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Recognition and catalytic hydrolysis of adenosine 5'-triphosphate by cadmium(II) and L-glutamic acid

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Interactions among Cd^{2+} , glutamic acid (Glu), and adenosine 5'-triphosphate (ATP) have been studied by potentiometric pH titration, IR, Raman, fluorescence, and NMR methods. In the Cd^{2+} –ATP binary system, the main interaction sites are the α -, β -, and γ -phosphate groups, N-1, and/or N-7. Cd^{2+} binds to the N-1 site at relatively low pH and binds to the N-7 site of adenosine ring of ATP with increasing pH. In the Cd^{2+} –Glu–ATP ternary system, ATP mainly binds to Cd^{2+} by the triphosphate chain. Oxygens of Glu coordinate with Cd^{2+} to form a complex to catalyze ATP hydrolysis. Hydrolysis of ATP catalyzed by the CdGlu complex was studied at pH 7.0 and 80°C by ^{31}P -NMR spectrometry. Kinetics studies showed that the rate constant of ATP hydrolysis was 0.0199 min^{-1} in the ternary system, which is 9.9-fold faster than that in the ATP solution ($2.01 \times 10^{-3}\text{ min}^{-1}$). Hydrolysis occurs through an addition–elimination reaction mechanism with Cd^{2+} regulating the recognition and catalytic hydrolysis of ATP; water participates in the hydrolysis reaction of ATP at different steps with different functions in the ternary system.

Keywords: ATP; Cd^{2+} ; L-Glutamic acid; Recognition; Catalytic hydrolysis

1. Introduction

Adenosine 5'-triphosphate is an important enzymatic compound and plays a fundamental role in bioenergetics of all living organisms by transferring its triphosphate chain [1, 2]. ATP hydrolysis is catalyzed by both ATPase and a divalent metal ion (Mg^{2+} , Cd^{2+} , etc.), which provides a binding site for ATP or serves as a cofactor that catalyzes the phosphoryl transfer process [3–8]. Because of the complicated nature of the biological system, the roles of ATPase and metal ions in the catalytic hydrolysis of ATP at the molecular level remain unknown. Therefore, non-enzymatic catalysis of ATP hydrolysis has attracted attention from both chemists and biologists. Although studies have been widely conducted on metal ions' impacts on ATP hydrolysis [8–11], the interactions between cadmium ions and ATP have been described only in a few studies [12–14] and the mechanisms for catalytic ATP hydrolysis involving cadmium ions have not been elucidated due to its known biotoxic effects [15, 16]. However, cadmium ions,

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which are as effective in activation of ATPase as magnesium ions [17], are involved in certain biological processes [18, 19] and can fully replace magnesium ions as metal cofactors for ATP hydrolysis. Thus, studying the structural organization and functional activity of ATPase *in vivo* is conducive to understanding the roles of cadmium ions in the catalytic hydrolysis of ATP.

ATP hydrolysis can be catalyzed not only by metal ions, but also other bioligands, such as amino acids or polyamines. Both natural polyamines and synthetic ligands, e.g., macrocyclic polyammonium, phenanthroline, and polyoxomolybdates, have been developed to elucidate mechanisms for reactions involving ATP and metal ions [8, 20–22]. However, there are only a few previous studies focusing on the roles of metal ions and amino acids in recognition and catalytic hydrolysis of ATP [23, 24]. Structural modifications or mutations of any of the amino acid residues could affect the functions of the living cells. For example, enzymes might lose catalytic activities when key amino acid residues are chemically modified or mutated to other amino acid residues. L-Glutamic acid, an important component in biological systems, has been found in the binding region of ATPase and plays an important role in ATP hydrolysis in living organisms [25, 26]. The ATPase activity decreases when the Glu residue is mutated to another amino acid residue in the ATPase pocket [27–29]. It is important to understand how Glu and a metal ion recognize ATP and then catalyze ATP hydrolysis. In this article, we present the results of the interactions among ATP, Glu, and Cd^{2+} to elucidate the reaction mechanism of ATP hydrolysis catalyzed by Cd^{2+} and Glu.

2. Experimental

2.1. Materials

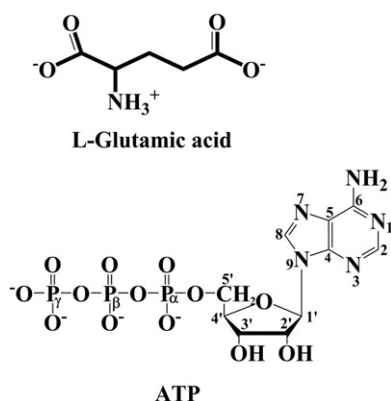
Adenosine 5'-triphosphate disodium salt (99%), adenosine-5'-diphosphate (ADP, 99%), adenosine-5'-monophosphate (AMP, 99.7%), and L (+)-glutamic acid (99%) were purchased from Acros Organics (USA). $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ was purchased from the Shanghai Hengxin Chemical Reagent Co. (China). Deuterium oxide (D_2O , 99.9%) was obtained from Cambridge Isotope Laboratories Inc. All these chemicals were used without purification. The structures of ATP and L-Glutamic acid are shown in scheme 1.

