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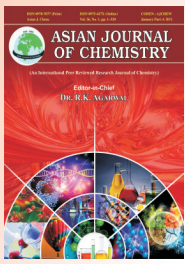

Effects of Metal Ions (Mg^{2+}, Ca^{2+}, Cd^{2+} and Zn^{2+}) on Adenosine Triphosphate Hydrolysis

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The interaction between metal ions (M: Mg^{2+}, Ca^{2+}, Cd^{2+} and Zn^{2+}), N_α-4-tosyl-*L*-arginine methyl ester hydrochloride (TAME) and adenosine triphosphate has been studied by NMR spectra. The catalytic influence of the combined effects on hydrolysis was examined using ^1H and ^{31}P NMR spectra. In M-ATP-TAME ternary systems, adenosine triphosphate interacts with the metal ions and TAME. The act sites are β, γ-phosphate groups and adenine ring. The interaction force are electrostatic-, cation-π and π-π stacking interactions. The rate constant of adenosine triphosphate hydrolysis in M-TAME-ATP ternary systems increased in the following order, $Zn^{2+} > Ca^{2+} > Cd^{2+} > Mg^{2+}$. Different catalytic behaviours were discussed in terms of the corresponding metal ion properties. At the same time, a reasonable mechanism has been proposed that adenosine triphosphate hydrolysis catalyzed by M-TAME occurs through an addition-elimination reaction sequence.

Keywords: N_α-4-tosyl-*L*-arginine methyl ester hydrochloride, Recognition interaction, Catalytic hydrolysis.

INTRODUCTION

Molecular catalysis, together with molecular recognition, self-assembling and translocation, is one of the corner-stones of supramolecular chemistry[1,2]. In this sense, it has given rise to tremendous interests is the catalytic cleavage of adenosine triphosphate (ATP) to give adenosine diphosphate (ADP) and inorganic phosphate, due to its ubiquitous participation in the bioenergetics of all living organisms[3]. Adenosine triphosphate plays a basic role in the bioenergetics of all living organisms, the center for chemical energy storage and transfer being its triphosphate chain. In nature, adenosine triphosphate hydrolysis is catalyzed by adenosine triphosphatease[4,5]. A divalent metal cation is involved in the catalytic mechanism, acting as binding site for adenosine triphosphate, favoring the phosphoryl transfer process[6].

Since complexes of adenosine triphosphate with metal ions acts as a very important function in many biological processes, the coordination chemistry of adenosine triphosphate has aroused much attention[7-9]. Sigel *et al.*[10] has carried out studies of metal ions and nucleotides in the biological systems for more than three decades. Recent studies of adenosine triphosphate with metal ion participation have involved the third ligand such as macrocyclic polyamine, phenanthroline and polyoxo-molybdates[11-14]. However, the role of amino acid on the adenosine triphosphate complexes is still unclear, especially, the role in the metal ion, adenosine triphosphate and ligand ternary systems. Carmona *et al.*[15] reported the effect of arginine-glutamic acid ion pair in the adenosine triphosphate hydrolysis, but the research did not involve the role of metal ions in that system.

Many divalent metal cations, such as Mg(II), Mn(II), Ca(II), Cd(II), Zn(II) and Co(II), have been shown to serve as essential cofactors of the F_1-ATPase for catalyzing adenosine triphosphate hydrolysis[16,17]. In non-enzymetic catalytic system of adenosine triphosphate hydrolysis, emphasis has been placed on the effect of many metal ions. Magnesium(II) and calcium(II) are reported as have the most biological significance[18]. Despite a large knowledge base of metalloenzyme structures, kinetic and binding data and extensive studies with model system, the detailed function of the metal ions play is still unclear. Current interest focuses still on the molecular recognition of nucleotides by the polyamine ligands and their metal complexes, but the effect of different metal ions as well as amino acid in the process of catalyzing adenosine triphosphate hydrolysis have not been studied thoroughly. So, it is necessary to investigate the effect of metal ions and amino acid on the adenosine triphosphate hydrolysis process.

