Interactions of a Nucleoside Cytidine with Metal Ions in Water Observed through Mass Spectrometry: Clustering Controlled by Electrostatic Interaction and Coordinating Interaction

Shunsuke Mochizuki[†] and Akihiro Wakisaka*

The National Institute of Advanced Industrial Science and Technology (AIST), Onogawa 16-1, Tsukuba, Ibaraki 305-8569, Japan

The effect of metal ions on the molecular clustering of cytidine in aqueous solution was studied by mass spectrometric analysis of clusters isolated from liquid droplets, and two types of cytidine clustering depending on the coexisting ions were observed. In the presence of alkali metal ion (Li^+, Na^+, K^+) or alkaline earth metal ion (Mg^{2+}, Ca^{2+}) in solution, cytidine molecules aggregated around these ions through the electrostatic interaction, which is similar to the solvation of ions by solvent molecules. In these cases, the predominant cytidine clusters were $M^{n+}(Cy)_a$, where $a = 1 \sim 4$ for alkali metal ion and $a = 1 \sim 8$ for alkaline earth metal ion, respectively. The number of cytidine molecules in the clusters had strong dependence on the charge density of the metal ion. On the other hand, in the presence of other divalent metal ions (Mn^{2+}, Zn^{2+}) having 3d electrons, the cytidine clusters had a specific structure represented by $M^{2+}Cl^-(Cy)_b$, where $b = 2$ or 3. This was characterized as coordination of cytidine to the metal ion, where the cytidine works as a bidentate ligand. These showed that the clustering of cytidine was controlled by the interaction with the coexisting metal ion in a solution. This would concern the role of a metal ion on the biological processes.

Introduction

It is well-known that metal ions play an important role in various biochemical reactions.[1−3] As the most representative case, the effect of metal ions on the DNA replication has been studied vigorously.[4,5] For example, by means of PCR (polymerase chain reaction), the DNA replication was promoted by the presence of Mg^{2+}, but not by the presence of Mn^{2+}.[6−9] However, due to the difficulty of the direct observation of ion–molecular interaction in a solution, the studies on the role of ions in the DNA replication cannot exceed the scope of the speculation, even though the effect of ions on the biological processes is obvious.

Recently, we have reported that mass spectrometric analysis of clusters, produced by the fragmentation of liquid droplets including electrolyte, can provide direct information on ion–molecule and ion–counterion interaction.[10] By means of this mass spectrometry, we have already reported the salt effect on the clustering of a nucleoside (cytidine, Figure 1) in water.[11] The cytidine molecules preferentially formed clusters with metal ions, such as Na^+, K^+, and Mg^{2+}. This clustering was mainly controlled by the charge density of the positive ions, and the Mg^{2+} promoted the cytidine clustering more efficiently than the others. Here we would like to report that there is another kind of ion–cytidine interaction, that is, the cytidine works as a bidentate ligand for Mn^{2+} and Zn^{2+}. It is shown directly that the clustering of cytidine is strongly influenced by the kind of the coexisting metal ion, which will provide a new insight into the study on the role of ions in DNA replication. Furthermore, the observed clustering will also suggest that the difference
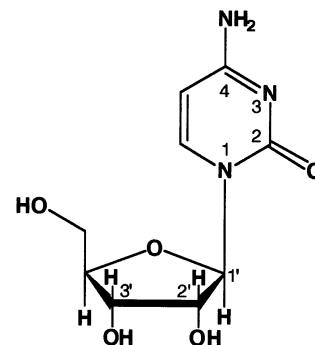

Figure 1. Structure of cytidine.

between the solvated ion and the complex ion can be observed directly by our mass spectrometry.

As for the clusters observed through the specially designed mass spectrometry, we have confirmed that the observed cluster structures reflect intermolecular, ion–molecule and ion–counterion interactions in solutions.[10−20] For electrolyte solutions, it was demonstrated that the clustering structures, including ions, were determined by the balance between ion–solvent and ion–counterion interactions.[10,13] For nonelectrolyte solutions, it was observed that self-association of hydrophobic molecules was remarkably promoted by the presence of water, methanol, or acetonitrile.[14−17,20] These observed clustering structures are determined by the balance of plural interactions, which represent the clustering peculiar to the solution.[10,13] However, it should be noted that the relatively weakly interacting molecules or ions were not included in the observed clusters, but these weak interactions play an important role to determine the balance of interactions in the solution.

* Corresponding author. E-mail: akihiro-wakisaka@aist.go.jp.
† Research fellow of New Energy and Industrial Technology Development Organization, Japan.