2.2. Potentiometric pH titration

A solution of ATP, metal ion, and/or secondary ligand glutamic acid in a molar ratio of 1:1 or 1:1:1 was titrated with a NaOH solution at $25 \pm 0.1^\circ\text{C}$ under an argon atmosphere. Constant ionic strength was maintained with $0.1 \text{ mol L}^{-1} \text{ NaNO}_3$ and a total volume of 25 mL used in each titration. The calibration of the composite electrode was checked using standard buffer solutions with pH values of 4.00 and 9.18. The calculations were carried out by SCMAR program [30] (Newton–Gauss nonlinear least-squares) on an IBM compatible computer.

2.3. Fluorescent, IR, and Raman measurements

Fluorescence spectra were recorded on a F-7000 spectrofluorophotometer (Hitachi, Japan), using 5/5 nm slit widths, with an excitation wavelength of 281 nm and the



Scheme 1. Structures of ATP and L-glutamic acid.

emission was read from 300 to 550 nm. Solution (3.0 mL) containing an appropriate concentration of ATP, ADP, and AMP was titrated by successive additions of Cd^{2+} or Glu to generate a final concentration of $0\text{--}1.0 \times 10^{-3} \text{ mol L}^{-1}$ of Cd^{2+} and $0\text{--}1.0 \times 10^{-3} \text{ mol L}^{-1}$ of Glu at pH 3.0. IR and Raman spectra were measured with a Nicolet Nexus 670 FT-IR spectrometer (America) and a Nicolet 950 FT-Raman spectrometer (America), respectively.

2.4. NMR measurements

^1H - and ^{31}P -NMR spectra were recorded on an Inova 400 MHz spectrometer. ^{31}P -NMR chemical shifts were referenced to 85% phosphoric acid. The pH of the solution was adjusted to the desired value using 0.1 mol L^{-1} NaOH or HCl at room temperature.

2.5. Kinetics

Kinetic studies were performed at 80°C by following the time-dependent change in the integrals from the resolved ^{31}P -NMR signals for T_α , T_β , and T_γ of ATP, D_α and D_β for ADP, the peak OP for the inorganic phosphate, and PN for intermediate species.

For NMR measurements, solution (0.5 mL 10% D_2O) containing ATP with and without Cd^{2+} and/or glutamic acid was placed in a 5 mm NMR tube and all the concentrations of ATP, Glu, and Cd^{2+} were $0.0363 \text{ mol L}^{-1}$.

3. Results and discussion

3.1. Potentiometric pH titration

Table 1 presents the overall stability constants ($\log \beta$) of the molecular complexes appearing in the ATP/ Cd^{2+} /Glu binary and ternary systems, determined from computer analysis of the potentiometric titration data. The species distribution diagrams of the complexes are shown in figure 1.

Table 1. Overall stability constants ($\log \beta$) for the complex of ATP with Cd^{2+} or Glu and ATP with Cd^{2+} and Glu.

Species	$\log \beta$	Species	$\log \beta$
HATP	6.5891 ± 0.07	H_2GluATP	20.2777
H_2ATP	10.2955 ± 0.06	H_3GluATP	24.8169
HGlu	9.6455	H_4GluATP	27.9636
H_2Glu	13.9818	CdGluATPOH	13.0958 ± 0.0056
H_3Glu	16.15	HCdGluATP	22.2123 ± 0.0057
CdATP	5.188 ± 0.0001	$\text{H}_2\text{CdGluATP}$	27.1415 ± 0.0057
HCdATP	8.3371 ± 0.0002	$\text{H}_3\text{CdGluATP}$	31.2567 ± 0.0058
HGluATP	13.2887	$\text{H}_4\text{CdGluATP}$	34.3496 ± 0.0059