In the past few years, we have been engaged in the studies on the coordination capabilities of *L*-arginine and one metal

ion with adenosine species[19-22]. Among these studies the physicochemical character and conformational variation of *L*-arginine have shown very interesting behaviour both in the recognition of magnesium ion and their interaction with adenosine triphosphate. In a preceding paper, we described different effects of Mg and Ca on the recognition and catalysis in adenosine triphosphate hydrolysis[23,24]. Aiming at further knowledge on the series metal ions of the coordination chemistry of adenosine triphosphate, our group reports the results of binary and ternary metal ion (Mg^{2+}, Ca^{2+}, Cd^{2+} and Zn^{2+}) complexes with adenosine triphosphate and N_α-4-tosyl-*L*-arginine methyl ester hydrochloride (TAME).

EXPERIMENTAL

Adenosine-5'-triphosphate disodium salt (adenosine triphosphate) and N_α-4-tosyl-L-arginine methyl ester hydrochloride were purchased from Acros organics (USA). Their structures are taken from the paper of Ma and Lu[23]. $MgCl_2{\cdot}6H_2O$ and other metal ion salt were purchased from Shanghai Chemical reagent Ltd. (China). Mg^{2+} was recrystallized two times in distilled water and subsequently dried at room temperature for 24 h. Deuterium oxide (D, 99.9 %) is from Cambridge Isotope Laboratories, Inc. Other chemicals used in our experiments were of analytical grade and were used without further purification.

NMR methods: ^1H and ^{31}P NMR spectra were recorded on an Inova 400 MHz spectrometer. The chemical shifts of ^{31}P NMR spectra in ppm were relative to an external reference of 85 % H_3PO_4. The pH value of the solution was recorded at room temperature with a Markson 6200 pH Meter, adjusted to the desired pH values using 1 mol L^{-1} NaOH or HCl. In a typical experiment, a 0.5 mL solution contains 0.07 mol L^{-1} adenosine triphosphate, TAME and metal ions (except for the concentration of Zn^{2+} is 0.01 mol L^{-1}), respectively. 20 % D_2O/H_2O was placed in a 5 mm NMR tube.

Kinetic studies were performed by following the time-dependent change in the integrals from the resolved ^{31}P NMR signals of T_α, T_β and T_γ of adenosine triphosphate, D_α, D_β of adenosine diphosphate and the peak OP for inorganic phosphate at 60 °C and pH 7.

RESULTS AND DISCUSSION

Adenosine triphosphate-TAME binary system: Interaction between TAME and adenosine triphosphate was detected by ^1H and ^{31}P NMR spectra. The variation of the chemical shifts for H2 and H8 proton of adenosine triphosphate as a function of pH at 298 K is plotted in Fig. 1. The ^1H chemical shifts of H2 and H8 are shifted upfield in the whole pH range when TAME is added. The result shows that the adenine ring of adenosine triphosphate takes part in the interaction with TAME. The change of shifts is attributed to be π-π stacking interaction between adenine ring of adenosine triphosphate and benzene ring moiety of TAME[25-27]. This interaction increases the electron cloud density of adenine ring of adenosine triphosphate and leads to the upfield shifting. The upfield shifts of H2 and H8 reflect the proton ionization at the N1 and N7 sites of adenosine triphosphate, which indicate that TAME interacts with N1 and N7 groups under this experimental condition[28,29]. The ^1H NMR spectra of ATP-TAME binary

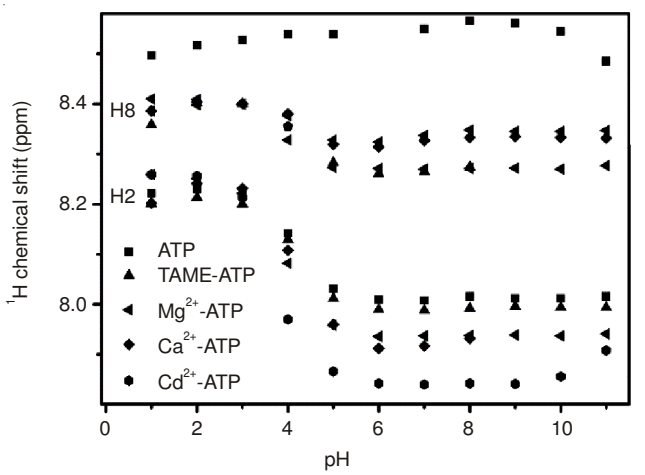

Fig. 1. Plot of the ^1H chemical shifts of H2 and H8 of adenosine triphosphate (0.07 mol L^{-1}) in the presence of TAME (0.07 mol L^{-1}) or metal ions (Mg^{2+}, Ca^{2+} and Cd^{2+}) (0.07 mol L^{-1}), Zn^{2+} (0.01 mol L^{-1}) binary systems as a function of pH at 298 K

system at different pH values provide unambiguous evidence for the involvement of an adenine moiety in π-stacking interactions with the benzene ring moiety of TAME.

