Reactivities and Site-Selectivities of Hydrolyses of ATP and UTP Promoted by Metal Complexes of Adenine-Linked Di-2-pyridylamine Derivatives

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The reactivities and the site-selectivities of the hydrolyses of ATP and UTP by the catalysts of the metal complexes of adenine-linked di-2-pyridylamine ligands, $(Py)_2N-(CH_2)_n-Ade$ (L: $n = 3$, 4, 5, and 6), were examined. It was found that these adenine–dipyridylamine coordinated Cu^{2+} complexes (with 2 : 1 ratios of $[Cu^{II}(L)]^{2+}$: ATP or $[Cu^{II}(L)]^{2+}$: UTP) were more reactive for the hydrolyses of ATP and UTP than the complexes of ligands containing other metal ions (Mg^{2+}, Ni^{2+}, and Zn^{2+}) at 40 °C and pH 7.3 (HEPES buffer), as reflected in much higher product ratios of ADP/AMP and UDP/UMP than those of Cu^{2+} alone. The observed high reactivity and selectivity are interpreted in terms of the base-base stackings between an adenine moiety of ATP or an uracil moiety of UTP and an adenine of the ligands, and of the selective coordination of Cu^{2+} to oxide ions in phosphate residues in the ternary complexes of ligand–Cu^{2+}–ATP. The Cu^{2+}-complex of di-2-pyridylamine having no adenine moiety, which is the active center of $[Cu^{II}(L)]^{2+}$, promoted the hydrolyses of ATP and UTP less efficiently than the aquacopper(II) ion. The number of spacer methylene groups of the ligands influenced the hydrolytic activity of the Cu^{2+}-complexes of these adenine–dipyridylamine ligands. The complexes of $[Cu^{II}(L\text{-}4)]^{2+}$ and $[Cu^{II}(L\text{-}5)]^{2+}$ were the most reactive and site-selective for the hydrolyses of ATP and UTP, respectively.

The phosphoric esters are most important metabolic intermediates in vivo. Adenosine 5′-triphosphate (ATP) is referred to as a high-energy phosphate, in which the cleavage of phosphate bonds is an important pathway for energy transfer in biological systems. ATP may undergo loss of either an orthophosphate (Pi) or a diphosphate (PPi) residue during its utilization in biosynthetic reactions catalyzed by the corresponding enzymes, to form ADP or AMP,[1] respectively. Other nucleoside 5′-triphosphates such as GTP, UTP, or CTP also participate as a carrier of high-energy phosphate residues, which they channel into specific biosynthetic routes.

In the enzymatic reactions of ATP, a metal ion is usually involved as a cofactor.[2—6] Numerous metallo-enzyme models have been designed and studied on hydrolyses of mono-, di-, and triphosphates by Sigel and co-workers.[7—14] It is considered that a) the structures of metal ion : ligand : substrate ternary complexes and/or of their transition states and b) the pK_as of coordinated H_2O, should play important roles for hydrolyses of carboxylic and phosphoric esters.[15] The work on the influence of various metal ions on the hydrolysis of ATP has shown that Cu^{2+} is particularly effective.[12] In most cases the 2 : 1 ratio of M^{2+}/ATP is more effective in promoting the hydrolysis than the 1 : 1 ratio, but in the case of Cu^{2+}/ATP, the 1 : 1 ratio is also active.[12] The efficacy of the metal ion to promote ATP dephosphorylation decreases in the order of $Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+} > Mn^{2+} > Mg^{2+}$. For model studies in this area, it has been shown that Co^{3+} complexes of *cis*-$[(N_4)Co^{III}(OH)(OH_2)]^{2+}$, where N_4 are tetraamine ligands, are very effective in promoting hydrolysis of ATP.[16] However, these reactions produce not only ADP but also AMP because of the ready dephosphorylation of ADP. In the neutral pH regions, $[Co(N_4)]$/ATP complexes show higher rates in production of Pi and ADP, than of PPi and AMP.[17] However, an addition of Cu^{2+} or Ca^{2+} to preformed $[Co(N_4)]$/ATP complex leads to increased rates in the production of PPi as well as Pi. The production of PPi is due to the coordination of Cu^{2+} to the adenine moiety (N-7) of $[Co(N_4)]/\beta,\gamma$-ATP. In the CTP and UTP systems, the metal ion/nucleic base interaction may not be significant, but Cu^{2+}/ATP exists in form of a macrochelate resulting from the coordination of Cu^{2+} to the β,γ-phosphate residues and to the adenine moiety (N-7).[9,10] Zinc and copper ion were found to catalyze the transphosphorylation of 1,10-phenanthroline-2-methanol by ATP.[18,19] In a ternary complex of bipyridine with Cu^{2+} and ATP, $[Cu^{II}(bpy)(atp)]$, the phosphate bonds are protected from dephosphorylation.[7] Meanwhile, the complex formation of di-2-pyridylamine (dpa) with Cu^{2+} ion is known to be quite strong in comparison with that with other metal ions and the $[Cu^{II}(dpa)]^{2+}$ complex can catalyze the hydrolysis of methyl acetate efficiently at pH 7.0.[20]

The theoretical consideration,[21] X-ray structural,[22,23] and the NMR studies[9,10] revealed the self-stacking of nucleoside 5′-triphosphates and their metal complexes. The molecular assemblies of adenine and thymine residues having lipophilic alkyl chains in micellar solutions indicated the 1 : 1 stoichio-

metric base-pairing caused by hydrogen bonding within the hydrophobic micelles, but in the absence of micelles the base-stacking occurred in preference to base-pairing.[24] Yet the information is limited as for aromatic stacking of nucleotide bases in water.[25] Lehn and co-wokers reported a study of the multifunctional receptor molecule containing a macrocyclic polyamine linked to an acridine moiety capable of catalyzing hydrolyses of nucleotide polyphosphates like as ATP and ADP, partially caused by stacking between its acridine and adenine moieties.[26] Acridine derivatives could associate with the bases of nucleotides and nucleic acids by stacking.[27] Recent observations demonstrated that a chimeric Rec-A nucleoprotein implicates non-Watson–Crick interactions in the recognition of homologous bases in duplex DNA.[28—30]

Herein we report that the metal complexes of di-2-pyridylamine (dpa) derivatives having adenyl moiety with different lengths of methylene spacers on their side chains (abbrevated to ade-dpa or L, hereafter) promoted the site-selective hydrolyses of ATP and UTP (Fig. 1). In these designed molecules, a metal complex of dpa could expect to work an active site for nucleotide polyphosphate hydrolysis and an adenine moiety could associate with nucleic bases by stacking. The hydrolytic reactivity and the site-selectivity of these complexes-promoted hydrolyses of ATP and UTP are discussed in terms of metal ion-coordination and base-base stacking, where a methylene spacer linking dpa and adenine may serve to locate the reacting partners on proper positions in a reactive ternary complex.

Results and Discussion

Preparation of ade-dpa Ligands. The ade-dpa ligands L-3, L-4, L-5, and L-6 were prepared according to the following methods (Fig. 2), where these ligands were obtained by the reactions of *N*-acetyladenine with α,ω-dibromoalkanes followed by the reaction with dipyridylamine (dpa). L-3, L-4, L-5, and L-6 have dpa groups attached to an adenine base with three to six methylene groups as a spacer. All these ligands were characterized by ^{1}H- and ^{13}C NMR spectrometry.

A Survey of Activities of Metal Ions. The hydrolysis of ATP by the complexes of these ligands with various metal ions was monitored by an anion-exchange HPLC, and the amounts of ATP, ADP, and AMP from the reaction mixtures were measured directly after incubation for 24 h at 40 °C in the presence or in the absence of metal ions and ligands.