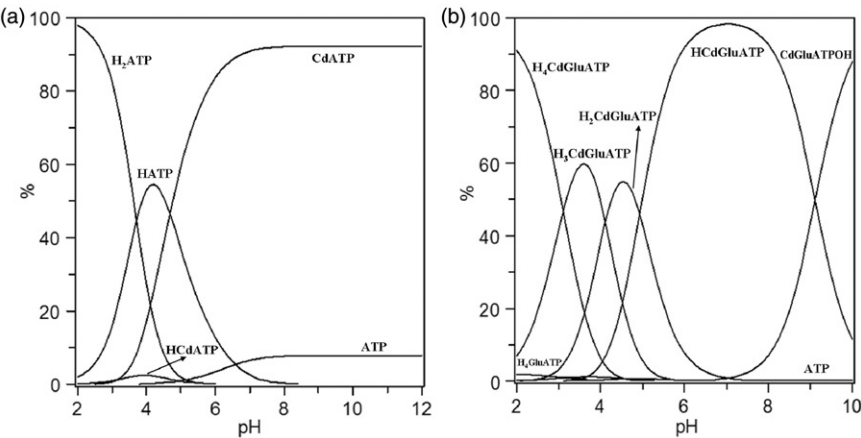


Figure 1. Species percentage distribution diagrams for the binary and ternary systems: (a) the ATP-Cd^{2+} binary system and (b) the $\text{ATP-Cd}^{2+}\text{-Glu}$ ternary system.

Stability constants of ATP and ATP-Cd^{2+} complexes, listed in table 1, are consistent with literature values [12, 31]. Figure 1(a) shows the distribution of the various forms reported for an ATP-Cd^{2+} solution. HCdATP was dominant in the pH range of 2–6 and up to a maximum at pH 4.0. The CdATP species forms when $\text{pH} > 2.5$ with maximum concentration at pH 7.0, the ideal pH level for bioactivity in the biological systems. The complex H_4GluATP appeared at pH below 5.0. H_3GluATP , H_2GluATP , and HGluATP were dominant in the pH ranges 2.0–7.0, 2.0–10.0, and 4.0–12.0, respectively, and reached a maximum at pH 4.0, 6.0, and 8.0, respectively (figure 1 of Supplementary Material). In the ternary $\text{ATP-Cd}^{2+}\text{-Glu}$ system, the following protonated complexes were found (figure 1b): $\text{H}_4\text{CdGluATP}$, $\text{H}_3\text{CdGluATP}$, $\text{H}_2\text{CdGluATP}$, and HCdGluATP . $\text{H}_4\text{CdGluATP}$ species was already observed to form at pH below 2.0 and the other complexes, $\text{H}_3\text{CdGluATP}$, $\text{H}_2\text{CdGluATP}$, and HCdGluATP , formed stepwise as pH increased, and reached a maximum at pH 3.5, 4.5, and 7.0, respectively. All the species are predominant in the pH range and their maximum amounts were higher than 60%. HCdGluATP got to its maximum concentration ($>90\%$) at pH 7.0, which is the ideal pH for bioactivity.

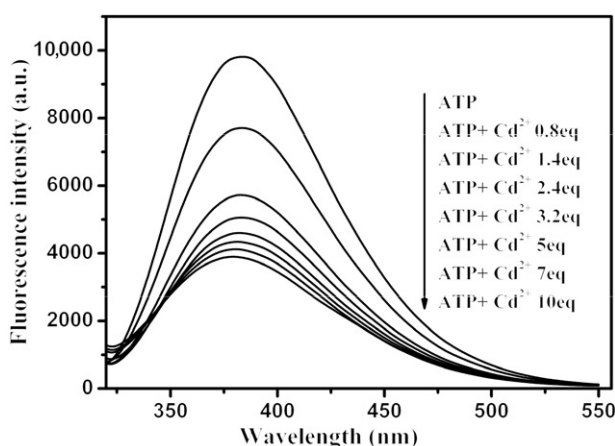


Figure 2. Changes in fluorescence spectra of ATP with gradual addition of Cd^{2+} in an aqueous solution (pH 3.0): $[\text{ATP}] = 1.0 \times 10^{-4} \text{ mol L}^{-1}$, $[\text{Cd}^{2+}] = 0\text{--}1.0 \times 10^{-3} \text{ mol L}^{-1}$; excitation wavelength: 281 nm.

3.2. Fluorescence

Fluorescent character of ATP only exists in a narrow wavelength range in aqueous solution. ATP emits light in the 310–500 nm range when the excitation wavelength is 281 nm. ADP showed less fluorescence intensity and AMP displayed a very weak signal compared with that of ATP in the same conditions (figure 2 of Supplementary Material). Adenine showed a very weak emission from 310 to 500 nm with completely different characteristics than those for AMP, ADP, and ATP. Adenosine almost has no fluorescence compared with that of nucleotide [32]. The number of phosphates in the nucleotide is able to shift the light emission and modify its intensity. Quenched spectra can be used to study the molecular environment in the vicinity of the chromophore molecule [32–34].