The ^{31}P chemical shifts of adenosine triphosphate and ATP-TAME system at different pH, presented in Fig. 2, provide information about the interaction between the phosphate chain of adenosine triphosphate and guanidinium group of TAME by electrostatic interaction. The difference of three P_α, P_β and P_γ chemcical shifts between ATP-TAME binary system and free adenosine triphosphate has minor effect in all binary systems. This indicates that the interaction between TAME and phosphate chain is relative weaker than the interaction between metal ions and phosphate chain[30].

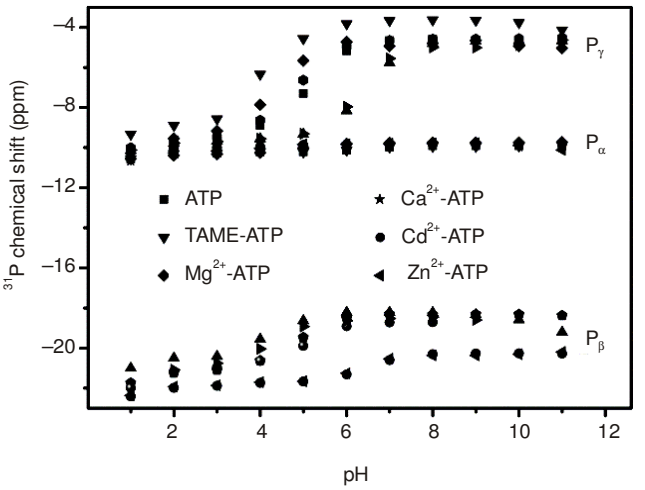

Fig. 2. Plot of the ^{31}P chemical shifts of adenosine triphosphate (0.07 mol L^{-1}) in the presence of TAME (0.07 mol L^{-1}) or metal ions (Mg^{2+}, Ca^{2+} and Cd^{2+}) (0.07 mol L^{-1}), Zn^{2+} (0.01 mol L^{-1}) binary systems as a function of pH at 298 K

Metal-ATP binary system: Mg^{2+} complexation study previously performed on the binary adenosine triphosphate system indicated the formation of a 1:1 complex[23]. Analogous techniques were used in this study to determine binary and ternary systems complexation tendencies, *i.e.*, by monitoring ^1H and ^{31}P chemical shifts with the different metal ions. The

results indicate that 1:1:1 M-TAME-ATP complex can be formed in the presence of the 1:1 complex between TAME or metal ions and adenosine triphosphate.

Fig. 1 showed that the chemical shifts of H2 and H8 are shifted upfield in all M-ATP binary systems expect for the H8 in Cd^{2+}-ATP and Zn^{2+}-ATP binary systems. The ^1H NMR experiments indicate that the divalent metal ions (Mg^{2+}, Ca^{2+}, Cd^{2+} and Zn^{2+}) can promote stacking of the adenine ring[31]. In Mg^{2+}-ATP and Ca^{2+}-ATP systems, ^1H chemical shifts of H2 and H8 move to upfield in the whole pH range. It indicates that Mg^{2+} and Ca^{2+} promote stacking of purine bases[32,33]. In addition, upfield shifts inflect the proton ionization at the N1 and N7 sites of the adenine ring. The signals of H8 in the Cd^{2+}-ATP and Zn^{2+}-ATP systems are shifted downfield in the whole pH range. The observed deshielding upon pH increases is, therefore, a direct result of the ionization of the phosphate group on the H8 proton. Two conformational possibilities exist for the purine ring with respect to its contiguous ribose. There are the so-called *syn* and *anti* conformers[7-9]. The results of Cd^{2+}-ATP and Zn^{2+}-ATP curves in Fig. 1 show an increased deshielding effect of H8 as the degree of phosphate deprotonation increases, demonstrateing that the phosphate deprotonation increases the preference for the *anti* conformation in adenosine triphosphate with cadmium ions and zinc ions[30]. For the adenosine triphosphate complexes with Cd^{2+} and Zn^{2+} the self-association tendency is much larger than for the mentioned corresponding Mg^{2+} and Ca^{2+}-ATP systems. Here, the important point is that metal ions like Zn^{2+} or Cd^{2+} favor the formation of dimers by bridging the phosphate moiety and N7 sites of adenosine triphosphate[6,34,35]. Because the phosphate chains are folded in such a way, which the terminal phosphate groups in adenosine triphosphate are spatially apart from the N7 site. Chemical shift of H8 are directly affected only by α-phosphorus of adenosine triphosphate, since α-phosphate group is in close proximity to N7 of the adenine ring in an *anti* conformation[28]. Another reason is that an intermolecular interaction of this type will occur the stacking, *i.e.*, that the metal ion coordinates to N7 of the adenine ring. Such an intermolecular interaction is expected to shift the resonance of H8 downfield[36]. The change of ^1H chemical shifts as a function of pH of Cd^{2+}-ATP is in accordance with the Sigel's report[37]. Above discusses improve that metal binds to the purine ring of adenosine triphosphate by cation-π interaction[38,39].