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Experimental Section

The mass spectra of clusters isolated from electrolyte solutions were measured by the specially designed mass spectrometer using electrospray interface, and the details have already been reported elsewhere.[10−13] The sample solution was injected into a five-stage differentially pumped vacuum chamber by an HPLC pump with a flow rate of 0.01 mL min^{-1} through a fused silica capillary tube (i.d. 0.1 mm). The high electric voltages were supplied to nozzle (+3.8 ∼ 4.5 kV) and skimmers (+210 ∼ 290 V) for the electrospray.[21,22] The multicharged liquid droplets including excess cations were generated through this homemade electrospray interface. The resulting multicharged liquid droplets fly from the first chamber (730 Torr) to the fifth chamber (10^{-7} Torr) through the pressure difference and a gradient of the electric field. During the flight of the liquid droplets, they are fragmented into clusters, molecules, or ions via adiabatic expansion and electrostatic repulsion. The resulting clusters having charges of ions were analyzed by a quadrupole mass spectrometer (Extrel C50) without any external ionization in the fifth chamber.

By this method, the clustering of cytidine with a metal ion was measured for the following metal ions: Li$^+$, Na$^+$, K$^+$, Mg^{2+}, Ca^{2+}, Mn^{2+}, and Zn^{2+}. All chemicals (cytidine and metal chloride) were purchased from WAKO (Osaka, Japan) and used as received.

Results and Discussion

1. Electrostatic Interaction of Cytidine with Metal Ions. *(i) Alkali Metal Ion.* Figure 2 shows the mass spectra of cytidine clusters generated from the aqueous solutions including an alkali metal salt (LiCl, NaCl, or KCl) and cytidine. In the presence of LiCl (Figure 2a), Li$^+$ interacting with water molecules (Li$^+$(H$_2$O)$_x$; $x = 1 \sim 6$) and with cytidine molecules (Li$^+$(Cy)$_a$; $a = 1 \sim 4$) were clearly observed as clusters. It is clear that Li$^+$ interacts with the cytidine as well as with the water. Considering that the cytidine concentration is much lower than the water concentration, the cytidine has strong preference to the Li$^+$. The same kind of cluster structure was observed in the presence of Na$^+$ (Figure 2b) and K$^+$ (Figure 2c), too. With increase of the ionic radius of the alkali metal ions, the number of the hydrated water molecules (hydration number) is decreased from 6 (for Li$^+$) to 4 (for K$^+$) due to the decrease of the charge density. However, the number of cytidine molecules around the alkali metal ion is not as sensitive as the hydration number. This suggests that the hydrophobic effects will also contribute to the cytidine clustering around the alkali metal ions.