Fluorescence emission spectra of $1 \times 10^{-4} \text{ mol L}^{-1}$ ATP with and without Cd^{2+} in aqueous solutions at pH 3.0 are shown in figure 2. Obviously, ATP emitted a strong fluorescence band at 384 nm when the excitation wavelength was 281 nm. Intensities of the emission peaks of ATP gradually decrease as Cd^{2+} is added into the ATP solution. Chemical quenching is ruled out as the cause of weakening fluorescence intensity of ATP after addition of Cd^{2+} because Cd^{2+} did not compete for the excitation photon, since Cd^{2+} does not absorb in the spectral range (300–550 nm). Dilution quenching is also ruled out because the volume and concentration of ATP were kept constant. Thus, it is reasonable to suggest that cadmium ions bound to oxygen of phosphate of ATP formed a CdATP complex and thus modified the fluorescence intensity of ATP.

Further research on the interactions between ATP and Cd^{2+} have been carried out by comparing fluorescence intensities of ATP, ADP, and AMP containing aqueous solutions with addition of Cd^{2+} (figure 3). Fluorescence intensities of ATP and ADP decrease with the addition of Cd^{2+} , while those of AMP were nearly unchanged. These results indicate that Cd^{2+} mainly binds β - and γ -phosphate groups while interactions between the α -phosphate of ATP and Cd^{2+} were very weak, which will be confirmed by NMR spectra in section 3.4. In figure 3 of the Supplementary Material, the fluorescence intensities of nucleotide (ATP, ADP, and AMP) were nearly unchanged with addition

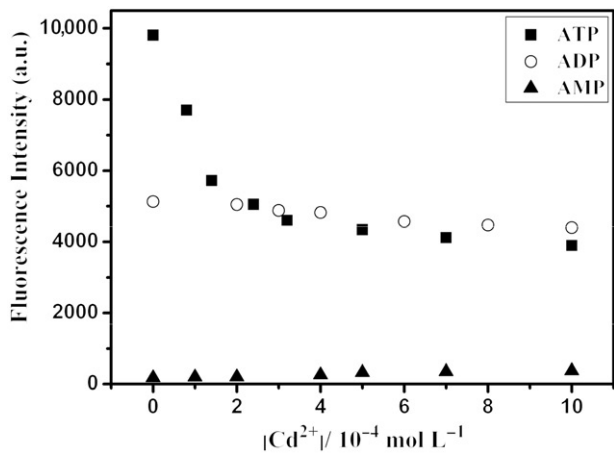


Figure 3. Relative fluorescence intensity of ATP, ADP, and AMP obtained by titration with Cd²⁺; [ATP]=[ADP]=[AMP]=1.0 × 10⁻⁴ mol L⁻¹, [Cd²⁺] from 0 to 1.0 × 10⁻³ mol L⁻¹.

Table 2. Characteristic IR bands (cm⁻¹) of the spectra of the complexes in the different ATP–Cd²⁺–Glu systems and free ATP.

Compound	N–H	C–N(N-7)	$\nu_{\text{ass}}\text{PO}(\alpha \text{ and } \beta)$	P–O–C	$\nu_{\text{s}}\text{PO}(\gamma)$	P–O–P
ATP	1648	1495	1230	1050	968	902
ATP + Cd ²⁺	1641	1512	1221	1081	992	920
ATP + Cd ²⁺ + Glu	1640	1513	1249	1079	992	913

of Glu into the nucleotide–Cd²⁺ solution, suggesting that the phosphate groups of nucleotide mainly interact with Cd²⁺ and the interactions between the phosphate groups of nucleotide and Glu were very weak in the ternary systems.

3.3. IR and Raman spectra

IR and Raman spectra of ATP and its complexes are shown in figures 4 and 5 of the Supplementary Material. The wavenumbers and positions [35, 36] are summarized in table 2. Small shifts in the bands, observed from 4000 to 1700 cm⁻¹, may be due to rearrangement of the strong hydrogen bonding network of free ATP upon complexation [37]. The absorption at 1495 cm⁻¹ assigned to the purine ring vibration of ATP showed a shift of about 15 cm⁻¹ for its complexes, suggesting N-7 binding [37]. The band at 968 cm⁻¹ assigned to the symmetric stretch of the γ -phosphate in free ATP [36–38] shifted to 992 cm⁻¹ and its intensity was greatly reduced upon complexation. In all cases, IR spectra showed a shift of *ca* 24 cm⁻¹ for these absorptions. The intense band at 1249 cm⁻¹, attributed to asymmetric stretch of the α - and β -phosphates of free ATP [36, 38], shifted to 1220–1230 cm⁻¹. Thus, oxygens of α -, β -, and γ -phosphates of ATP participate in coordination for all compounds.