The ^{31}P chemical shifts of adenosine triphosphate and metal-ATP systems presented in Fig. 2 provide information about the interaction between the phosphate chain and the different metal ions. All experimental data of metal-ATP are similar to the Mg^{2+}-ATP binary system analyzed in the previous work in detail[23]. All the conclusions outlined for Mg^{2+}-ATP are also valid for Ca^{2+}-ATP, Cd^{2+}-ATP and Zn^{2+}-ATP binary systems. In another word, the two terminal phosphorus are the primary active sites of adenosine triphosphate with different metal ions by electrostatic interaction[40].

In the metal-ATP binary systems, the interaction strength between cadmium ions and adenosine triphosphate is larger than that between magnesium, calcium, zinc ions and adenosine triphosphate in the P_γ site. Only TAME does not affect the ^{31}P chemical shifts of the adenosine triphosphate, while magnesium and calcium ions have the obvious effect on P_β and P_γ. According to the large chemical shifts noted for adenosine triphosphate in the binary systems, it indicated there exists strong interactions between the metal ions and phosphate chain, which is in the order: $Cd^{2+} > Zn^{2+} > Mg^{2+}, Ca^{2+} >$ TAME. In conclusion, all metal ions (Mg^{2+}, Ca^{2+}, Cd^{2+} and Zn^{2+}) in this study are able to form a complex with adenosine triphosphate by coordinating not only to the phosphate chain but also to the adenine ring[41].

Metal-TAME-ATP ternary systems: To shed further light on the structural characteristics of the ternary systems, we decided to perform ^1H and ^{31}P measurements on metal ions and TAME in the adenosine triphosphate aqueous solution at the different pH values. Figs. 3 and 4 display the pH dependence of ^1H and ^{31}P signals of adenosine triphosphate in the metal-ATP-TAME ternary systems.

The ^1H NMR spectra of the ternary systems at different pH values provide obvious evidence for the involvement of an adenine ring of adenosine triphosphate in π-stacking interaction with metal ions and benzene ring moiety of TAME[42]. Similar to adenosine triphosphate coordination binary systems,

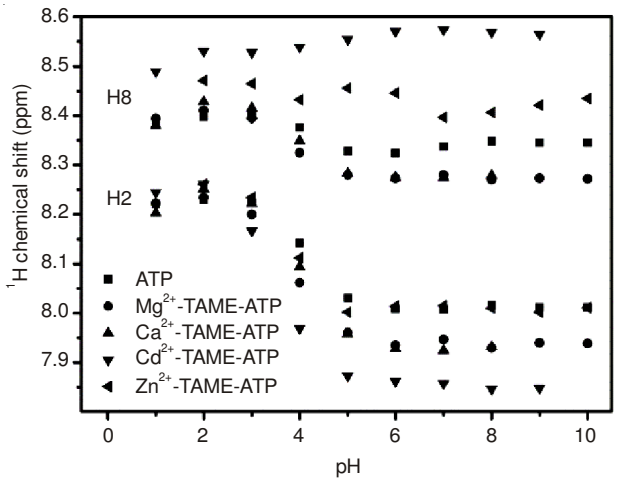

Fig. 3. Plot of the ^1H chemical shifts of H2 and H8 of adenosine triphosphate (0.07 mol L^{-1}) in the presence of TAME (0.07 mol L^{-1}) and metal ions (Mg^{2+}, Ca^{2+} and Cd^{2+}) (0.07 mol L^{-1}), Zn^{2+} (0.01 mol L^{-1}) ternary systems as a function of pH at 298 K