(ii) Alkaline Earth Metal Ion. When the alkaline earth metal ions (Mg^{2+} and Ca^{2+}) were used instead of the Li$^+$, Na$^+$, and K$^+$, the clustering of cytidine molecules around the positive ions was promoted obviously. Figure 3 shows the mass spectra of cytidine clusters generated from the aqueous solution including an alkaline earth metal salt (MgCl$_2$, CaCl$_2$) and cytidine. In the presence of Mg^{2+} (Figure 3a and a′), hydrated Mg^{2+} (Mg^{2+}(H$_2$O)$_x$; $x = 3 \sim 14$) and cytidine clusters with Mg^{2+} (Mg^{2+}(Cy)$_d$; $d = 1 \sim 9$) are observed. It is clear that the numbers of the water and the cytidine forming cluster with Mg^{2+} are drastically increased in comparison with the case of alkali metal ion, which will be due to the strong electrostatic interaction of Mg^{2+} with water and cytidine. Mg^{2+}Cl$^-$(Cy)$_e$ are also observed in Figure 3a, but with intensities much lower than Mg^{2+}(Cy)$_d$ clusters. The positive charge density of Mg^{2+} is partially neutralized by negative Cl$^-$, which results in the decrease of the electrostatic interaction between the ion pair Mg^{2+}Cl$^-$ and cytidine.
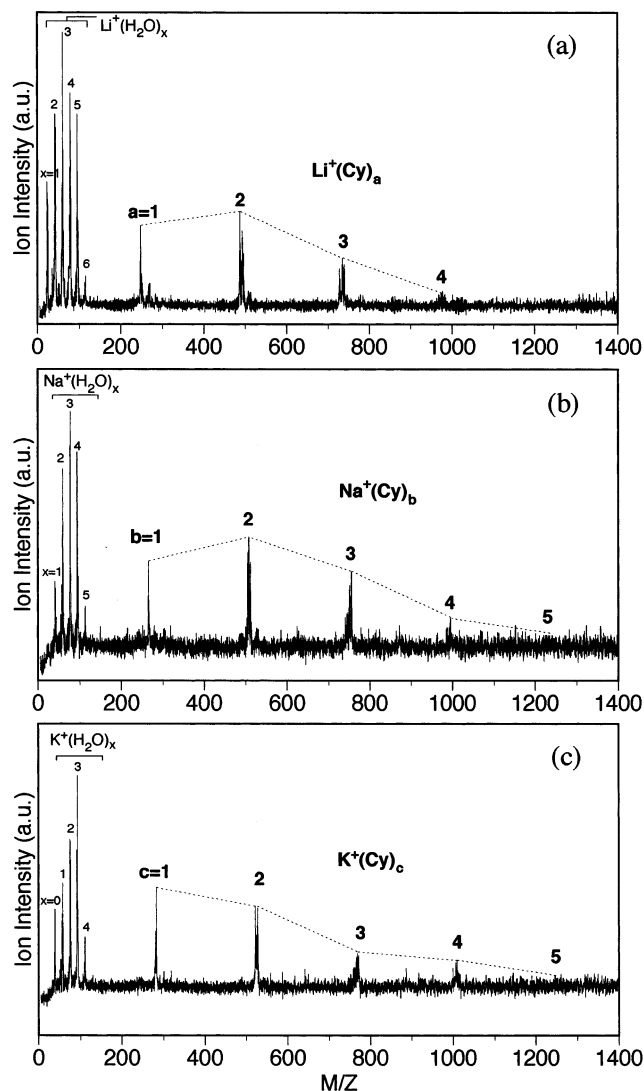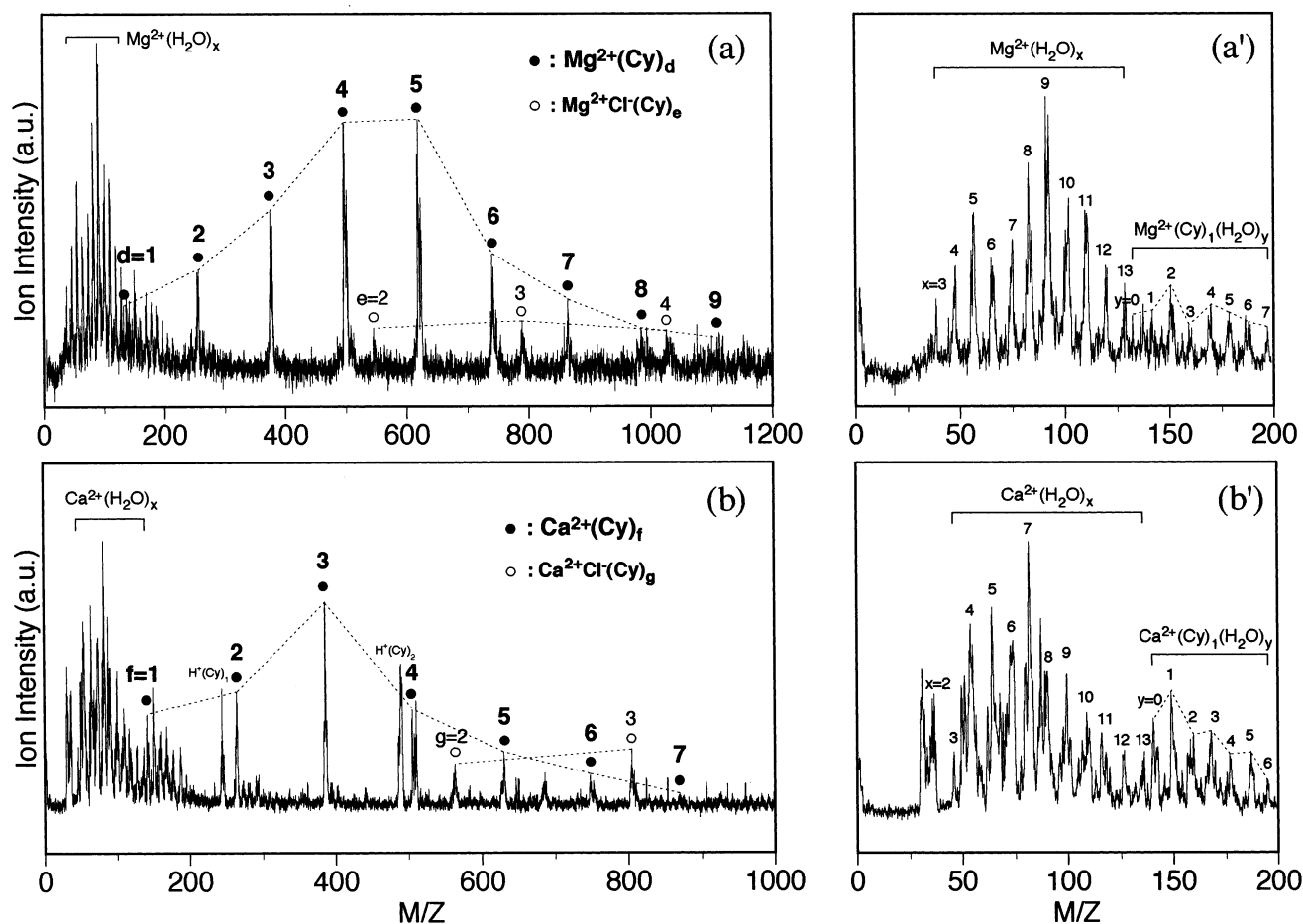

