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Effect of Alkaline Earth Metal Ions on the Phosphodiester Hydrolysis of RNA

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Hydrolyses of di- and triribonucleotides assisted by alkaline earth metal ions were investigated at pH 7.3 and 50 °C. Mg^{2+} was the most effective for the phosphodiester hydrolysis, and the rate exibited first-order dependence on the concentration of Mg^{2+} in a range of 0.1–1.0 mol dm^{-3}.

Magnesium ion is an essential factor in a number of biological catalysts for the phosphodiester hydrolysis of nucleic acids, such as ribozymes[1] and nucleases.[2] Magnesium ion is known to play two distinct roles: (1) a catalytic factor, which provides acid and/or base catalysts, and (2) a structural factor, which is essential in constructing a specific three-dimensional structure required for the reaction. Generally, it is difficult to observe these two distinct roles of metal ions separately in complex biological catalysts, because the structural factor closely relates to the efficiency of the catalysis.[1,2] Although many elegant works have disclosed details of roles of Mg^{2+},[1–3] the basic chemistry concerning catalytic effects of alkaline metal ions on the phosphodiester hydrolysis is not known well.[4] It should be useful to study the metal-ion-assisted hydrolysis of RNA by using a simple substrate in order to gain knowledge about the catalytic role of the metal ion. In this study, effects of Mg^{2+} on the hydrolysis of oligoribonucleotides were studied, along with those of other alkaline earth and alkaline metal ions.

Results and Discussion

Hydrolysis of adenylyl(3′-5′)adenosine (ApA) (0.1 mmol dm^{-3}) was conducted in the presence of alkaline or alkaline earth metal ions (100 mmol dm^{-3}) at pH 7.3 (HEPES 50 mmol dm^{-3}) and 50 °C. The conversions after 90 h reaction, calculated on the basis of the decrease of ApA and the amount of the resulting products, are summarized in Table 1.

The products, except those of the Ca^{2+}-assisted reaction, were adenosine 3′-monophosphate (3′-AMP), adenosine 2′-monophosphate (2′-AMP), adenosine 2′,3′-cyclicmonophosphate (2′,3′-cAMP), and adenosine, indicating that the hydrolysis of the phosphodiester bond selectively occurred. On the basis of a generally accepted reaction mechanism for a non-enzymatic RNA hydrolysis,[1–5] the 5′-terminal moiety of ApA should be converted into a mixture of 3′-AMP, 2′-AMP, and 2′,3′-cAMP, whereas the 3′-terminal moiety of ApA should result in the formation of adenosine. Consistently, in the Mg^{2+}-assisted reaction, the amount of adenosine was equal to the total amount of 3′-AMP, 2′-AMP, and 2′,3′-cAMP. In the case of Ca^{2+}, however, the formation of adenosine was unusually low, compared with other products, i.e. 3′-AMP, 2′-AMP, and 2′,3′-cAMP. Instead, adenine was formed. The formation of adenine indicates that an *N*-glycoside cleavage occurred along with the phosphodiester hydrolysis by the Ca^{2+} assisted reaction.

Among metal ions tested, the phosphodiester hydrolysis was most efficiently promoted by Mg^{2+} and Ca^{2+}, and less efficiently by Sr^{2+} and Ba^{2+}. In contrast, the effect of alkaline metal ions, Na^+ and K^+, was very low. Although Lönnberg et al. have reported that the acceleration effect of Mg^{2+} (10 mmol dm^{-3}) on the hydrolysis of uridylyl(3′-5′)uridine (UpU) was very small,[4] a notable effect of Mg^{2+} was observed in this study, because we have chosen a higher Mg^{2+} concentration, i.e. 100 mmol dm^{-3}.

In the presence of excess NaCl (1.0 mol dm^{-3}), the rate of the phosphodiester hydrolysis assisted by Mg^{2+} was about one third of that in the absence of NaCl. Such a result indicates that a competitive binding between Na^+ and Mg^{2+} to the substrate occurs and that the binding of Na^+ prevents the Mg^{2+}-assisted hydrolysis.

The hydrolysis of di- or triribonucleotides, ApA, UpA, CpC, ApUp, and ApCpC, assisted by Mg^{2+} was investigated at pH 7.3 (HEPES 50 mmol dm^{-3}) and 50 °C. The rate of hydrolysis was the greatest for ApUp ($k_{obs} = 11 \times 10^{-3}\ h^{-1}$). The rates of hydrolyses of ApA ($k_{obs} = 1.7 \times 10^{-3}\ h^{-1}$), UpA ($k_{obs} = 1.8 \times 10^{-3}\ h^{-1}$), and CpC ($k_{obs} = 1.3 \times 10^{-3}\ h^{-1}$) were the lowest, but these three did not show significant differences from each other. ApCpC ($k_{obs} = 3.7 \times 10^{-3}\ h^{-1}$) showed a medium hydrolysis rate. The hydrolysis rate was mainly affected by the backbone structure, and was in the order of the total negative charge of the molecule, i.e. ApUp (-3) > ApCpC (-2) > ApA, UpA, and CpC (-1). In particular, a diribonucleotide having a 3′-terminal phosphate group was found to be hydrolyzed faster than that having no 3′-terminal phosphate group. The acceleration effect of the 3′-terminal phosphate group of oligoribonucleotides has also been found in the Zn^{2+}-assisted hydrolysis.[5] On the other hand, the effect of nucleic acid bases on the Mg^{2+}-assisted hydrolysis[1a] was relatively small.