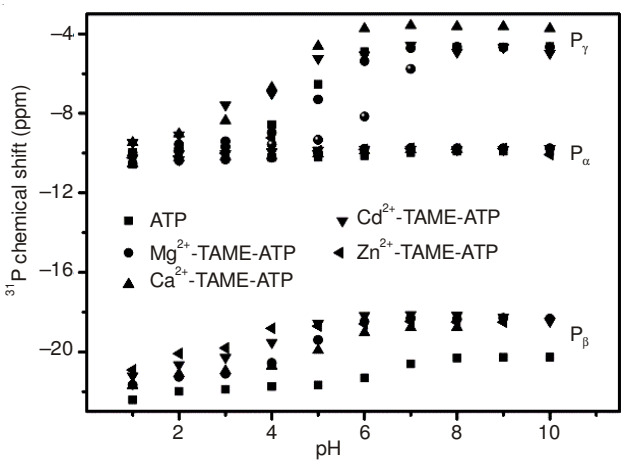

Fig. 4. Plot of the ^{31}P chemical shifts of adenosine triphosphate (0.07 mol L^{-1}) in the presence of TAME (0.07 mol L^{-1}) and metal ions (Mg^{2+}, Ca^{2+} and Cd^{2+}) (0.07 mol L^{-1}), Zn^{2+} (0.01 mol L^{-1}) ternary systems as a function of pH at 298 K

all signals of H_2 and H_8 of adenosine triphosphate in the Mg^{2+}, Ca^{2+}-ATP-TAME system shift upfield, which indicates that the adenine ring of adenosine triphosphate has π-stacking interactions with the benzene ring moiety of TAME and cation ions[43,44]. In the above discussion, there are cation-π stacking interaction between metal ions and adenine ring of adenosine triphosphate as well as ion-paring electrostatic interaction between metal cation and phosphate chain. They are determined by comparison of the ^{31}P chemical shift of adenosine triphosphate before and after the addition of the metal ion to the adenosine triphosphate systems.

As shown in Fig. 4, the ^{31}P signals of α, β and γ-phosphorus of adenosine triphosphate in Ca^{2+}-ATP-TAME ternary system are downfield shifted by 0.147, 1.837 and 1.049 ppm, respectively. Whereas the shifts of adenosine triphosphate in the Ca^{2+}-ATP system are 0.173, 1.876 and 1.102 ppm for α, β and γ-phosphorus, respectively. The signal of the terminal γ-phosphorus shows the largest downfield shift upon complexation, suggesting a stronger involvement of this phosphate chain in charge-charge and hydrogen bonding interactions with the cation. The results indicate that the mixed ligands compete to bind Ca^{2+} to some extent at neutral pH. Both the ^{1}H and ^{31}P NMR data indicate that adenosine triphosphate is not only strongly bound to metal ions (Mg^{2+}, Ca^{2+}, Cd^{2+} and Zn^{2+}) but also to TAME in the ternary systems. As shown in Figs. 3 and 4, ^{31}P chemical shifts are similar to those of the corresponding metal ion-ATP binary systems. The Cd^{2+} and Zn^{2+}-ATP-TAME systems are exceptions, with the downfield shift of H8 in the whole pH range. It indicates that the nucleoside residue of adenosine triphosphate undergoes stacking interactions with benzene ring moiety of TAME. The electron-withdrawing effect of positive charges metal ions bound to the substrate increases the susceptibility of the P_γ center and enhances its activity toward nucleophilic attack[45]. If the recognition process occurs in a sterically controlled way, the metal ion becomes a "messenger" between the receptor and the substrate[44]. Meanwhile, the guanidinium group of TAME and phosphate chain of adenosine triphosphate can form a salt bridge via $N\text{-}H^+ \cdots {}^-O\text{-}P$ hydrogen bonds. The salt bridge favors the proton transfer process from the tertiary nitrogens to the secondary ones, assumption is consistent with as actually suggested by the ^{1}H and ^{31}P NMR measurements[46].