The Raman spectrum of ATP between 600 and 1600 cm⁻¹ is important for analytical studies. The main features of the Raman spectra of ATP (figure 5 of

Supplementary Material) are in general agreement with previous reports [39]. Most observed bands can be assigned to vibrations of phosphate and adenine, which predominantly interacted with metal ions [40–42]. Detectable variations were found in the phosphate vibration region at 1121 cm^{-1} where a shift of 11 cm^{-1} was observed in the ATP– Cd^{2+} binary system and a shift of 3 cm^{-1} in the ATP– Cd^{2+} –Glu ternary system. Ring vibrations of the five-membered ring at 1498 cm^{-1} shifted and their intensities were greatly reduced in both the ATP– Cd^{2+} binary and ATP– Cd^{2+} –Glu ternary systems. The intensity reductions at 1309 cm^{-1} and the line broadening at 724 cm^{-1} indicate that the N-7 site of the adenine ring was the site of interaction between ATP and Cd^{2+} and/or Glu, since these two vibrations can be attributed to either five- or six-membered rings. Thus, the triphosphate groups and the adenine ring engage in ATP's interactions with Cd^{2+} and/or Glu in the ATP– Cd^{2+} binary system and ATP– Cd^{2+} –Glu ternary system, respectively.

3.4. NMR

3.4.1. ATP– Cd^{2+} binary system. Interactions between Cd^{2+} and ATP were investigated by ^1H - and ^{31}P -NMR spectrometry. The change in ^1H chemical shift as a function of pH is in agreement with that reported by Wang *et al.* [43]. Figure 6 of the Supplementary Material shows the normal proton NMR spectrum of the adenine ring of ATP, which serves as the reference for other spectra. The two peaks at 8.008 and 8.337 ppm were assigned to H-2 and H-8 protons of ATP at pH 7.0, respectively. The upfield shifts of H-2 in the pH range 3–5 reflect proton ionization at the N-1 site [43]. The signals of H-2 and H-8 shift downfield at $\text{pH} \geq 6$, indicating that the terminal phosphate group and the N-1 site of ATP were deprotonated [43].

The chemical shifts of H-2 and H-8 of the adenine ring of ATP changed significantly in the ATP– Cd^{2+} binary system compared with those in the ATP solution (figure 6 of Supplementary Material). Two conformations, the so-called *syn* (the base and sugar are on the same side with respect to the glycosidic bond) and *anti*-conformers [44], exist in the ATP molecule. At relatively low pH, ATP takes a *syn* conformation, while it takes an *anti*-conformer when $\text{pH} > 5$ [45]. As shown in figure 6 of the Supplementary Material, the chemical shifts of H-2 were downfield in the binary system when $\text{pH} \leq 3$. It is well known that coordination of a metal ion to a binding site deshields neighboring protons and the resonances of such protons are shifted downfield [43, 46, 47]. Protons, however, are added to the N-1 site to form zwitterion species $^+\text{H}_2(\text{ATP})^{3-}$, with the N-1 site being positively charged when $\text{pH} \leq 3$. Compared with H^+ , Cd^{2+} has the advantage of smaller charges. Thus, Cd^{2+} can at least partially replace the proton to bind to the N-1 site at low pH [48] and the resonance signals of H-2 were shifted downfield compared with those in free ATP solution. However, with increasing pH, the chemical shifts of H-2 were upfield, indicating that Cd^{2+} does not interact with the N-1 site of ATP at $\text{pH} > 3$ because ATP becomes an *anti*-conformer and no geometric advantage exists between the N-1 site of ATP and Cd^{2+} . The corresponding chemical shifts of H-2 were upfield, probably due to the known Cd^{2+} promoted self-association of ATP [49]. Figure 6 of the Supplementary Material shows that the chemical shifts of H-8 were downfield in the whole pH range, due to Cd^{2+} directly coordinating with the N-7 site of adenine ring of ATP, which was also supported by previous studies [12, 48–50].

The ATP molecule can interact with metal ions not only by nitrogens of the purine nucleus, but also by oxygens of the triphosphate chain. In ^{31}P -NMR spectra of Cd^{2+} –ATP binary system (figure 7 of Supplementary Material), the chemical shifts of all the phosphorus atoms changed with the addition of Cd^{2+} into ATP solution, which, together with the results of the fluorescence, IR, and Raman studies, indicate that all phosphate groups participated in this reaction. Compared with the ^{31}P chemical shifts of all the phosphate groups in free ATP solution, the change of signals of β - and γ -phosphates was much larger than that of the α -phosphate of ATP in the ATP– Cd^{2+} binary system. Cd^{2+} mainly interacted with β - and γ -phosphates and the interaction between Cd^{2+} and α -phosphate was weaker than that among Cd^{2+} , β -, and γ -phosphate groups in the whole pH range. The results are consistent with the findings from the molecular modeling calculations (figure 8 of Supplementary Material).

3.4.2. ATP– Cd^{2+} –Glu ternary system. The complexation of CdATP in the presence of Glu has been studied by ^1H - and ^{31}P -NMR spectrometry (figures 9 and 10 of Supplementary Material). The purpose was to find out whether an ATP– Cd^{2+} –Glu complex can be observed.