Figure 2. Mass spectra of clusters generated from aqueous solutions including alkali metal salt ((a) LiCl, (b) NaCl, (c) KCl; 1.0×10^{-3} mol dm^{-3}) and cytidine (2.0×10^{-3} mol dm^{-3}). The numerals on peaks represent the number of molecules in the clusters. A series of clusters composed of a metal ion and cytidine molecules is connected by the dotted line.

Figure 3b and b′ shows the clustering in the presence of Ca^{2+}: hydrated Ca^{2+} (Ca^{2+}(H$_2$O)$_x$; $x = 2 \sim 13$) and cytidine clusters with Ca^{2+} (Ca^{2+}(Cy)$_f$; $f = 1 \sim 7$ and Ca^{2+}Cl$^-$(Cy)$_g$; $g = 2 \sim 3$) are also observed. The cytidine clusters with Ca^{2+} are a little smaller than those with Mg^{2+}.

In comparing the positive charge densities of metal ions represented by the ratio of charge to ionic radius as shown in Table 1, they are decreasing in the following order: Mg^{2+} > Ca^{2+} > Li$^+$ > Na$^+$ > K$^+$. As Figures 2 and 3 show, the clustering of cytidine with these ions is strongly related with this positive charge density. This indicates that the clusterings of cytidine with alkali and alkaline earth metal ions are controlled by the electrostatic interaction.

2. Coordination of Cytidine to Metal Ions. When the metal ion Mn^{2+} or Zn^{2+}, which is capable of forming complex ion through coordination with ligands, is coexisting with cytidine, the clustering of cytidine with these ions was found to be quite different from that with alkali and alkaline earth metal ions. Figure 4 shows the mass spectra of clusters generated from aqueous solutions including metal salt (MnCl$_2$ or ZnCl$_2$) and cytidine.

Figure 3. Mass spectra of clusters generated from aqueous solutions including alkaline earth metal salt ((a) MgCl$_2$, (b) CaCl$_2$; 1.0×10^{-3} mol dm^{-3}) and cytidine (2.0×10^{-3} mol dm^{-3}). The numerals on peaks represent the number of molecules in the clusters. (a') and (b') correspond to the mass spectra expanded in the mass region 0−200 amu of (a) and (b), respectively. A series of clusters composed of a metal ion and cytidine molecules is connected by the dotted line.

TABLE 1: Ionic Radii and Charge Densities (the Ratio of Charge to Radius) for Metal Cations

	ionic radius/nm	ratio of charge to radius
Li$^+$	0.073	13.7
Na$^+$	0.116	8.6
K$^+$	0.152	6.6
Mg^{2+}	0.086	23.3
Ca^{2+}	0.114	17.5
Mn^{2+}	0.097 (high spin)	20.6 (high spin)
	0.081 (low spin)	24.7 (low spin)
Zn^{2+}	0.088	22.7