Adenosine triphosphate hydrolysis catalyzed by TAME: The supramolecular catalysis of ligand and metal ions on the adenosine triphosphate hydrolysis has also been studied in binary and ternary system by ^{31}P NMR spectra. The hydrolysis of adenosine triphosphate catalyzed by TAME was examined at 60 °C and pH 7 indicate that TAME has the large catalytic activity.

The course and products of the reaction have been recorded as illustrated in Fig. 5. adenosine triphosphate hydrolyzes to OP and adenosine diphosphate, which thereafter yields adenosine monophosphate and OP[47,48]. Its ^{31}P NMR signal is distinct from that of the different reactive time.

From Fig. 5, it can be seen that no release of pyrophosphate (PP) by ATP-cleavage between P_α and P_β. As reaction time increases, the peaks intensity of adenosine triphosphate went down and those of adenosine diphosphate and OP increased. The reaction medium contained 57.9 % of adenosine

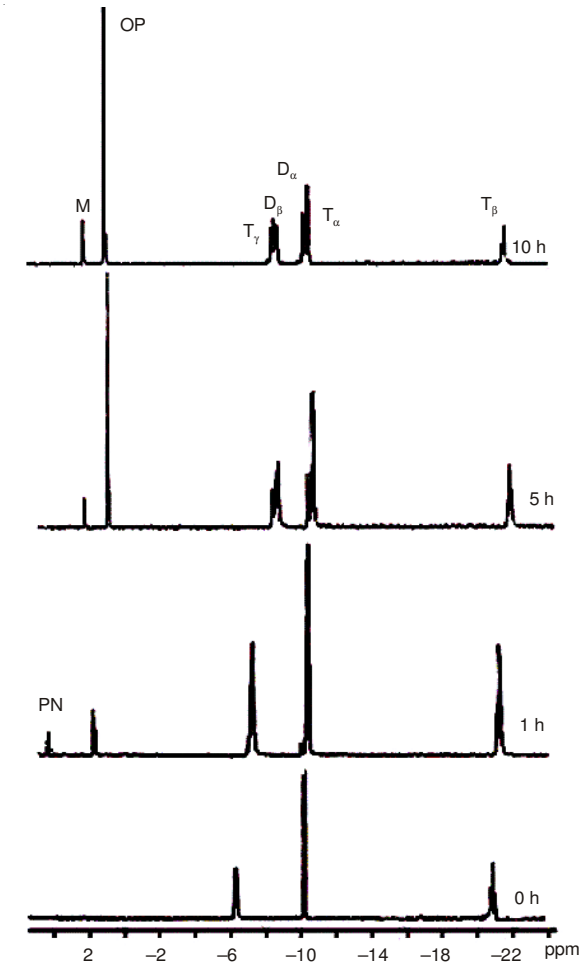

Fig. 5. Observation of adenosine triphosphate hydrolysis by ^{31}P NMR spectra as a function of time, ^{31}P NMR spectra of 0.07 mol L^{-1} adenosine triphosphate and TAME in D_2O/H_2O = 2:8 at 60 °C and pH 7 corresponds to a spectrum taken without heating; the signals are identified by the following symbols: T_α, T_β, T_γ for the α-, β-, γ-phosphorus of adenosine triphosphate; Da, Db for ADP; M for AMP; OP for inorganic phosphate and PN for intermediate

triphosphate, 20.6 % of adenosine diphosphate, 2.3 % of adenosine monophosphate and 17.5 % of Pi after 5 h hydrolysis. While in the system without TAME, there was only 3.5 % of adenosine diphosphate and 3.4 % of π in the same reaction time. The apparent rate constant is obtained from the plot of ln ($[ATP]_t/[ATP]_0$) as a function of time, in which $[ATP]_0$ and $[ATP]_t$ is the initial concentration and the concentration of adenosine triphosphate hydrolysis at the certain reactive time, respectively[49,50].

$$r = k_{obs}[ATP] = -d[ATP]/dt \quad (1)$$

The rate constant of adenosine triphosphate hydrolysis catalyzed by TAME was 5.4×10^{-2} h^{-1} and the rate constant without TAME was 8.5×10^{-3} h^{-1} under the same condition.