Addition of Glu into the ATP solution caused downfield shifts of the signals of H-2 and H-8 in the whole pH range (figure 9 of Supplementary Material). The N-1 site is a positive reaction center because N-1 is protonated when $\text{pH} < 5$ and the carboxyl group of Glu is a negative center of the interaction because it has a negative charge in the whole pH range. Therefore, the carboxyl of Glu interacted with the N-1 site of ATP by hydrogen bond and electrostatic interactions [51]. With increasing pH, N-1 is deprotonated, and an inverse interaction takes place with N-1 of ATP becoming a negative center and the protonated amine of Glu taking part in the interaction as a positive center. Intermolecular interactions between the carboxyl of Glu and ATP are impossible because there are no positive centers in the ATP molecule at relatively high pH. At the same time, the phosphate group of ATP also engaged in noncovalent interactions with Glu, supported by changes in the position of the ^{31}P -NMR signals. Compared with the ^{31}P chemical shifts of all the phosphate groups in free ATP solution, the presence of Glu resulted in downfield shifts of both β -P (0.04 ppm) and γ -P (0.15 ppm) and no shift occurred in α -P at pH 7.0. The results indicate that the terminal phosphate group is the primary active site of ATP with Glu by electrostatic interaction and hydrogen bonding [52], although the interaction between Glu and the triphosphate groups of ATP is very weak. Compared with those in the ATP solution, the ^{31}P signals of α -, β -, and γ -P of ATP in the ternary system were downfield shifted by 0.125, 2.33, and 1.69 ppm, respectively, and those in the Cd^{2+} –ATP binary system by 0.21, 2.45, and 1.75 ppm, respectively, at pH 7.0 (figure 10 of Supplementary Material). Cd^{2+} could coordinate with the oxygen of the carboxyl groups of Glu [53], leading to decreased activity of Cd^{2+} with the phosphate groups of ATP. Since the positive charge density of Cd^{2+} bonded to Glu, it decreased the electron-withdrawing effect of Cd^{2+} to oxygens of phosphate groups of ATP [53]. Thus, competition exists between ATP and Glu in binding to Cd^{2+} in the ternary Cd^{2+} –ATP–Glu system. One interesting aspect of ^{31}P signals for the ternary system is that the chemical shifts for all three phosphorus atoms of ATP were almost the same as those in the binary Cd^{2+} –ATP system, indicating that the major interaction of the phosphate groups of ATP was with cadmium in the ternary Cd^{2+} –ATP–Glu system.

3.4.3. Catalytic hydrolysis of ATP. Kinetic measurements of ATP hydrolysis were carried out by the following time-dependent changes of the integrals from the resolved ^{31}P -NMR signals of α -, β -, and γ -P of ATP and peaks of inorganic phosphates. The ATP hydrolysis catalyzed by Cd^{2+} and Glu was studied at 80°C and pH 7.0 using ^{31}P -NMR. The ^{31}P signals of ATP at the different hydrolysis times are illustrated in figure 4. ATP hydrolyzed to ADP and phosphate (OP), and then yielded AMP and OP. The observed rate constant k_{obs} (equation (1)) was obtained from the plot of $\ln([\text{ATP}]/[\text{ATP}]_0)$ as a function of time (figure 5), where $[\text{ATP}]_0$ and $[\text{ATP}]$ are the initial and actual concentrations of ATP, respectively.

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

From the kinetic study, Cd^{2+} and Cd^{2+} -Glu accelerate the reaction of ATP hydrolysis moderately with rate constants of $1.84 \times 10^{-2} \text{ min}^{-1}$ and $1.99 \times 10^{-2} \text{ min}^{-1}$,