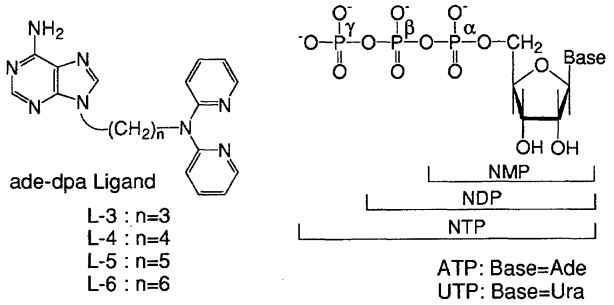

Fig. 1. Structures of ade-dpa ligands and NTP.

Table 1 indicates that the reactivity of divalent metal ions (M^{2+} : ATP=1 : 1) for the hydrolysis of ATP at pH 7.3 and 5.7 in the absence of ligand is in the order $Cu^{2+} > Zn^{2+} > Co^{2+}$. This order of the reactivity of metal ions agrees with the initial rate of dephosphorylation of ATP at pH 7.5 and may be reasonable if considered on the basis of the affinity of phosphate residues of ATP toward these metal ions.[12] On the other hand, the reactivities (hydrolysis %) and site-selectivities (ADP/AMP) were similar in the presence of ligands (L-3 and L-4) and metal ions (L : M^{2+}: ATP=1 : 1 : 1 ratio) as compared to those in the presence of M^{2+} alone (M^{2+} : ATP=1 : 1 ratio), the only clear exception being $Cu(L)^{2+}$ which showed high site-selectivity.

Since from the earlier studies[12] indicated that the most reactive species of M^{2+}/ATP systems are 2 : 1 compositions, $[M^{II}_2(atp)]$, we then examined at 2 : 2 : 1 compositions of M^{2+}/L/ATP at both pH 7.3 and 5.7, as shown in Table 2. Table 2 shows that the reactivity of M^{2+}-promoted hydrolysis of ATP is in the order of $Cu^{2+} > Zn^{2+} > Ni^{2+} \approx Mg^{2+} > Co^{2+}$ at pH 7.3, whereas that is in the order of $Cu^{2+} > Zn^{2+} > Mg^{2+} > Ni^{2+}$ at pH 5.7. The addition of dpa without adenine inhibited the activity of Cu^{2+} at pH 7.3, indicating that the $[Cu^{II}(dpa)]^{2+}$ complex was inactive, although it was active at pH 5.7. In the presence of both adenine and dpa, which are functional groups in these ligands, the inhibitory effect was somewhat reduced at pH 7.3, but its selectivity was still low and similar to the case of Cu^{2+} alone.

The aquacopper(II) ion was more reactive than $[Cu^{II}(L)]^{2+}$-complexes at both pHs 7.3 and 5.7, and the reactivities of M^{2+} and $[M^{II}(L)]^{2+}$-complexes were almost similar in the presence of the other metal ions. Consequently, Cu^{2+}-complexes were more reactive than the other metal ion complexes. The site-selectivities of $[Cu^{II}(L)]^{2+}$ at both pH 7.3 and 5.7 and of $[Zn^{II}(L)]^{2+}$ at pH 5.7 were higher than those of Cu^{2+} and Zn^{2+} alone, respectively. On the other hand, their site-selective hydrolyses of the other $[M^{II}(L)]^{2+}$ complexes were similar to

Table 1. Extents and Selectivities of the Hydrolysis of ATP at 1 : 1 : 1 Ratios of Metal Ion, Ligand, and ATP[a]

		pH 7.3		pH 5.7	
M^{2+}	Ligand	Hydrolysis (%)[b]	Ratio of ADP/AMP	Hydrolysis (%)[b]	Ratio of ADP/AMP
None	None	ca. 0	—	19.5	14.2
Cu^{2+}	None	65.2	4.2	64.7	4.8
Cu^{2+}	L-3	16.0	18.8	24.4	18.9
Cu^{2+}	L-4	17.4	18.1	24.5	22.3
Zn^{2+}	None	13.0	4.6	38.9	11.9
Zn^{2+}	L-3	11.5	6.7	30.1	18.0
Zn^{2+}	L-4	13.4	5.8	35.7	15.0
Co^{2+}	None	1.5	7.6	—	—
Co^{2+}	L-3	1.5	—[c]	—	—
Co^{2+}	L-4	0.2	—[c]	—	—

a) Experimental conditions; All reactions were run at 40 °C for 24 h in 20 mmol dm^{-3} HEPES (pH 7.3) and MES (pH 5.7), [M^{2+}], [L], [ATP]: 0.6 mmol dm^{-3} each. b) Accuracy of the hydrolysis of ATP was ±5% of its extent. c) Production of AMP was not observed.

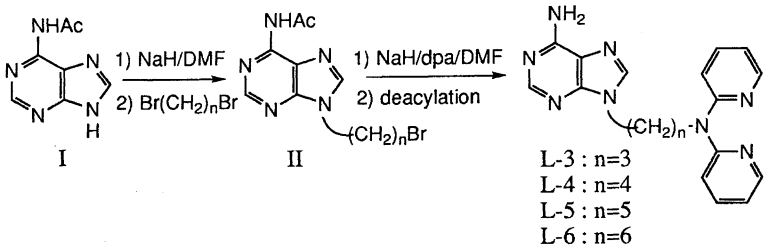

Fig. 2. Scheme of ade-dpa ligand syntheses.

Table 2. Extents and Selectivities of the Hydrolysis of ATP at 2 : 2 : 1 Ratios of Metal Ion, Ligand, and ATP[a)]

M^{2+}	Ligand	pH 7.3		pH 5.7	
		Hydrolysis (%)[b)]	Ratio of ADP/AMP	Hydrolysis (%)[b)]	Ratio of ADP/AMP
Cu^{2+}	None	72.3	2.4	92.4	0.8
Cu^{2+}	dpa	5.0	2.1	92.0	5.1
Cu^{2+}	Ade-dpa	41.6	1.9	—	—
Cu^{2+}	L-3	64.2	5.2	75.4	5.2
Cu^{2+}	L-4	67.1	6.6	84.1	4.6
Zn^{2+}	None	42.3	2.4	39.7	14.7
Zn^{2+}	L-3	46.0	2.2	29.4	20.2
Zn^{2+}	L-4	44.8	2.9	37.8	16.8
Co^{2+}	None	3.7	10.0	—	—
Co^{2+}	L-3	3.5	—[c)]	—	—
Co^{2+}	L-4	3.2	13.0	—	—
Mg^{2+}	None	12.5	8.9	5.0	8.7
Mg^{2+}	L-3	11.3	8.2	1.2	10.1
Mg^{2+}	L-4	9.3	8.4	1.4	34.2
Ni^{2+}	None	12.4	8.8	0.3	22.5
Ni^{2+}	L-3	11.1	9.1	1.4	22.5
Ni^{2+}	L-4	10.7	8.6	0.4	29.4

a) Experimental conditions; All reactions were run at 40 °C for 24 h in 20 mmol dm^{-3} HEPES (pH 7.3) and MES (pH 5.7), [M^{2+}], [L]: 1.2 mmol dm^{-3} each and [ATP]: 0.6 mmol dm^{-3}.
b) Accuracy of the hydrolysis of ATP was ±5% of its extent.
c) Production of AMP was not observed.

those of M^{2+} alone.

The high ratios of ADP/AMP in the presence of Mg^{2+}, Ni^{2+}, Co^{2+} and their $[M^{II}(L)]^{2+}$ complexes were the result of the low extent of the direct hydrolysis of ATP to AMP and of a slow rate of the hydrolysis of ADP to AMP (see below).

Reactivities and Site-Selectivities of $[Cu^{II}(L)]^{2+}$ Complexes for Hydrolysis of ATP. In order to delineate a more detailed feature of these systems, the Cu^{2+}-complex formation was examined by measuring the UV absorption spectra of a ade-dpa ligand (L-4) in the presence of various concentrations of Cu^{2+} ion as shown in Fig. 3, which indicates the formation of the 1 : 1 stoichiometric complex of $[Cu^{II}(L\text{-}4)]^{2+}$. Similarly, the formation of 1 : 1 complexes with Cu^{2+} was observed for the other ade-dpa ligands.