The effects of metal on the ^{31}P NMR signals of adenosine triphosphate are very similar to the Fig. 5. Therefore, the trend given above for the complexation of ATP-TAME can be extended to the metal-ATP binary systems and M-ATP-TAME ternary systems[51].

Adenosine triphosphate hydrolysis catalyzed by M-TAME complexes: A series of studies regarding metal ion catalysis of adenosine triphosphate hydrolysis were performed

under same conditions to obtain correlative values, with and without TAME added (Table-1).

TABLE-1
FIRST-ORDER RATE CONSTANTS (K_{obs}) FOR ADENOSINE TRIPHOSPHATE (0.07 mol L^{-1}) HYDROLYSIS IN THE PRESENCE OF TAME (0.07 mol L^{-1}) AND/OR METAL IONS (Mg^{2+}, Ca^{2+}, Cd^{2+}) (0.07 mol L^{-1}) (Zn^{2+}) (0.01 mol L^{-1}) at pH 7 and 60 ^{0}C

Substrate	TAME	Metal ion	Ratio	$10^3k_{obs}(h^{-1})$
Adenosine triphosphate	-	-	1	8.5
	+	-	1:1	54
	-	Mg^{2+}	1:0:1	18.3
	+	Mg^{2+}	1:1:1	21.6
	-	Ca^{2+}	1:0:1	95.4
	+	Ca^{2+}	1:1:1	103.5
	-	Cd^{2+}	1:0:1	30.9
	+	Cd^{2+}	1:1:1	47.5
	-	Zn^{2+}	1:0:1	116
	+	Zn^{2+}	1:1:1	115.4

In metal-ATP binary systems, the rates of adenosine triphosphate hydrolysis were 0.018, 0.095, 0.048 and 0.116 h^{-1} at the presence of magnesium(II), calcium(II), cadmium(II) and zinc(II) ions, respectively. Compared with the rate constant of adenosine triphosphate hydrolysis alone in aqueous solution, their catalytic activities were enhanced by 2.2, 11.2, 3.6 and 13.6 times, respectively. It should be noted that the catalytic activity of zinc(II) is much faster than that of others. Magnesium and cadmium ions appear to increase the rate constant, but the catalytic effect is relative lower than the calcium ions, zinc ions and TAME. Metal ions also influenced the amount of phosphoramidate intermediate, greater percentages being observed in reactions with magnesium, calcium, cadmium and zinc ions[52].

A similar relationship of rates was previously observed for the following five species: Adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, OP and phosphoramide (PN) in the presence TAME as well as added metal ions (Fig. 5). The analysis of the time dependence of ^{31}P NMR spectra in M-ATP-TAME ternary systems shows that the ternary systems hydrolyzed at a faster rate than the binary systems. Particulary, the largest rate constant of hydrolysis in all systems is the Zn(II)-ATP and Zn(II)-ATP-TAME systems. The catalytic activity enhanced more than 13 fold. The metal ion has dual functions. It acts to increase the observed percentage of the intermediate PN *via* stabilization of the P-N bond. Additionally, it is capable of complexation with the nucleotide[53]. Those results prove that the metal ions play the important role in the catalysis on hydrolysis of adenosine triphosphate[48,54].

According to ^{31}P NMR spectra in different time of hydrolysis of adenosine triphosphate, no signal for pyrophosphate (P-P) is observed. However, the signals for adenosine triphosphate, adenosine diphosphate, adenosine monophosphate and Pi, simply that the products of adenosine triphosphate hydrolysis are orthophosphate and adenosine diphosphate, which subsequently products adenosine monophosphate and Pi, *i.e.*, the hydrolysis process can be described as:

$$\text{ATP-Pi} \longrightarrow \text{ADP-Pi} \longrightarrow \text{AMP}$$

There is no evidence to demonstrate the hydrolysis of the produced adenosine monophosphate and the release of pyrophosphate, which indicates that these binary and ternary systems compounds can only catalyze the exo-cleavage of phosphoanahydride bond and can not catalyze intra-cleavage of phosphoanhydride and phosphoester bonds[55].

Tentative mechanism for adenosine triphosphate hydrolysis: The mechanism proposed for the adenosine triphosphate hydrolysis catalyzed by TAME in the presence of M (Mg^{2+}, Ca^{2+}, Cd^{2+} or Zn^{2+}) is shown in Fig. 6.