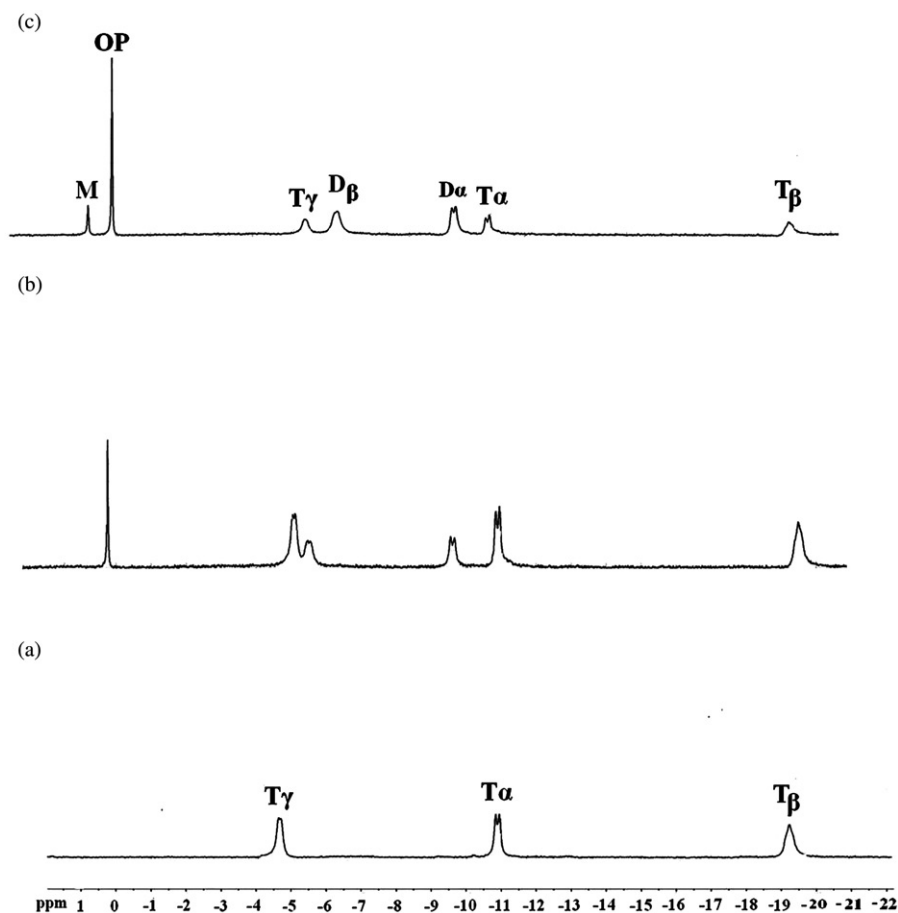


Figure 4. ^{31}P -NMR spectra of ATP hydrolysis at different reaction times in the ternary system. Concentrations of Cd^{2+} , ATP, and Glu are $0.0363 \text{ mol L}^{-1}$, $\text{D}_2\text{O}/\text{H}_2\text{O}=1:9$ at 80°C and pH=7.0. (a) $t=0$ h, (b) $t=0.5$ h, and (c) $t=2$ h. T_α , T_β , and T_γ for the α -, β - and γ -phosphorus of ATP; D_α and D_β for ADP; M for AMP and OP for inorganic phosphate.

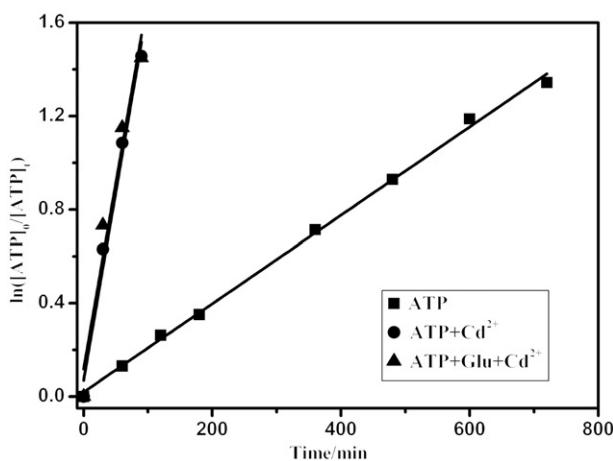
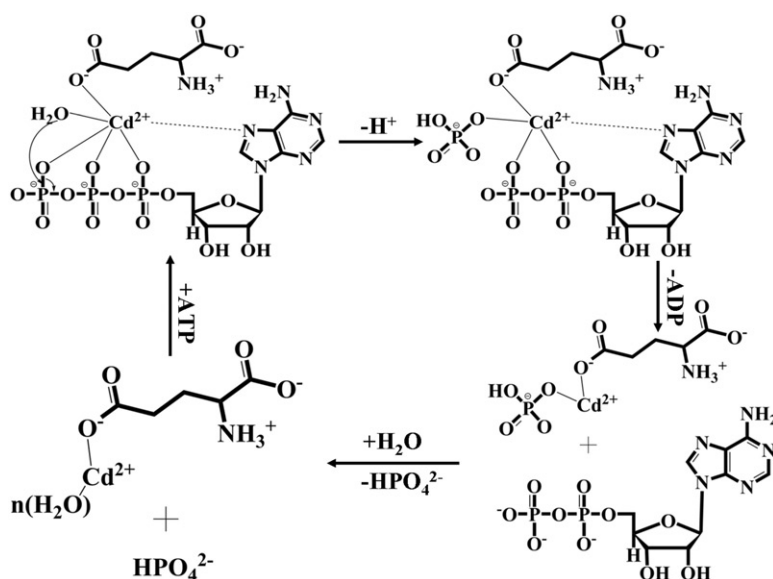