The dependence of ATP hydrolysis after 24 h on the ratios of $[Cu^{II}(L)]^{2+}$/[ATP] from 1 to 5 were examined at pH 7.3 and 5.7, as shown in Figs. 4 and 5. For other $[Cu^{II}(L)]^{2+}$ complexes, except for $[Cu^{II}(L\text{-}6)]^{2+}$, it is seen that the activity saturation occurred at 2:1 ratios of $[Cu^{II}(L)]^{2+}$/ATP at both

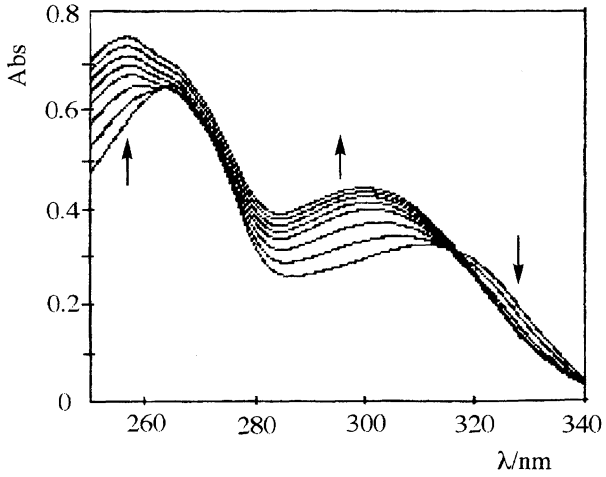

Fig. 3. UV spectral change of L-4 in the titration with $CuCl_2$ at pH 7.3, [L-4]: 3.6×10^{-5} mol dm^{-3}, [$CuCl_2$]: 0, 0.14, 0.29, 0.43, 0.57, 0.71, 0.88, and 1.0 equiv mol, respectively.

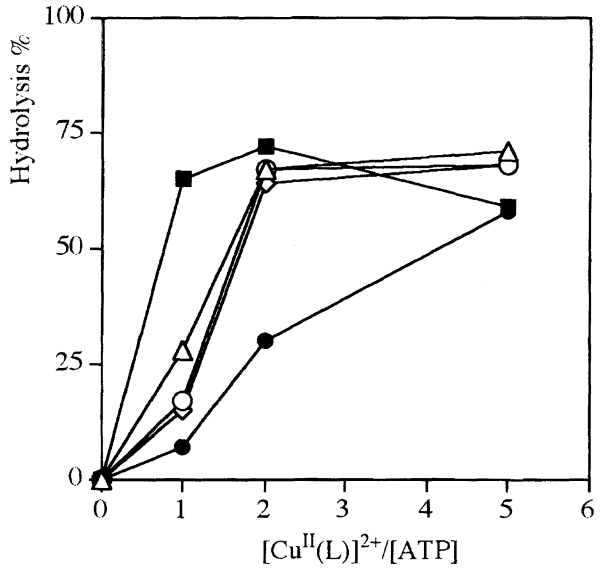

Fig. 4. Dependence of the percentages of ATP hydrolysis catalyzed by Cu^{2+} or $[Cu^{II}(L)]^{2+}$ on the ratios of $[Cu^{II}(L)]^{2+}$/[ATP]; [ATP]: 0.6 mmol dm^{-3} at 40 °C for 24 h in HEPES buffer (pH 7.3), ■: Cu^{2+}; ◇: $[Cu^{II}(L\text{-}3)]^{2+}$; ○: $[Cu^{II}(L\text{-}4)]^{2+}$; △: $[Cu^{II}(L\text{-}5)]^{2+}$; ●: $[Cu^{II}(L\text{-}6)]^{2+}$.

pHs 7.3 and 5.7. The figures also show that the conversions of ATP at pH 5.7 were higher than those at pH 7.3.

The time courses of the productions of ADP and AMP were examined for the $[Cu^{II}(L)]^{2+}$/ATP=2 : 1 systems, as shown in

Figs. 6 and 7. As can be seen in Fig. 6, the production of ADP occurred almost similarly with all systems at pH 7.3, while the production of AMP was much more reduced with $[Cu^{II}(L)]^{2+}$ complexes than with those of Cu^{2+} alone. It is noticed that, at pH 5.7 with Cu^{2+} alone, the production of ADP showed a maximum and then decreased with further time. Concomitantly, the production of AMP increased, indicating its successive formation from ADP in the case of Cu^{2+} alone. Lower production of AMP in the case of $[Cu^{II}(L)]$ at both pHs suggests the protection of $[Cu^{II}(L)]^{2+}$/ADP from further dephosphorylation.

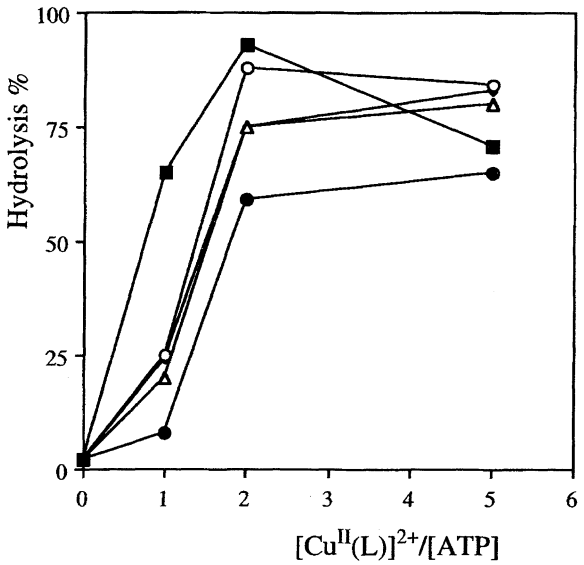

Fig. 5. Dependence of the percentages of ATP hydrolysis catalyzed by Cu^{2+} or $[Cu^{II}(L)]^{2+}$ on the ratios of $[Cu^{II}(L)]^{2+}$/[ATP]; [ATP]: 0.6 mmol dm^{-3} at 40 °C for 24 h in MES buffer (pH 5.7), ■: Cu^{2+}; ◇: $[Cu^{II}(L\text{-}3)]^{2+}$; ○: $[Cu^{II}(L\text{-}4)]^{2+}$; △: $[Cu^{II}(L\text{-}5)]^{2+}$; ●: $[Cu^{II}(L\text{-}6)]^{2+}$.

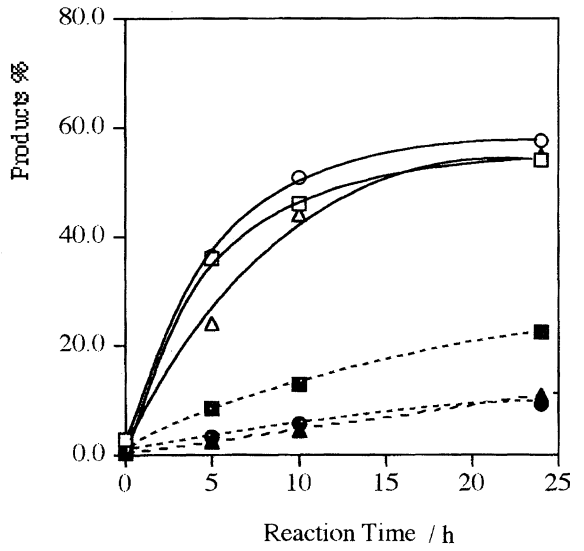

Fig. 6. Time course of the products of ADP and AMP from ATP hydrolysis catalyzed by Cu^{2+} or $[Cu^{II}(L)]^{2+}$ on $[Cu^{II}(L)]^{2+}$/[ATP]=2; [ATP]: 0.6 mmol dm^{-3} at 40 °C for 24 h in HEPES buffer (pH 7.3), □: ADP by Cu^{2+}; ○: ADP by $[Cu^{II}(L\text{-}3)]^{2+}$; △: ADP by $[Cu^{II}(L\text{-}4)]^{2+}$; ■: AMP by Cu^{2+}; ●: AMP by $[Cu^{II}(L\text{-}3)]^{2+}$; ▲: AMP by $[Cu^{II}(L\text{-}4)]^{2+}$.