Catalytic efficiency depends on defined structural requirements of the complex itself, involving protonation pattern, electrostatic and hydrogen bonding effects and spatial arrangement of the reacting species at the catalytic site. The recognition of adenosine triphosphate by protonated TAME, which is mediated by the bridging metal ions, is the first step of the reaction (Fig. 6a). ^{31}P NMR signal appears at 3.21, 3.08, 3.36 and 2.29 ppm after different adenosine triphosphate hydrolysis time, peaks were formed by phosphorylation of nucleophilic N atom, is assigned as the phosphaoramidate intermediate in different ternary systems of Mg^{2+}, Ca^{2+}, Cd^{2+} and Zn^{2+}-ATP-TAME, respectively. With the peak of intermediate disappeared at the different time of hydrolysis, the terminal P_γ will be cleaved. The role of metal ion is considered to be the most stable β, γ coordination of adenosine triphosphate (Fig. 6a). Stacking interactions in the ternary systems are of considerable importance since they contribute to stabilize the PN adduct and assist the nucleotide in achieving the correct inside the active sites. The second step involves the following intramolecular attack by unprotonated N in guanidinium group of TAME or by water on γ-phosphorus to form the intermediate. It is believed that adenosine triphosphate is bound to protonated TAME through electrostatic forces, hydrogen bonds and π-π stacking interaction. Hence, the electrophilicity of the terminal phosphate center is greatly enhanced and therefore the γ-phosphorous center is susceptible to nucleophilic attack from a water molecule[56]. The positive charge formed by the asymmetric cleavage of P_γ transfers to the hydrogen atom of guanidinium group of TAME. Thus, a proton is released to form a covalent N-PO_3^{2-} bond in the intermediate, which can be destroyed by the attack of water molecular in the next step.

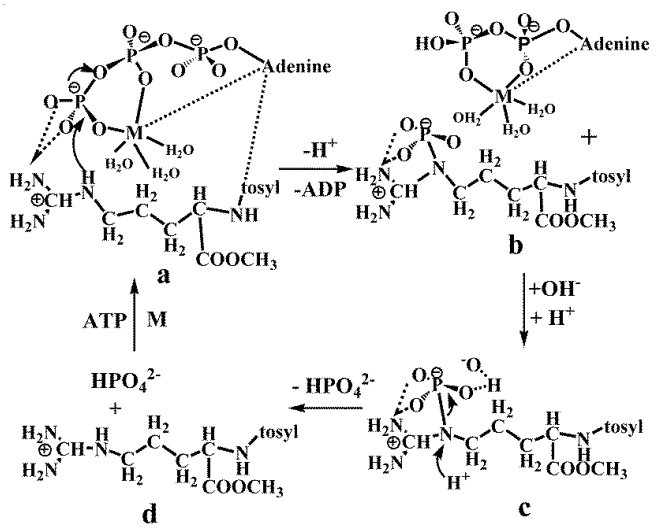

Fig. 6. Proposed mechanism of adenosine triphosphate hydrolysis catalyzed by metal-TAME

The hydroxyl unit of water coonects with PO_3^{2-} to give HPO_4^{2-} (Fig. 6b,c)[57]. Then, the hydrogen atom of water comes back to form TAME again (Fig. 6d).

In our model, the γ-phosphate is coordinated to the metal ion and guanidinium group, making an associative hydrolytic pathway available. Since the chemical shift values for the metal ions in the stacks, bridging by metal must occur *via* the phosphate chains[6]. This process has also proposed that metal ion may play a significant role as an enzyme cofactor through an electrostatic effect and π stacking coordinate bonds between the ion and substrate.

Conclusion

^1H and ^{31}P NMR spectra have indentified that metal ions and TAME can interact with adenosine triphosphate not only by hydrogen bonding and electrostatic interaction but also by cation-π and π-π stacking interaction in binary systems or ternary systems. In the case of M-ATP-TAME, weak forces (electrostatic interaction, cation-π and π-π stacking interaction) can lead to a higher stability in the ternary system. A more complex and challenging catalytic mechanism of adenosine triphosphate hydrolysis catalyzed by M-TAME is obtained. This is attributed to the role of the metal ions as "messenger" between the two terminal phosphates of adenosine triphosphate molecule and the guanidinium group of TAME through the addition-elimination reaction sequence.

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