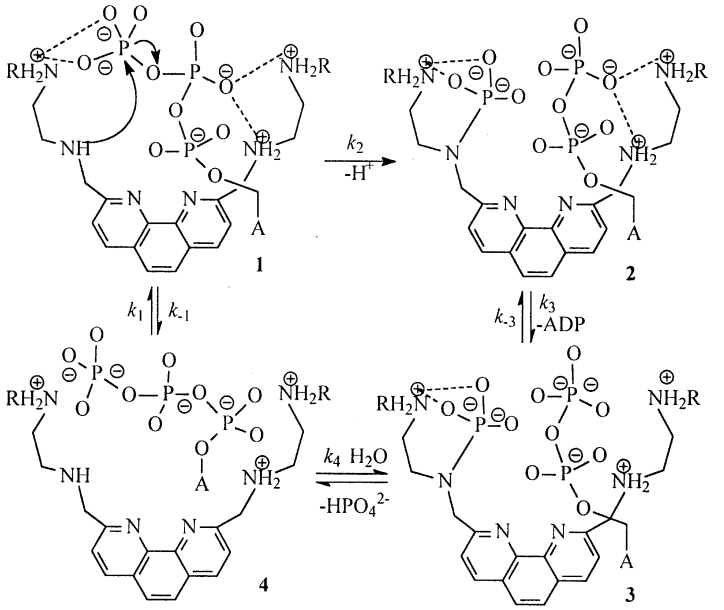
Previously studies have revealed that Zn^{2+}, Mg^{2+} and Ca^{2+} can form 1:1 complexes with ATP in the 1:1 metal/ATP stoichiometry [26,29]. In the Mg^{2+}(Ca^{2+})-ATP complexes ATP could be a mixture of α–β–γ tridentate and β–γ bidentate (mainly). Whereas, in Zn(II)/ATP system ATP uses only β and γ-phosphate to coordinate Zn(II). However, Zn(II) exhibits association with N-7 of adenosine ring [29] while the hard metal ion Mg or Ca has no tendency to interact with the N-7 of purine. As shown in Table 3, all the three metal ions have a common trend to cause the shifts of the ^{31}P signal of ATP in the metal/ATP systems, i.e. large downfield shifts for P_β (2.56–2.79 ppm) and P_γ (0.74–0.92 ppm) and small downfield shifts for P_α (0.33–0.40 ppm), respectively.

The facilitation of ATP hydrolysis by Zn^{2+} ion through a $(Zn_2ATP)_2(OH^-)$ dimer was previously studied [3]. One Zn^{2+} ion is able to coordinate to α- and β-phosphates of ATP, and direct Zn^{2+}/N-7 interaction occurs, together with the large tendency for another metal ion bound to γ-phosphate to form a hydroxyl complex that is a good nucleophile to attack the terminal phosphate of ATP at the physiological pH. The other part of the dimer is only to stabilize the active center of the catalyst. Mg^{2+} alone has small enhancement ability (4.5×10^{-4} min^{-1}) for ATP hydrolysis at neutral pH, which is attributed to the lack of the two factors of no metal ion/N-7 interaction and the low tendency to form a Mg^{2+}–OH^- species at pH 7 [6]. Ca^{2+} has higher efficiency (21.2×10^{-4} min^{-1}) in catalyzing ATP hydrolysis than Mg^{2+} does, due to the stronger binding of Ca^{2+} to the phosphate oxygens of ATP, which leads to the easier formation of $CaADP^-$ from $CaATP^{2-}$ than $MgADP^-$ from $MgATP^{2-}$ [6].