These results were supported by the independent experiments of the hydrolysis of ADP in 2 : 1 ratio of $[Cu^{II}(L)]^{2+}$/ADP systems, as shown in Table 3. Obviously the hydrolysis % of ADP was retarded by $[Cu^{II}(L)]^{2+}$ at pH 7.3.

The reason why $[Cu^{II}(bpy)]^{2+}$ protects ATP from the dephosphorylation at pH 7.3, is the coordination of oxide ion in β,γ-phosphate residues to the Cu^{2+} and to the coordination of adenine(N-7), together with an intramolecular stacking between the pyridyl and adenine moiety,[7,8] although at pH 5.7 both the reactivity and the selectivity of $[Cu^{II}(dpa)]^{2+}$ are almost similar to those of $[Cu^{II}(L)]^{2+}$. Table 2 indicates that the reduced reactivity of $[Cu^{II}(dpa)]^{2+}$ was restored partially by the addition of adenine in the $[Cu^{II}(dpa)(atp)]$ system, but its site-selectivity remains at a low level being almost the same as those of Cu^{2+} only and $[Cu^{II}(dpa)]^{2+}$.

Four hydroxyl groups of ATP phosphate bonds are almost

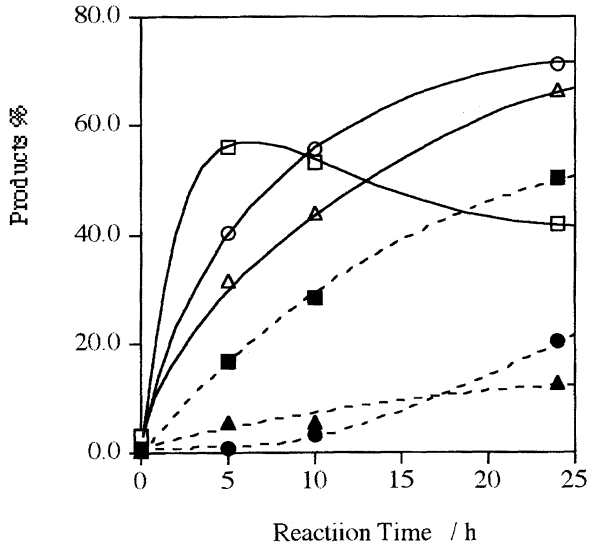

Fig. 7. Time course of the products of ADP and AMP from ATP hydrolysis catalyzed by Cu^{2+} or $[Cu^{II}(L)]^{2+}$ on $[Cu^{II}(L)]^{2+}$/[ATP]=2; [ATP]: 0.6 mmol dm^{-3} at 40 °C for 24 h in MES buffer (pH 5.7), □: ADP by Cu^{2+}; ○: ADP by $[Cu^{II}(L\text{-}3)]^{2+}$; △: ADP by $[Cu^{II}(L\text{-}4)]^{2+}$; ■: AMP by Cu^{2+}; ●: AMP by $[Cu^{II}(L\text{-}3)]^{2+}$; ▲: AMP by $[Cu^{II}(L\text{-}4)]^{2+}$.

Table 3. Extents of the Hydrolysis of ADP at 2 : 2 : 1 Ratios of Cu^{2+}, Ligand, and ADP[a]

Cu^{2+}/lignad	Hydrolysis (%), pH 7.3	Hydrolysis (%), pH 5.7
Cu^{2+} alone	43.9	77.6
Cu^{2+}/dpa	5.7	59.9
Cu^{2+}/L-3	27.6	65.9
Cu^{2+}/L-4	9.4	26.8
Cu^{2+}/L-5	27.9	44.9
Cu^{2+}/L-6	12.7	20.0

a) Experimental conditions; All reactions were run at 40 °C for 24 h in 20 mmol dm^{-3} HEPES (pH 7.3) or MES (pH 5.7) buffer, $[Cu^{2+}]$, [L]: 1.2 mmol dm^{-3} each and [ADP]: 0.6 mmol dm^{-3}.

ionized at pH 7.3, but at pH 5.7 the fourth hydroxyl group is not ionized since it has a pK of 6.95. With this reason, β,γ-phosphate residue coordinated tightly to $[Cu^{II}(dpa)]^{2+}$ cooperated by adenine–pyridine stacking interaction in the ternary complex of $[Cu^{II}_2(L)_2(atp)]$ at pH 7.3, but weakly with the α phosphate and thus it is able to protect ATP from the hydrolysis, as illustrated in Fig. 13. The reactive species may arise when 2 : 1 complexes are formed, i.e. $[Cu^{II}_2(L)_2(atp)]$, in which the γ-phosphate residue coordinates to the second metal ion forcing the first metal ion to shift onto α,β-position to cause labilization of the γ-phosphate residue.

$$Cu^{2+} + ATP^{4-} \rightleftharpoons [Cu^{II}(atp)]^{2-} \xrightarrow{H_2O} [Cu^{II}(adp)]^{2-} + Pi$$
$$[Cu^{II}(adp)]^{2-} \xrightarrow{H_2O} [Cu^{II}(amp)]^{2-} + Pi \quad (1)$$

$$2\,Cu^{2+} + ATP^{3-} \rightleftharpoons [Cu^{II}_2(atp)]^{+} \xrightarrow{H_2O} [Cu^{II}_n(adp)]^{+} + Pi$$
$$[Cu^{II}_n(adp)]^{+} \xrightarrow{H_2O} [Cu^{II}_n(amp)]^{+} + Pi \quad n=1 \text{ or } 2 \quad (2)$$

$$Cu^{2+} + L \rightleftharpoons [Cu^{II}(L)]^{2+} \quad (3)$$

$$2\,[Cu^{II}(L)]^{2+} + ATP^{4-} \rightleftharpoons [Cu^{II}_2(L)_2(atp)] \xrightarrow{H_2O} [Cu^{II}_2(L)_2(adp)] + Pi \quad (4)$$

$$2\,[Cu^{II}(L)]^{2+} + ATP^{3-} \rightleftharpoons [Cu^{II}_2(L)_2(atp)]^{+} \xrightarrow{H_2O} [Cu^{II}_2(L)_2(adp)]^{+} + Pi \quad (5)$$

The above equilibria and reaction pathways can be represented by the following equations (Eqs. 1, 2, 3, 4, and 5):

In the absence of ligands, Cu^{2+} forms 1 : 1 or 2 : 1 complexes with ATP at pH 7.3 (Eq. 1) or 5.7 (Eq. 2), respectively, which then undergo successive hydrolysis to the complexes of ADP and AMP. The direct productions of AMP and diphosphate (PPi) from ATP are also conceivable, but the amount of AMP at the initial stages was observed to be quite small.

In the presence of the ade-dpa ligands L, the Cu^{2+}-complexes form 2 : 1 ternary complexes with ATP at both pH 7.3 and 5.7 (Eqs. 4 and 5). Here, the hydrolyzed complexes $[(Cu^{II}_2(L)_2(adp)]$ and $[(Cu^{II}_2(L)_2(adp)]^{+}$ are fairly stable, resisting the further hydrolysis to AMP complexes.