For the clustering of cytidine with metal ions (Mn^{2+} and Zn^{2+}), M^{2+}Cl$^-$(Cy)$_n$: M^{2+} = Mn^{2+} or Zn^{2+}, $n = 1 \sim 3$ clusters are more favorable than M^{2+}(Cy)$_m$: $m = 1 \sim 8$ clusters, as shown in Figure 4. This is in good contrast with the case for using alkaline earth metal ion, Mg^{2+} or Ca^{2+}, even though the positive charge densities are comparable, as shown in Table 1. This represents the difference between coordinating and electrostatic interactions, which is clearly attributed to the presence or absence of 3d electrons of metal ion. In the series of M^{2+}Cl$^-$(Cy)$_n$ clusters, the clusters with $n \geq 4$ are hardly formed, and the clusters with $n = 2$ or 3 are observed as predominant peaks. This will be reasonably explained if the cytidine works as a bidentate ligand for Mn^{2+} and Zn^{2+}. In fact, cytidine has possible two coordinating sites at N(3) and O as shown in Figure 1, which are attractive at neutral pH region.[25] Since the most probable coordination numbers of Mn^{2+} and Zn^{2+} are 6 and 4, respectively, Mn^{2+}Cl$^-$(Cy)$_3$ and Zn^{2+}Cl$^-$(Cy)$_2$ would be ob-

served as stable complex ions. Furthermore, Sigel et al. reported the stability constants of the 1:1 complexes between metal ions and nucleoside (mono- or di-) phosphate, as shown in Table 2.[23,24] This indicates that the coordination ability of cytidine to Mn^{2+} is comparable with that to Zn^{2+}, and that they are much larger than that to Mg^{2+}.

In a series of the coordination-type clusters, the positive charge of the metal ions (Mn^{2+}, Zn^{2+}) is not completely shielded by two or three of bidentate-ligand cytidine, which would result in the Cl$^-$ inclusion. This is also in good contrast with the clusterings of cytidine with alkali and alkaline earth metal ions, where the positive charges are completely shielded by the cytidine molecules. It should be noted that the conformations of cytidine molecules around the metal ions are quite different between [Li$^+$, Na$^+$, K$^+$, Mg^{2+}, or Ca^{2+}] and [Mn^{2+} or Zn^{2+}].

As for the hydrated metal ions observed in Figure 4a' and b', the hydration numbers are found to be dependent on the positive charge density and are similar to the hydration for Mg^{2+} and Ca^{2+} as observed in Figure 3a' and b'.

Conclusion

The mass spectrometric analysis of clusters, generated through fragmentation of liquid droplets, showed that a nucleoside, cytidine, interacted with the metal cations favorably in water, even though the nucleoside does not have a phosphate group. Two types of interactions between the cytidine molecules and