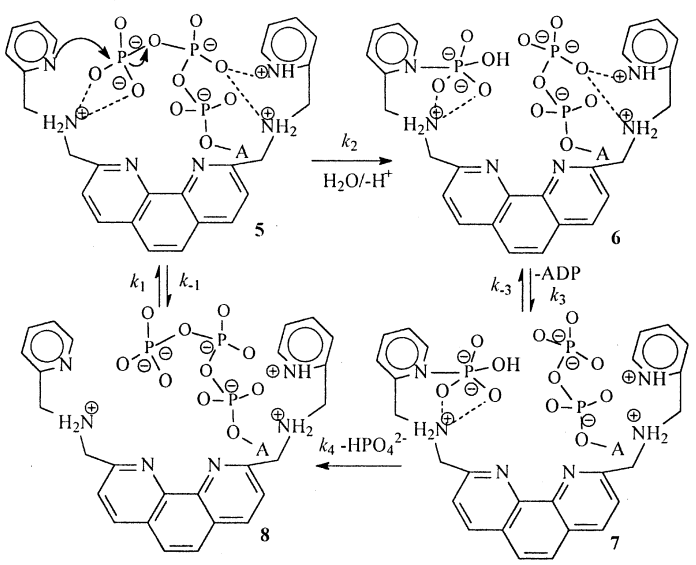
The hydrolysis of ATP at 70 °C catalyzed by the protonated L1–L3 were carried out at $I=0.1$ mol·dm^{-3} KNO_3 using ^{31}P NMR spectra. The observed rate constant k_{obs} Eq. (3) was obtained from plot of log ([ATP]/[ATP]$_0$) as a function of time, in which [ATP]$_0$ and [ATP] are the initial concentration and the certain concentration of ATP, respectively. The data for ADP hydrolysis were not directly measured by using ADP, but resulted from the hydrolysis of the product ADP, which hydrolyzes following the cleavage of ATP. The kinetic study shows that the protonated receptors moderately accelerate the reaction of ATP hydrolysis with the rates 11.8, 30.8, 3.5×10^{-4} min^{-1} for L1, L2 and L3, respectively.

$$r=k_{obs}[ATP]=-d[ATP]/dt \quad (5)$$

The dephosphorylation of ATP catalyzed by protonated ligands keeps to an ‘addition–elimination’ mechanism [13] (Scheme 2) of the initial



(a)



(b)

Scheme 2. Postulated mechanism for ATP hydrolysis catalyzed by (a) L1 (R=Et); (b) L2. The purine ring and/or ribose group has stacking interactions with phenanthroline (and/or pyridyls of L2) of L.

formation of a supramolecular complex **1** (**5**) between the negatively charged ATP and the protonated ligand. The ^{31}P NMR spectra of L/ATP (L = L1, L2) show that at pH 7.6 the ^{31}P signals of P_β and P_γShift 0.35–0.4 ppm and 1.05–1.25 ppm corresponding to those of free ATP, respectively, while P_α signal almost does not shift. This indicates that only P_γ and P_β participate in the coordination. The chemical shift upon the binding of ATP by protonated L is induced mainly by the electrostatic interactions and the formation of hydrogen bonds between the negatively charged oxygen atoms of ATP and the positively charged ammonium ions of L. Moreover, the marked π-stacking interactions of phen (and/or pyridyls of L2) with the nucleoside residue of nucleotide also contribute to recognition of the nucleobase.

The second step involves the following intramolecular attack by amine or intermolecular nucleophilic attack by water on P_γ to form the intermediate **2** (**6**) and the release of ADP **3** (**7**). It is interesting that at pH 7.6 an additional ^{31}P NMR signal appeared at 2.88 ppm in the L1/ATP system, formed by phosphorylation of the nucleophilic N atom, may be assigned as the phosphoramidate intermediate [12–14,24]. Finally, PO_3^{2-} in the intermediate captures a water molecule and gives HPO_4^{2-} and the catalyst **4** (**8**). It is worth noting that the reference of our ^{31}P systems is downfield by 5.63 ppm according to other studies, so that the signal of the intermediate found at 2.88 ppm should add 5.63 ppm when comparing with other models [12–14,24].

Comparing Scheme 2a with b, the water molecule takes part in the catalytic process in different steps with different functions. When L1 and L3 catalyze (Scheme 2a) ATP-hydrolysis, the positive charge (on P_γ of ATP) formed by the asymmetric cleavage of terminal γ-phosphate transfers to the hydrogen atom of –NH– group (free amine nitrogen) in **1**. Thus, a proton releases to form a covalent N–PO_3^{2-} bond in the intermediate **2**, which can only be destroyed by the attack of a water molecule in the last step (k_4). Then the hydroxyl unit of water connects with PO_3^{2-} to give HPO_4^{2-} while the H comes back to the nucleophile (N atom) to form the catalyst again. But the L2/ATP system (Scheme 2b), the positive charge formed by the cleavage of P_γ–P_β is substituted by a water molecule which then releases a proton to form the intermediate **6**. Since the pyridyl is an aromatic group, the positive charge cannot be transferred to the nitrogen atom of pyridine and then the bond formed between pyridyl nitrogen and the γ-phosphate is covalent. By comparison with the covalent bond formed in the intermediate **2**, the formation of the N(pyridyl)–PO_3(OH) coordination bond facilitates the release of HPO_4^{2-} in the last step. However, if the nucleophile in the two mechanisms is water molecule in the solution, the hydrolysis reaction will occur through a penta–covalent oxyphosphorane intermediate [30].