The reactivities and selectivities of Cu^{2+}-complexes are compared in Fig. 8 under the conditions of $[Cu^{II}(L)]^{2+}/[ATP]=2$ and pH 7.3 (see Fig. 4). As seen in the solid circles, the extents of the hydrolysis after 24 h are in the order of complexes of none > L-4 ≥ L-5 ≥ L-3 > dpa-Ade > L-6 ≫dpa. Here remarkable features include a low activity of the dpa complex and a comparable activity of the complexes of L-3, L-4, and L-5 as compared with that of none (Cu^{2+} alone). Much more interesting are the high selectivities of L-3, L-4, and L-5. The selectivity values as measured by the ratio of ADP/AMP are 2.4—1.9 for the three

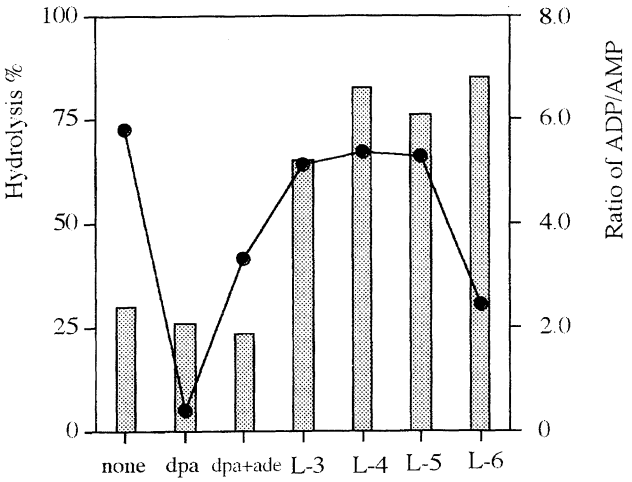

Fig. 8. Extent (lines) and selectivity (bars) of ATP hydrolysis catalyzed by Cu^{2+} and $[Cu^{II}(L)]^{2+}$ at 2 : 1 ratio of $[Cu^{II}(L)]^{2+}/[ATP]$ at 40 °C for 14 h in 20 mmol dm^{-3} HEPES (pH 7.3), $[Cu^{2+}]$, [L]: 1.2 mmol dm^{-3} each and [ATP]: 0.6 mmol dm^{-3}.

complexes of none, dpa, and dpa-Ade, and 5.2—6.8 for the other four complexes of L-3, L-4, L-5, and L-6. As can been seen from the data in Figs. 6 and 7 and Table 3, it is obvious that a higher selectivity of the latter complexes is the result of a slow rate of production of AMP. In other words, it is due to a high stability of the complexes $[Cu^{II}_2(L)_2(adp)]$.

Reactivities and Selectivities of $[Cu^{II}(L)]^{2+}$ Complexes for the Hydrolysis of UTP. It is likely that the above high reactivity and selectivity of the complexes of adenine-containing ade-dpa ligands L as compared with those of a non-adenine-containing ligand dpa, are due to some specific interaction(s) between the two adenine rings of ATP and ade-dpa ligands. In order to learn more about such a specific interaction, the hydrolysis of UTP was also examined under the similar conditions to those in the ATP hydrolysis.

As shown in Fig. 9, the results were widely different from those of ATP. In the absence of ade-dpa ligand, the reactivity of Cu^{2+} alone was much lower for UTP than for ATP. The reactivities of the other $[Cu^{II}(L)]^{2+}$ complexes were also much lower than those in the cases of ATP. This appears partly due to a lower interaction between uracil ring and Cu^{2+} ion, and the lack of such an interaction between adenine N-7 and Cu^{2+}. However, it is interesting to note that both the reactivities and the site-selectivities of the complexes of an adenine-containing ade-dpa ligands were higher than those of non-adenine-containing complexes (none and dpa). Particularly interesting were the much higher reactivity and the selectivity of the complex of L-5, i.e. 34.2 % hydrolysis and UDP/UMP=12.2, than those of the other complexes.

The effects of ratios of $[Cu^{II}(L)]^{2+}/[UTP]$ on the extent of hydrolysis are shown in Fig. 10. Here, no saturation phenomena as shown in Figs. 4 and 5 were observed. Hydrolysis % continued to increase even by using five molar excess of complexes over UTP. This may simply reflect a much weaker complexation between $[Cu^{II}(L)]^{2+}$ and UTP than in the cases of ATP. Noteworthy are the selectivities observed

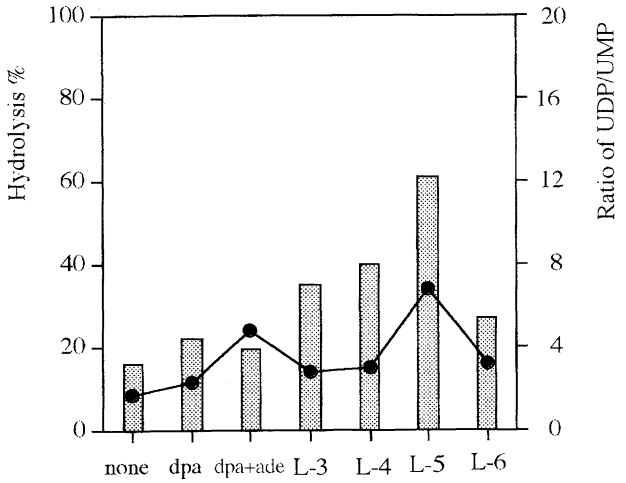

Fig. 9. Extent (lines) and selectivity (bars) of UTP hydrolysis catalyzed by Cu^{2+} and $[Cu^{II}(L)]^{2+}$ at 2:1 ratio of $[Cu^{II}(L)]^{2+}$/[UTP] at 40 °C for 14 h in 20 mmol dm^{-3} HEPES (pH 7.3), $[Cu^{2+}]$, [L]: 1.2 mmol dm^{-3} each and [UTP]: 0.6 mmol dm^{-3}.

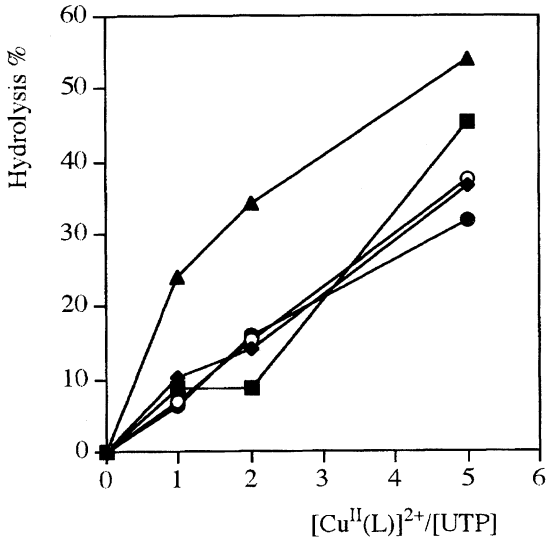

Fig. 10. Dependence of the percentages of UTP hydrolysis catalyzed by Cu^{2+} or $[Cu^{II}(L)]^{2+}$ on the ratios of $[Cu^{II}(L)]^{2+}$/[UTP]; [UTP]: 0.6 mmol dm^{-3} at 40 °C for 24 h in HEPES buffer (pH 7.3), ■: Cu^{2+}; ◆: $[Cu^{II}(L\text{-}3)]^{2+}$; ○: $[Cu^{II}(L\text{-}4)]^{2+}$; ▲: $[Cu^{II}(L\text{-}5)]^{2+}$; ●: $[Cu^{II}(L\text{-}6)]^{2+}$.

with the use of $[Cu^{II}(L)]^{2+}$/[UTP]=5 which are still much higher with adenine-containing ligands than with none and dpa; none (hydrolysis % and ratio of UTP/UDP; 45.4 and 3.0), dpa (67.5 and 2.3), L-3 (36.7 and 4.3), L-4 (37.5 and 5.6), L-5 (54.0 and 6.2), and L-6 (31.9 and 4.7), respectively.

These methylene spacer-dependent effects of adenine containing ade-dpa ligands on the hydrolysis of UTP could suggest that some specific interaction between an adenine and an uracil moiety is unlikely. A more likely possibility is the stacking of a base pair as is known in self-association of nucleotides. It is also known that the stacking association is much stronger between adenine–adenine than adenine–uracil in water.

^{1}H NMR Studies for Base Stacking. As shown in Fig. 8, $[Cu^{II}(L\text{-}4)]^{2+}$ was the most effective in the hydrolysis of ATP, so that the possibility of base-stacking was examined for the L-4/ATP pair by NMR spectroscopy. Since Cu^{2+} is not appropriate for NMR study, Zn^{2+} ion was used instead for the complexation. As can be seen in Table 2, Zn^{2+} ion is also effective in promoting the hydrolysis of ATP.