Figure 5. Rate constants for ATP hydrolysis in different systems. ATP (■), Cd^{2+} -ATP (●), and Cd^{2+} -ATP-Glu (▲).

respectively, at pH 7.0 (figure 5), which were about 9.15- and 9.9-fold faster than in the ATP solution ($2.01 \times 10^{-3} \text{ min}^{-1}$), respectively. Cd^{2+} not only directly interacts with the N-7 site and coordinates with α -, β - and γ -P of ATP but also coordinates with water to form a complex [53], which is a good nucleophile to attack the terminal phosphate of ATP. Thus, the catalysis of cadmium ions is greater than that of calcium and magnesium ions [11, 54, 55]. For efficient catalytic hydrolysis of ATP, strong binding of the substrate by the catalyst is required. The binding of ATP by the Glu-metal complex has the following dual effects on the rate acceleration: (a) neutralization of the negative charge of the substrate and (b) an increase in the susceptibility of the phosphorus center of ATP [56].

All these experimental results allowed us to find the reaction mechanism for catalysis of ATP hydrolysis by Cd^{2+} and Glu in the ternary Cd^{2+} -ATP-Glu system. Catalytic efficiency depends on the structural requirements of the complex itself, which involves a protonation pattern, electrostatic interaction, hydrogen bonding effects, and spatial arrangement of the reacting species at the catalytic site [57]. According to an addition-elimination mechanism [58], we propose that ATP hydrolysis was catalyzed by Cd^{2+} and Glu in the ATP- Cd^{2+} -Glu ternary system, as shown in scheme 2. The hydrolysis of ATP has been divided into three steps. The first step is recognition and binding among ATP, Cd^{2+} , and Glu, as discussed in previous sections. The second step involves an intermolecular nucleophilic attack on γ -P of ATP by $\text{HCdGlu-H}_2\text{O}$, the formation of an intermediate, and the release of ADP. The cadmium binds to the α -, β -, and γ -phosphate groups of ATP and the oxygen of the carboxyl groups of Glu. $\text{CdGlu-H}_2\text{O}$ acts as a nucleophile to attack the terminal phosphate in ATP- Cd^{2+} -Glu ternary system to form a phosphorylated intermediate. The phosphorylated intermediate was found in ^{31}P -NMR spectra in other ATP-metal ion-amino acid ternary systems [55]. However, the intermediate has not been observed in the Cd^{2+} -Glu-ATP ternary system since formation of the phosphoramidate intermediate is related to pH, reaction time, ATP concentration, etc. The final step of the mechanism at neutral pH involves the capture of a water and the coordination bond $\text{CdGlu-PO}_3(\text{OH})$ cleavages to give



Scheme 2. Postulated mechanism for ATP hydrolysis catalyzed by Cd^{2+} -Glu.

HPO_4^{2-} and regenerate the catalyst. The hydrolysis of ATP in the ternary system is faster than that of free ATP, from a combination of the stronger nucleophile and the electrostatic catalysis.

4. Conclusions

Both the N-1 and/or N-7 of the adenosine ring and all the triphosphate groups of ATP are reaction sites in the Cd^{2+} -ATP binary system. At relatively low pH, Cd^{2+} can partially or fully replace the proton to interact with N-1 site of adenosine; the efficiency of the interactions of N-1 with Cd^{2+} decreases with increasing pH as N-1 is deprotonated. Cd^{2+} directly interacts with the N-7 site of the adenosine ring in the whole pH range and promotes self-association of ATP at relatively high pH. Cd^{2+} coordinates with the α -, β -, and γ -phosphate groups of ATP in the whole pH range, although the interactions between α -phosphate and Cd^{2+} are weaker than those of β - and γ -phosphate groups. In the Cd^{2+} -Glu-ATP ternary system, ATP mainly binds to the cadmium ion. Interactions between ATP and Glu are very weak, negligible compared with that between ATP and Cd^{2+} . The oxygens of the carboxyl groups of Glu coordinate with Cd^{2+} to form a complex to catalyze ATP hydrolysis.

The hydrolysis of ATP catalyzed by Cd^{2+} and Glu in the Cd^{2+} -Glu-ATP ternary system has been investigated by ^{31}P -NMR spectra. Cd^{2+} and Cd^{2+} -Glu accelerate the rate of ATP hydrolysis and the roles of Cd^{2+} -Glu in the ternary system are analyzed. The possible mechanism of ATP hydrolysis catalyzed by Cd^{2+} -Glu has been proposed on the basis of an addition-elimination reaction mechanism. The results show that metal ions can regulate the hydrolysis reaction of ATP and water participates in the

ATP hydrolysis process at different steps with different functions. This is useful to obtain more information about the key amino acid residues and metal ions that serve as cofactors in the ATPase effect on the ATP synthesis and hydrolysis at the molecular level.

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