As shown in Table 5, the catalytic hydrolysis of ATP performs a pH-dependent profile. The rates of L1/ATP, L2/ATP and L3/ATP systems vary in the range 23.0–51.9 $\times 10^{-4}$ min^{-1} at pH 2.5 and the hydrolysis rate of ATP decreases with the increasing pH. But in the neutral solution, the L2/ATP system has the largest rate 30.8×10^{-4} min^{-1}, since the pyridyl N atom of L2 attacks P_γ with more freedom than the benzylic N atom of L1 or L3 leading to the most efficiency of protonated L2 at pH 7.6. The catalytic efficiency L1 > L2 at all the pH indicates that a more efficient recognition of the nucleotides leads to a faster hydrolysis rate.

The reaction stoichiometry of M/L/ATP favors 1:1:1, which was determined by the ^{31}P titrations of 1:1 [L/ATP] as a function of [M^{2+}]. The rates of ATP or ADP hydrolysis catalyzed by polyaza ligands in the presence of various metal ions are listed in Table 2. The Mg^{2+}/L/ATP and Ca^{2+}/L/ATP systems have higher hydrolysis rates than that of Mg^{2+}/ATP and Ca^{2+}/ATP, respectively. With regard to Zn^{2+}/L/ATP, although ATP hydrolyzes faster than that of L/ATP or free ATP, the rate is significantly retarded comparing with that of Zn^{2+}/ATP.

Since different affinities of zinc(II) ion and 'hard' metal ions to the receptor and the substrate leads to different roles that metal ions play in the hydrolysis process, ATP hydrolysis occurs through different mechanisms in the ternary systems, including the steps of recognition of the substrate, nucleophilic attack toward P_γ, stabilization of the

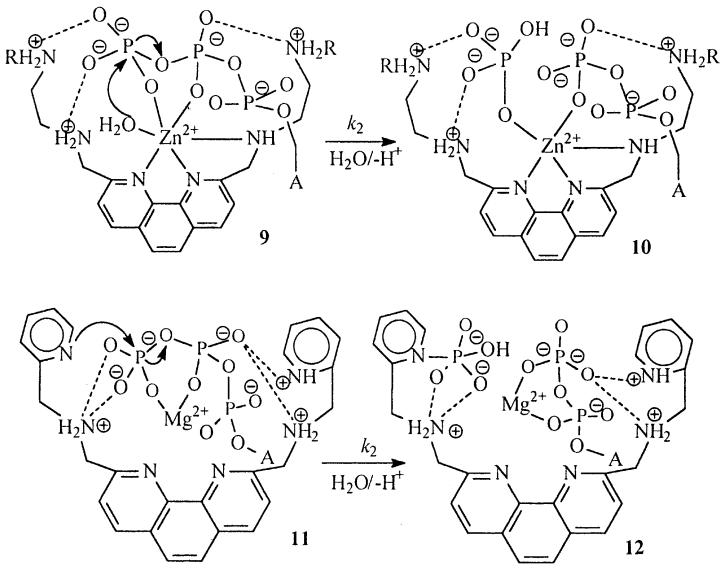
Table 5
k_{obs} (10^{-4} min^{-1}) of 0.01 mol·dm^{-3} ATP or ADP hydrolysis determined in the presence of ligand L (0.01 mol·dm^{-3}) and/or various metal ions Mg^{2+}, Ca^{2+}, Zn^{2+} (0.01 mol·dm^{-3}) at pH 7.6 and 70 °C, I=0.1 mol·dm^{-3} KNO_3