The protons to be examined were H-2 and H-8 protons on the adenine ring of ATP and H′-1 proton of ribose of ATP and H-2″ and H-8″ of the adenine of L-4. The 6 combinations of ATP, L-4, ATP/L-4, ATP/Zn^{2+}, L-4/Zn^{2+}, and ATP/Zn^{2+}/L-4 were examined, by varying the concentration of each combination from 1—8 mmol dm^{-3}, in D_2O–methanol-d_4 (10:1 v/v ratio, pD=7.0) containing tetramethylammonium ion as an internal standard (δ=3.188). The results are shown in Tables 4 and 5 and Fig. 11.

Tables 4 and 5 show that the concentration-dependent upfield shifts of all protons of the ternary complex of $[Zn^{II}(L\text{-}4)(atp)]$, i.e. H-2 of the adenine ring of ATP and H′-1 of ribose of ATP and H-2″ and H-8″ of the adenine ring of L-4, are larger than those of $[Zn^{II}(atp)]$, $[Zn^{II}(L\text{-}4)]^{2+}$ and ATP/L-4 combinations. The largest upfield shifts were those of H-2″ and H-8″ protons of the L-4 combination, which suggests the self-stacking of an adenine ring of L-4. However, such upfield shift was reduced in the binary combination with Zn^{2+} or ATP, and again enhanced in the ternary combination. For example, in the case of the same total concentrations of the adenine moieties of the ade-dpa ligand and/or ATP, H-2″ of the adenine of L-4 (8 mmol), $[Zn^{II}(L\text{-}4)]^{2+}$ (8 mmol each), and L-4/ATP (4 mmol each) were appeared at 7.836, 7.837, 7.834 ppm, respectively, whereas that of $[Zn^{II}(L\text{-}4)(atp)]$ (4 mmol each) was appeared at 7.826 ppm and thus the largest up-field shift of H-2″ was observed in the presence of these three components, Zn^{2+}, L-4, and ATP. Thus the adenine–adenine interaction, presumably by stacking, seems

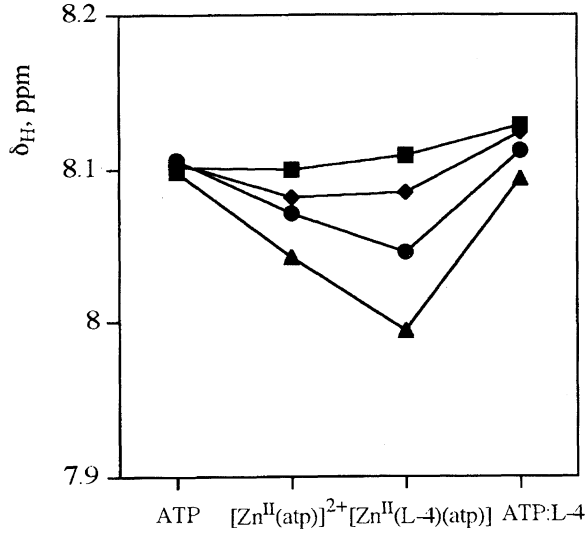

Fig. 11. ^{1}H NMR chemical shifts of H-2 of adenine ring of ATP in the presence of L-4, Zn^{2+} and $[Zn^{II}(L\text{-}4)]^{2+}$ at pD=7.0, [ATP], $[Zn^{2+}]$, [L-4]: ■: 1 mmol dm^{-3}, ◆: 2 mmol dm^{-3}, ●: 4 mmol dm^{-3}, ▲: 8 mmol dm^{-3}.

Table 4. Chemical Shifts (δ_H/ppm) of the Protons of Adenine and Sugar Moeities of ATP of $[Zn^{II}(L\text{-}4)]^{2+}$: ATP Systems[a)]

	H-2				H-8				H-1′			
System	1	2	4	8	1	2	4	8	1	2	4	8
ATP	8.101	8.104	8.106	8.098	8.375	8.383	8.381	8.381	5.985	5.994	5.994	5.986
ATP/Zn^{2+}	8.099	8.081	8.071	8.042	8.455	8.461	8.463	8.476	6.001	5.975	5.976	5.961
ATP/L-4	8.128	8.124	8.112	8.094	8.408	8.407	8.404	8.402	6.016	6.011	6.011	5.991
ATP/Zn^{2+}/L-4	8.109	8.084	8.045	7.994	8.469	8.461	8.456	8.455	6.013	5.988	5.988	5.937

a) All systems were at [ATP], [Zn^{2+}], [L-4]: mmol dm^{-3} each.

Table 5. Chemical Shifts (δ_H/ppm) of the Protons of an Adenine Ring of L-4 of $[Zn^{II}(L\text{-}4)]^{2+}$: ATP Systems[a)]

	H-2				H-8			
System[a)]	1	2	4	8	1	2	4	8
L-4	7.854	7.849	7.838	7.836	7.956	7.953	7.940	7.934
L-4/Zn^{2+}	7.854	7.852	7.842	7.837	7.956	7.955	7.942	7.932
L-4/ATP	7.851	7.844	7.834	7.820	7.952	7.947	7.935	7.919
L-4/Zn^{2+}/ATP	7.840	7.840	7.826	7.819	7.949	7.945	7.926	7.906

a) All systems were at [ATP], [Zn^{2+}], [L-4]: mmol dm^{-3} each.

to become important in the ternary complex.

The effects of reaction components on the ^{31}P NMR spectra of ATP were also examined at pH 7.0 with an external standard of 1 mmol dm^{-3} H_3PO_4,[28] as shown in Fig. 12. As indicated, the signals of two β- and γ-phosphorus atoms show a down-field shift from those of free ATP in the presence of Zn^{2+} ion. Thus the major sites of Zn^{2+} coordination are on the oxide ions of β- and γ-phosphate residues, leaving α-phosphate residue relatively free. However, in the presence of $[Zn^{II}(L\text{-}4)]^{2+}$, the signal of α-phosphorus shifted more down field than that in the presence of Zn^{2+} or L-4.

Reaction Mechanism. Several features of the present reactions can be summarized as follows: (1) In all the cases including the case of Cu^{2+} alone, the major pathway for the hydrolysis of ATP is stepwise to form ADP first, then followed by the hydrolysis of ADP to form AMP (Figs. 6 and 7). (2) The Cu^{2+}- complex of dipyridylamine (dpa) is ineffective, but becomes effective when linked to adenine through a methylene-spacer (Table 2 and Figs. 8 and 9) at pH 7.3. (3) The selectivity defined by ADP/AMP or UDP/UMP is widely different between the reaction promoted by Cu^{2+} alone and those of $[Cu^{II}(L)]^{2+}$, being much higher in the latter than in the former cases (Figs. 8 and 9). (4) The ratio of $[Cu^{II}(L)]^{2+}{}_n$/ATP is important in determining the reactivity and selectivity. At pH 7.3, the activity of Cu^{2+} alone is saturated when $n \geq 1$, but it must be $n \geq 2$ for the complexes of the ade-dpa ligands containing adenine moiety for the saturation of the activity (Figs. 4 and 5). (5) There is an optimum length of methylene-spacer linking dpa and adenine moieties in determining the reactivity and selectivity (Figs. 8 and 9). (6) The Cu^{2+} alone is the most effective for the hydrolysis of ATP, but the least effective for that of UTP. (7) The ^1H NMR spectra support the adenine-adenine base stacking in the ternary complex of $[Zn^{II}(L\text{-}4)]^{2+}$/ATP. The

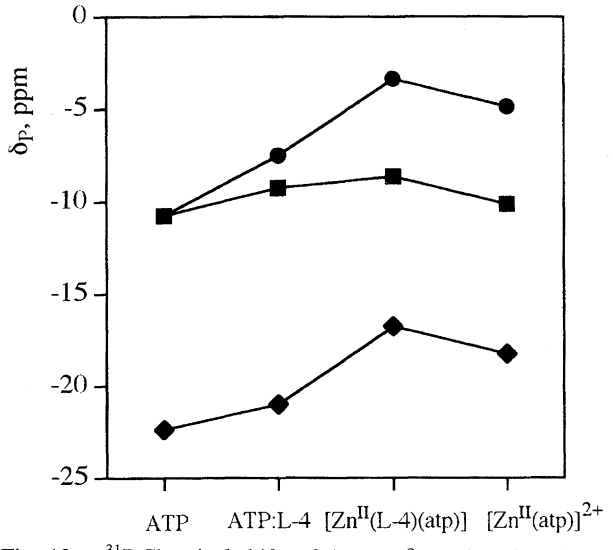

Fig. 12. ^{31}P Chemical shifts of the α-, β-, and γ-phosphorus of ATP in the presence of Zn^{2+} and $[Zn^{II}(L\text{-}4)]^{2+}$, [ATP], [$Zn^{2+}$], [L-4]: 4.0 mmol dm^{-3} each, pD=7.0, ■: α-P, ◆: β-P, ●: γ-P.