The dependence of the rate constants for the hydrolysis of oligoribonucleotides on the concentration of Mg^{2+} was investigated at pH 7.3 (HEPES 50 mmol dm^{-3}) and 50 °C. Figure 1 shows the results. The log–log plots demonstrate linear dependencies with the slope of unity for all substrates investigated. Such results indicate that the rate of the phosphodiester hydrolysis is affected by one metal ion in the range of $[Mg^{2+}] = 0.1$–1.0 mol dm^{-3}. ^1H NMR spectroscopic studies (270 MHz, D_2O, pD 7) of ApA (0.1 mmol dm^{-3}) with $MgCl_2$ (0, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mol dm^{-3}) indicated that no significant conformational change of ApA depending on the concentration

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Table 1. Conversion and Product Distribution for the Hydrolysis of ApA Assisted by Alkaline Earth and Alkaline Metal Salts after the Reaction at pH 7.3 (HEPES 50 mmol dm^{-3}) and 50 °C for 90 h[1)]

Metal ion	Conv./%	Product ratio/%				
		3′-AMP	2′-AMP	2′,3′-cAMP	Adenosine	Adenine
Mg^{2+}	15	32	14	4	50	—[2)]
Ca^{2+}	15	28	13	8	3	48
Sr^{2+}	2	10	—[2)]	40	50	—[2)]
Ba^{2+}	4	10	—[2)]	40	50	—[2)]
Na^{+}	0.8	—[2)]	—[2)]	50	50	—[2)]
K^{+}	0.7	—[2)]	—[2)]	50	50	—[2)]
none	—[2)]					

1) [metal ion] = 100 mmol dm^{-3} (chloride), $[ApA]_0$ = 0.1 mmol dm^{-3}. 2) Under detection.

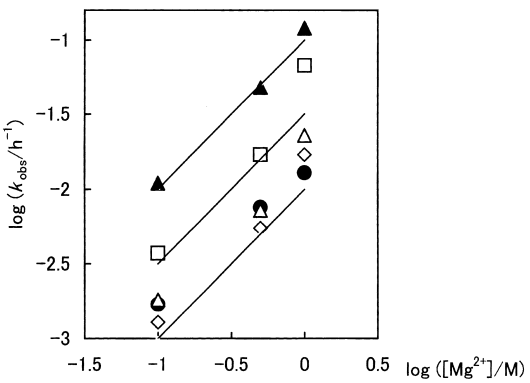

Fig. 1. Dependence of the pseudo-first-order rate constant for the oligoribonucleotide hydrolysis on the concentration of Mg^{2+} at pH 7.3 (HEPES 50 mmol dm^{-3}) and 50 °C. ●: ApA, △ : UpA, ◇: CpC, ▲: ApUp, □: ApCpC. The lines show the unit slope.

of $MgCl_2$ occurred. Therefore, the observed linear dependency of the rate constants on the Mg^{2+} concentration can be ascribed to the binding of Mg^{2+}, which assists the hydrolysis as a catalytic factor, to ApA. Its binding constant is estimated to be very small ($\log K < 0$), because otherwise the concentration profile should show a saturation in this concentration range. This result can be interpreted as meaning either (1) the binding constant for Mg^{2+} and ApA is indeed small, and seems to be outstandingly small when compared with the binding constant for Mg^{2+} and 3′-AMP ($\log K = 1.89$),[6] or (2) the actual binding of Mg^{2+} is not so weak, but the observed enhancement of the rate is due to the binding of the second metal ion, which should bind much more weakly than the first one. The latter interpretation means that the hydrolysis is efficiently promoted only when the second Mg^{2+} binds to the substrate. This can be supported by several examples of the participation of two metal ions in the phosphodiester hydrolysis both in biological catalysts[1,2] and synthetic ones.[5,7,8] Whatever the interpretation is, to facilitate the binding of Mg^{2+}, which was found to be very weak in this study, is suggested to be one of the important factors for the high activity of the biological catalysts for phosphodiester hydrolysis using Mg^{2+}.

Experimental

All reagents were purchased from commercial sources and used as received.

In a typical experiment, the solution of ApA (0.1 mmol dm^{-3}), $MgCl_2$ (100 mmol dm^{-3}), and HEPES (50 mmol dm^{-3}) was adjusted to pH 7.3 and heated at 50 °C. The reaction mixture was periodically analyzed by the HPLC (column: Merck LiChrospher 100 RP-18(e) ODS-column; eluent: pH 5.0 water/acetonitrile 92:8 v/v mixture containing phosphate (3 mmol dm^{-3}) and acetate (40 mmol dm^{-3}); flow rate: 0.5 mL min^{-1}; detection: 260 nm). The products, were confirmed by coinjection of the reaction mixture and standard samples. The reactions could be analyzed by pseudo-first-order kinetics. During the entire process, great care was taken in order to avoid any contamination, which may cause enzymatic hydrolysis.

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