L	M^{2+}	Substrate	pH	k_{obs}	L	M^{2+}	Substrate	pH	k_{obs}
–	–	ATP	2.5	17.2	L3	–	ATP	2.5	23.0
–	–	ATP	5.0	4.8	L3	–	ATP	7.6	3.5
–	–	ATP	7.6	2.0	L3	–	ADP	7.6	2.0
–	–	ATP	10.0	0	L1	Zn^{2+}	ATP	7.6	18.4
–	–	ADP	2.5	6.0	L1	Mg^{2+}	ATP	7.6	11.0
–	–	ADP	7.6	1.8	L1	Ca^{2+}	ATP	7.6	50.2
	Zn^{2+}	ATP	7.6	82.0[a]	L1	Zn^{2+}	ADP	7.6	9.7
	Mg^{2+}	ATP	7.6	4.5[b]	L1	Mg^{2+}	ADP	7.6	7.6
	Ca^{2+}	ATP	7.6	21.2[c]	L1	Ca^{2+}	ADP	7.6	15.3
L1	–	ATP	2.5	33.9	L2	Zn^{2+}	ATP	7.6	16.7
L1	–	ATP	5.0	20.7	L2	Mg^{2+}	ATP	7.6	10.5
L1	–	ATP	7.6	11.8	L2	Ca^{2+}	ATP	7.6	62.8
L1	–	ATP	10.1	1.1	L2	Zn^{2+}	ADP	7.6	3.4
L1	–	ADP	7.6	5.1	L2	Mg^{2+}	ADP	7.6	9.6
L2	–	ATP	2.5	51.9	L3	Zn^{2+}	ATP	7.6	24.4
L2	–	ATP	5.2	35.3	L3	Mg^{2+}	ATP	7.6	12.5
L2	–	ATP	7.6	30.8	L3	Ca^{2+}	ATP	7.6	27.7
L2	–	ADP	7.6	5.5	L3	Ca^{2+}	ADP	7.6	8.3

[a] 0.001 Mol·$dm-^{3}$ ATP in water solution, I=0.1 M $NaClO_4$, pH 7.5, r=d[PO_4]/dt=0.25×10^{-8} mol^{1}·dm^{3}·min^{-1}, see Ref. [3].
[b] 0.01 Mol·dm^{-3} ATP in 1:1 (v/v) $(CH_3)_2CO$, I=0.2 M $(CH_3)_4N^+$, pH 7.0, r=d[ATP]/dt=3.8×10^{-4} min^{-1}, see Ref. [6].
[c] 0.01 Mol·dm^{-3} ATP in 1:1 (v/v) $(CH_3)_2CO$, I=0.2 M $(CH_3)_4N^+$, pH 7.0, r=d[ATP]/dt=20.8×10^{-4} min^{-1}, see Ref. [6].

negatively charged leaving group, and regeneration of the catalyst.

The mechanisms proposed for ATP hydrolysis catalyzed by Zn(L1)$ATPH_3$ and Mg(L2)$ATPH_3$ are drawn in Scheme 3a and b, respectively. The recognition of ATP by the protonated ligand mediated by the 'bridging' zinc(II) ion **9** or the hard metal ion Mg^{2+}(Ca^{2+}) **11** is the first step. As



Scheme 3. The different mechanisms for ATP hydrolysis in (a) Zn(II)/L1/ATP; (b) Mg(II)/L2/ATP.

shown in Table 3, the Zn^{2+}, Mg^{2+} and Ca^{2+} ions cause similar ^{31}P chemical shifts for the phosphate groups of ATP. By comparison with the Zn(II)/ATP system, the addition of Zn^{2+} to the 1:1 L/ATP system (L=L1, L2) caused large downfield shifts for all the ^{31}P signals of P_α (0.62, 0.44 ppm), P_β (2.09, 1.60 ppm) and P_γ (0.44, 0.28 ppm), respectively. But in the $Mg^{2+}(Ca^{2+})$L/ATP system, P_α, P_β and P_γ only shift slightly comparing with the corresponding M^{2+}/ATP systems. The results indicate that competition exists between L and ATP in the binding of Zn^{2+} in the Zn^{2+}/L/ATP system, while this competition becomes weak or even disappears in the $Mg^{2+}(Ca^{2+})$/L/ATP system. However, the coordination of Zn^{2+} to the amino nitrogen(s) of the receptor leads to the decrease in activity of the phosphate group of the substrate toward nucleophilic attack. Since the positive charge of Zn^{2+} fluxes to the amino nitrogen(s) of L, the electron-withdrawing effect of Zn^{2+} to the phosphate oxygen atom(s) decreases. However, due to the higher affinity to oxygen atoms, Mg^{2+} and Ca^{2+} have the ability in 'structuring' ATP and then makes it well recognized by the protonated L through ion-pairing, H-bonding and stacking interactions. On the other hand, Zn^{2+}/N-7 [2] interaction occurs to some extent in the Zn^{2+}/L/ATP system, while the Mg^{2+} and Ca^{2+} have no tendency to interact with this site of coordination [6,26]. In a word, the anchoring process occurs in a sterically orientated way that the metal ion take a controlling factor to the formation of the active center.