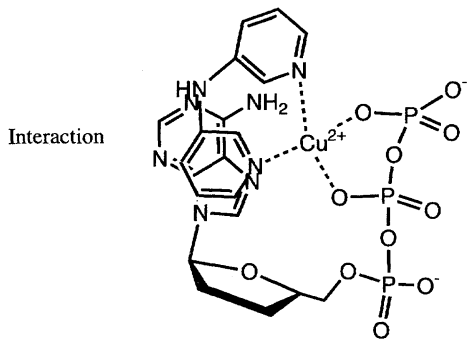

Fig. 13. A possible structure of the ternary complex of $[Cu^{II}(dpa)(atp)]$ by stacking of adenine and dpa.

^{31}P NMR spectra support the interaction of Zn^{2+} ion with β- and γ-phosphate residues.[26] These features allow us to propose the following mechanisms for the effects of Cu^{2+} and the ade-dpa ligands, as illustrated in Figs. 13, 14, and 15.

The fact that Cu^{2+} is active for ATP but not for UTP hydrolysis is explained as due to its coordination with N-7 in the former ATP. Such coordination would be hindered by a much stronger chelation with dpa, in which an adenine-pyridine base stacking plays a role as illustrated in Fig. 13. Similar complexation was also proposed in the $[Cu^{II}(bpy)]^{2+}$/ATP complex,[12] in which the coordination sites are considered to be β- and γ-phosphate groups. A 2 : 1 complex of $[Cu^{II}(dpa)_2]^{2+}$ may also be involved in the inhibition.

The modification of dpa with adenine restored the activity of Cu^{2+} ion. The most likely possibility is that the adenine moiety of the ade-dpa ligands replaces the inhibitory stacking of pyridine with adenine in the manner shown in Fig. 13, allowing dpa/Cu^{2+} moiety to adopt a better coordination structure with phosphate residues for the hydrolysis. Two cases of 1 : 1 and 2 : 1 complexation are conceivable. In the first

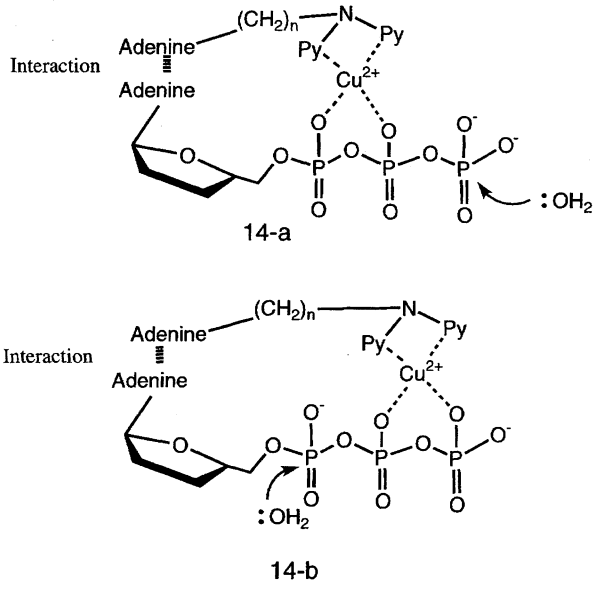

Fig. 14. Two possible structures of the ternary complexes of $[Cu^{II}(L)(atp)]$.

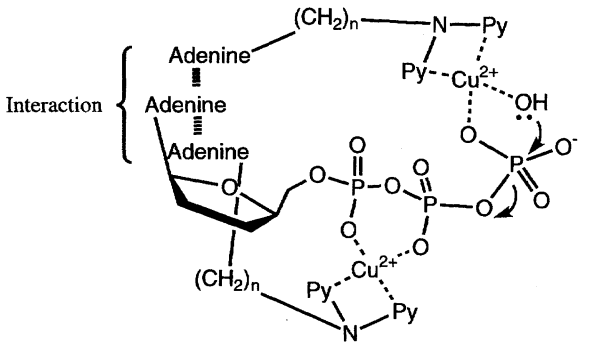

Fig. 15. An oversimplified structure of the ternary complex of $[Cu^{II}_2(L)_2(atp)(OH)]^-$.

1 : 1 complexation, it is likely that ATP and $[Cu^{II}(L)]^{2+}$ interact to form an adenine–adenine base stacking with either coordination of α- and β-phosphate or with β- and γ-phosphate residues to Cu^{2+}, or both, as illustrated in Figs. 14a and 14b, respectively. In either case, the nucleophile attacking, presumably on the γ- or on the α-phosphate residue must be an unactivated water molecule with a low reactivity in accordance with the experimental observations. In the second 2 : 1 complexation, a likely structure which accounts for the experimental observations appears to that shown in Fig. 15. The three adenine bases are involved in the base stacking, and the α- and β-phosphate residues coordinates to one $[Cu^{II}(L)]^{2+}$, and the γ-phosphate residue coordinates to the second $[Cu^{II}(L)]^{2+}$. Here it is important to notice that the second Cu^{2+} has a free coordination site for an activated water molecule (e.g. OH^-) which selectively attacks the γ-phosphate residue forming a stabilized ADP-complex.

The complexes of $[Cu^{II}(L\text{-}4)]^{2+}$ and $[Cu^{II}(L\text{-}5)]^{2+}$ were the most reactive and site-selective for the hydrolysis of ATP and UTP, respectively. This result suggests the existence of an ideal length of the spacer for the best base–base stacking and metal ion coordination with phosphate residues, although a methylene-spacer is rather flexible to adopt such a precise length. The choice of base-stacking may also be important, although the information from the present study is limited.

Finally, the present study presents significant information for the design of site-selective reagents for the reactions of phosphoric esters like nucleic acids.

Experimental

Materials. Adenosine 5′-triphosphate disodium salt (ATP, Kishida), adenosine 5′-diphosphate sodium salt (ADP, Sigma), adenosine 5′-monophosphate sodium salt (AMP, Sigma), and uridine 5′-triphosphate trisodium salt (UTP, Wako) were used without further purification. All the materials were obtained commercially (guaranteed reagent grade) and used without further purification. Organic solvents were used after distillation. $CuCl_2 \cdot 2H_2O$, 0.1 mmol dm^{-3} aqueous solution of $ZnCl_2$, and other metal salts used as the source of Cu^{2+}, Zn^{2+}, and other M^{2+} for the reactions and buffers were commercial extra-pure reagents. The buffers were 2-morpholinoethanesulfonic acid (MES)/NaOH (pH 5.7) and 4-hydroxypiperizine-1-ethanesulfonic acid (HEPES) (pH 7.3). Hitachi–Horiba pH meter (F-8) and Shindengen pH BOY-P2 were used for the pH determination and control. UV spectra were measured using a Shimadzu UV-160A. NMR spectra were recorded using a JEOL JNM-A400 FT NMR spectrometer.