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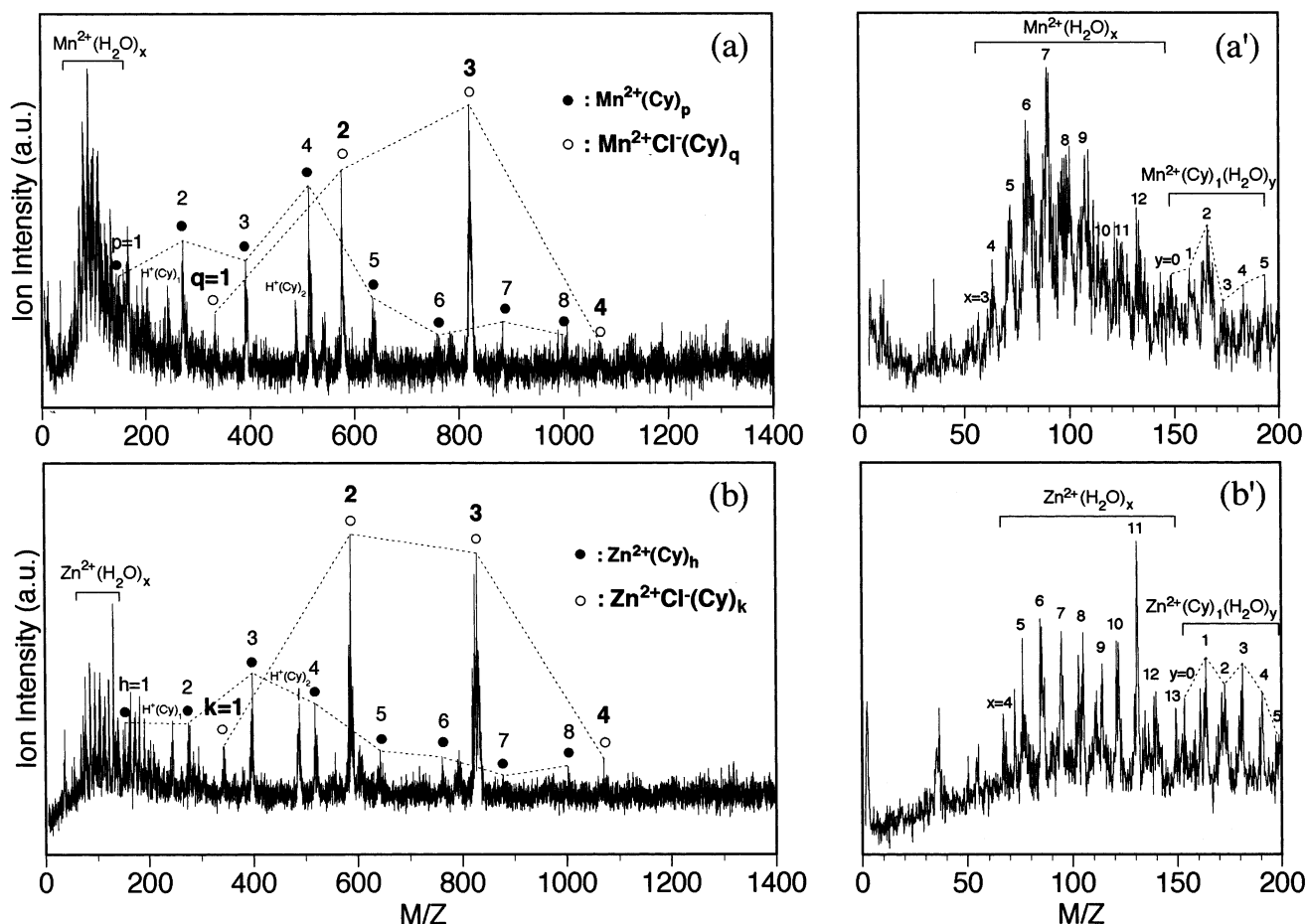
Figure 4. Mass spectra of clusters generated from aqueous solutions including metal salt ((a) MnCl$_2$ (b) ZnCl$_2$; 1.0×10^{-3} mol dm^{-3}) and cytidine (2.0×10^{-3} mol dm^{-3}). The numerals on peaks represent the number of molecules in the clusters. (a′) and (b′) correspond to the mass spectra expanded in the mass region 0−200 amu of (a) and (b), respectively. A series of clusters composed of a metal ion and cytidine molecules is connected by the dotted line.

TABLE 2: Logarithms of the Stability Constants of the Complexes for Nucleoside Mono- or Diphosphate in Aqueous Solution at 25 °C and Ionic Strength: 0.1 mol dm^{-3} (NaNO$_3$)

	AMP^{2-} ^{a,f}	GMP^{2-} ^{b,f}	UDP^{3-} ^{c,g}	CDP^{3-} ^{d,g}	dTDP^{3-} ^{e,g}
Mg^{2+}	1.60 ± 0.02	1.70 ± 0.02	3.32 ± 0.05	3.25 ± 0.03	3.34 ± 0.06
Mn^{2+}	2.23 ± 0.01	2.39 ± 0.02	4.07 ± 0.05	4.09 ± 0.04	4.18 ± 0.03
Zn^{2+}	2.38 ± 0.07	2.69 ± 0.02	4.07 ± 0.05	4.10 ± 0.06	4.15 ± 0.04

^{a} AMP^{2-}: adenosine 5′-monophosphate. ^{b} GMP^{2-}: guanosine 5′-monophosphate. ^{c} UDP^{3-}: uridine 5′-diphosphate. ^{d} CDP^{3-}: cytidine 5′-diphosphate. ^{e} dTDP^{3-}: thymidine 5′-diphosphate. ^{f} Ref 23. ^{g} Ref 24.

the metal ions were characterized as "electrostatic interaction" and "coordinating interaction". The clustering of cytidine with an alkali metal ion or an alkaline earth metal ion was controlled by the electrostatic interaction, and the number of cytidine molecules around the metal ion was dependent on the charge density. On the other hand, the cytidine functioned as a bidentate ligand for Mn^{2+} and Zn^{2+}, which was characterized as coordinating interaction. This coordination was restricted by the 3d orbital of the metal ion, therefore, the coordination number was different from the number of cytidine molecules in the clusters controlled by the electrostatic interaction.

The DNA replication is promoted by the presence of Mg^{2+}, but not by the presence of Mn^{2+} through the PCR method. The observed difference between the cytidine−Mg^{2+} and the cytidine−Mn^{2+} clustering will provide a new insight into the study on the role of metal ions in the DNA replication.

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