Subsequently, the zinc(II)-coordinated hydroxyl (or $ZnLH_2–H_2O$) and pyridyl nitrogen act as nucleophile to attack the P_γ center to form the intermediates **10** and **12** in the Zn^{2+}/L/ATP and the $Mg^{2+}(Ca^{2+})$/L/ATP systems, respectively. The existence of the positively charged metal ion in the center of the intermediate facilitates the release of ADP by neutralizing the charge density of its terminal phosphate. However, due to the different affinities of Zn^{2+} and $Mg^{2+}(Ca^{2+})$ to amino nitrogen atom or oxygen atom, the products might be free ADP and $Mg^{2+}(Ca^{2+})$-ADP in the Zn^{2+}/L/ATP and $Mg^{2+}(Ca^{2+})$/L/ATP systems, respectively. The final step involves the capture of a water molecule by the negatively charged PO_3^{2-} and the leaving of HPO_4^{2-} from the intermediate and the regeneration of the catalysts, i.e. Zn–L complexes in the Zn^{2+}/L/ATP system and protonated L in the $Mg^{2+}(Ca^{2+})$/L/ATP system, respectively.

The mechanism of the Zn^{2+}/L2/ATP system is similar to that of Zn^{2+}/L1/ATP that the nucleophile is probably $Zn^{2+}–H_2O$ (OH^-). But if there is free pyridyl nitrogen, the attack step will be analogous to that of $Mg^{2+}(Ca^{2+})$/L2/ATP with the formation of a pyrdiyl–N–PO_3(OH) **12** coordination bond. In the $Mg^{2+}(Ca^{2+})$/L1/ATP system, the nucleophile is the free benzylic N2 or water, and the corresponding intermediate is similar to that in complex **6** or oxyphosphorane [30], respectively. Indeed, the ^{31}P signal of this type of intermediate, observed at 2.88 ppm (should add 5.63 ppm when compared with others) in the L1/ATP system, was not found in the M(M=Zn^{2+}, Mg^{2+} and Ca^{2+})/L/ATP systems. Since the appearance of the intermediate is affected by several factors, such as pH, time, the concentration of ATP and so on.

Water molecule also participates in the hydrolysis reactions with different functions. As shown in Scheme 3, the zinc(II) ion in the complex $ZnLATPH_3$ **9** is bound to β,γ-phosphates of ATP, phen and one benzylic N atom of L1 or one pyridine of L2, so that the water or $–OH^-$ at the sixth coordination site acts as a nucleophile. The intermediate **10** has a Zn^{2+}-bound penta-covalent oxyphosphorane center [30]. Subsequently, the coordination bond Zn(II)–O–PO_2(OH) in **10** cleavages to give HPO_4^{2-} in aqueous solution. However, in the presence of 'hard' metal ions and L2 the complex **11** changes to **12** under the initial attack of a free N atom of pyridine ring and the participation of a water molecule and the following release of a proton. The decomposition of complex **12** is similar to that of **6**. Despite the difference discussed above, the phosphate center in all the intermediates is penta-covalent [30].

The hydrolysis of ATP in the ternary system is faster than that of free ATP, which may be the combination of the stronger nucleophile (N atom or $Zn^{2+}–H_2O$ or $Zn^{2+}–OH$ vs. H_2O) and the more sufficient acid catalysis and electrostatic catalysis (the additional positive charge of Mg^{2+},

Ca^{2+}, Zn^{2+} on ATP in the ternary complexes). By comparison with the Mg^{2+} (Ca^{2+})/ATP system, the existence of hard metal ion not only makes the anionic substrate to be more favored by the chain of polyammonium, but also increases the activity of phosphate center of the intermediate, therefore, all these facilitate the nucleophilic attack and the release of the products. With regard to the Zn^{2+}/L/ATP system, the competition of the mixed ligands primarily attributes to the decrease in rate, compared with that of Zn(II)/ATP.

In this paper the interactions of nucleotides (ATP, ADP) with the protonated ligands L1–L3 and/or metal ion (Zn^{2+}, Mg^{2+} and Ca^{2+}) were determined by pH titrations and NMR spectra. Furthermore, the catalytic hydrolysis of ATP by protonated L and/or metal ions was investigated. The results show that metal ion not only promotes the recognition of the substrates by the receptor, but also regulates the mechanism of the hydrolysis reaction, in which the water molecule participates in the hydrolysis process at different steps with different functions. The broad range of the dephosphorylation rates of ATP including retardation or acceleration, compared with M/ATP, depends on the properties of metal ions. To achieve more active molecular catalysts, ligands containing more recognition sites or ligands can bear multi-ions [31] is required, and the work of this viewpoint is undergoing.

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