N^6-Acetyl-9-[4-(di-2-pyridylamino)butyl]adenine (L-4): (see Fig. 2). To a stirred solution of N_6-acetyladenine I (29.2 g, 165 mmol) in 100 cm^3 of DMF was added sodium hydride (8.0 g, 60% in oil, 200 mmol) for 3 h, following by the dropwise addition of 1, 4-dibromobutane (40 g, 185 mmol) in 30 cm^3 of DMF at 0—5 °C. After stirring for 18 h at room temperature, the solvent was removed in vacuo. The solution was quenched by the addition of water and was extracted with dichloromethane (2×100 cm^3). The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The resulting partial solid was purified by chromatography on silica gel. Elution with chloroform–MeOH (30 : 1) afforded 13.5 g (26.3 %) of N^6-acetyl-9-(4-bromobutyl)adenine II (n=4). To a stirred solution of di-2-pyridylamine (7.0 g, 40 mmol) in 40 cm^3

of DMF was added sodium hydride (1.92 g, 60% in oil, 40 cm^3) for 3 h, following by the dropwise addition of II (12.5 g, 40 mmol) in 100 cm^3 of DMF. After stirring for 18 h at room temperature, the solvent was removed in vacuo. The solution was quenched by the addition of water and was extracted with dichloromethane (2×60 cm^3). The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The resulting partial solid was purified by chromatography on silica gel. Elution with chloroform–MeOH (30 : 1) afforded 3.93 g (24.4 %) of N^6-acetyl-9-[4-(di-2-pyridylamino)butyl]adenine. To a stirred solution of 50 cm^3 of MeONa–MeOH (0.6 M, 1 M=1 mol dm^{-3}) was added the above product (3.93 g, 9.8 mmol). After refluxing for 0.5 h and then cooling, the resulting solution was neutralized with 1 M HCl and most of the solvent was removed in vacuo. The reaction mixture was diluted with water and extracted with chloroform (3×30 cm^3). The combined extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved with a small portion of chloroform (ca. 10 cm^3), following by an addition of 50 cm^3 of ether to afford the desired ligand L-4, (2.8 g, 79.3 %), mp 148—150 °C, ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ=8.34 (s, 1H), 8.34 (d, J=5.4 Hz, 2H), 7.83 (s, 1H), 7.49 (dd, J=8.4 and 7.2 Hz, 2H), 7.05 (d, J=8.4 Hz, 2H), 6.86 (dd, J=7.2 and 5.2 Hz, 2H), 5.76 (br s, 2H), 4.28 (t, J=8.0 Hz, 2H), 4.27 (t, J=7.2 Hz, 2H), 2.02—1.94 (m, 2H), and 1.83—1.73 (m, 2H); ^{13}C NMR δ=157.2, 155.5, 152.8, 150.0, 148.4, 140.4, 137.2, 119.6, 117.1, 114.6, 46.9, 43.4, 27.3, and 25.0. Found: C, 63.40; H, 5.62; N, 31.08%. Calcd for $C_{19}H_{20}N_8$: C, 63.32; H, 5.59; N, 31.09%. The other ligands were prepared by a method similar to that for L-4;

9-[3-(di-2-pyridylamino)propyl]adenine (L-3); mp 174—176 °C, ^{1}H NMR δ=8.34 (d, J=4.8 Hz, 2H), 8.33 (s, 1H), 7.98 (s, 1H), 7.49 (dd, J=8.4 and 7.2 Hz, 2H), 7.05 (d, J=8.4 Hz, 2H), 6.87 (dd, J=7.2 and 4.8 Hz, 2H), 5.76 (br s, 2H), 4.34 (t, J=6.8 Hz, 2H), 4.32 (t, J=6.8 Hz, 2H), and 2.38—2.33 (m, 2H); ^{13}C NMR δ=157.1, 156.5, 152.7, 150.2, 148.4, 141.0, 137.3, 119.7, 117.3, 114.6, 45.1, 41.9, and 28.4. Found: C, 62.52; H, 5.16; N, 32.33%. Calcd for $C_{18}H_{18}N_8$: C, 62.41; H, 5.24; N, 32.35%.

9-[5-(di-2-pyridylamino)pentyl]adenine (L-5); mp 181—183 °C, ^{1}H NMR δ=8.34 (s, 1H), 8.32 (dd, J=4.8 and 1.6 Hz, 2H), 7.78 (s, 1H), 7.50 (ddd, J=8.4, 7.2, and 1.6 Hz, 2H), 7.05 (d, J=8.4 Hz, 2H), 6.85 (dd, J=7.2 and 4.8 Hz, 2H), 6.09 (br s, 2H), 4.20—4.16 (m, 4H), 1.94—1.91 (m, 2H), 1.77—1.75 (m, 2H), and 1.41—1.338 (m, 2H); ^{13}C NMR δ=157.2, 155.4, 152.4, 149.9, 148.2, 140.5, 137.1, 119.5, 116.9, 114.5, 47.6, 43.7, 29.6, 27.5, and 23.9. Found: C, 64.11; H, 6.04; N, 29.85%. Calcd for $C_{20}H_{22}N_8$: C, 64.15; H, 5.92; N, 29.92%.

9-[6-(di-2-pyridylamino)hexyl]adenine (L-6); mp 142—143 °C, ^{1}H NMR δ=8.33 (s, 1H), 8.30 (dd, J=4.8 and 2.0 Hz, 2H), 7.75 (s, 1H), 7.49—7.45 (m, 2H), 7.03 (d, J=8.4 Hz, 2H), 6.83—6.80 (m, 2H), 5.76 (br s, 2H), 4.14 (dt, J=7.2 Hz, 4H), 1.90—1.83 (m, 2H), 1.70—1.63 (m, 2H), and 1.42—1.33 (m, 4H); ^{13}C NMR δ=157.4, 155.5, 152.8, 150.0, 148.3, 140.4, 137.1, 119.6, 116.9, 114.6, 47.9, 43.8, 29.9, 28.0, 26.4, and 26.3. Found: C, 64.91; H, 6.21; N, 28.81%. Calcd for $C_{21}H_{24}N_8$: C, 64.93; H, 6.23; N, 28.84%.

Hydrolysis of ATP and UTP. a) Typical Run: All hydrolysis reactions were run in 20 mmol dm^{-3} HEPES buffer (pH 7.3) or MES buffer (pH 5.7) at 40 °C. A stock solution of [ligand]=40 mmol dm^{-3} was prepared by dissolving in 50 % (v/v) aqueous methanol solution. In the typical reaction, the 2 : 1 complex of ATP or UTP was formed as follows: The assay solution contained, in a total volume of 1.0 cm^3, 0.6 mmol dm^{-3} of nucleoside triphosphate (NTP), 1.2 mmol dm^{-3} of ligand, 1.2 mmol dm^{-3} of $CuCl_2$, and 20 mmol dm^{-3} of HEPES buffer (pH 7.3). The reaction mixture was incubated at 40 °C, using an Advantec CI-310 constant temperature incubator for 24 h, after which time 20 μl aliquot was assayed by ion-exchange HPLC.

b) Product Analysis by HPLC: The product analyses were performed by high-pressure liquid chromatography (HPLC) using a Shimadzu Model LC-6A/G-1 system equipped with a Shimpack WAX-1 ion-exchange column with the following elution gradient: for 0—5 min with KH_2PO_4 (20 mmol dm^{-3}, pH 7.0) and then for 5—25 min with a 0—100% linear gradient of KH_2PO_4 (20 mmol dm^{-3}, pH 7.0 and 480 mmol dm^{-3}, pH 6.8) in a flow rate of 1.0 dm^3 min^{-1}. The retention times of ATP, ADP, and AMP were 23.9, 17.5, and 10.6 min, respectively. And those of UTP, UDP, and UMP were 20.2, 14.4, and 3.7 min, respectively.

References

1) Abbreviations and definitions: (a) AMP, ADP or ATP, adenosine 5′-mono-, -di- or -triphosphate; dpa, di-2-pyridylamine; bpy, 2,2′-bipyridyl; UMP, UDP, UTP, uridine 5′-mono-, -di- or -triphosphate; M^{2+}, metal ion; tn, trimethylenediamine=1,3-propanediamine. (b) The phosphate residues in nucleoside 5′-triphosphates are labeled α, β, and γ, where the latter refers to the terminal phosphate residue.

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