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Efficient Cleavages of Ribonucleoside 2',3'-Cyclic Phosphates and Ribonucleotide Dimers Catalyzed by β-Cyclodextrin Attached with Diethylenetriamine-Zinc(II) Complex

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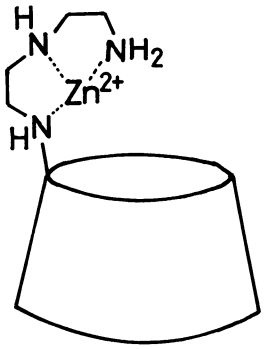
The complex between a modified β-cyclodextrin, which is attached with a diethylenetriamine residue at the primary hydroxyl side, and zinc(II) ion effectively and homogeneously catalyzes the cleavages of the 2',3'-cyclic monophosphates of ribonucleosides and the ribonucleotide dimers. The magnitude of the catalysis is highly dependent on the kind of the nucleic base in the substrates.

Recently cleavages of deoxyribonucleic acids have been achieved by several non-enzymatic systems.[1] However, information on the selective and effective cleavages of ribonucleic acids by artificial systems has been rather scarce. The scheme for the fission of the phosphodiester linkages in ribonucleic acids, which proceeds via 2',3'-cyclic phosphates as intermediates,[2,3] is entirely different from that for the fission in deoxyribonucleic acids.

In previous papers,[4,5] one of the present authors showed that cyclodextrins (CyDs), cyclic oligomers of glucose,[6] exhibit regioselective catalyses in the cleavages of the 2',3'-cyclic monophosphates of ribonucleosides.

This paper reports that the complex between a modified β-CyD (I), which is attached with a diethylenetriamine residue, and zinc(II) ion is quite effective for the cleavages of ribonucleoside 2',3'-cyclic phosphates and ribonucleotide dimers. Significant dependence of the catalytic activity on the nucleic bases in the substrates is shown.

The preparation of the modified β-CyD (I) by the introduction of a diethylenetriamine residue to the primary hydroxyl side of the cavity was accomplished by the method in the literature.[7] The structure and the purity were confirmed by ^{1}H-NMR spectroscopy. The I-Zn^{2+} complex was prepared in situ by mixing I and zinc(II) hydroxide in water at the concentrations 10^{-2} mol dm^{-3}.



I-Zn^{2+} complex

The cleavages of the 2',3'-cyclic monophosphates of adenosine, guanosine, cytosine, and uridine (IIa-d) were carried out at pH 9.5 and 20 °C, whereas the cleavages of adenylyl-3',5'-adenosine (ApA), adenylyl-3',5'-cytidine (ApC), cytidylyl-3',5'-adenosine (CpA), and uridylyl-3',5'-adenosine (UpA) were effected at pH 10.0 or 11.0 and 40 °C. The initial concentrations of the substrates were around 10^{-4} mol dm^{-3}. The reaction mixtures were periodically analyzed by HPLC (Merck LiChrosorb RP-18(e) columns). All the reactions proceeded homogeneously, and satisfactorily followed pseudo first-order kinetics.

Table 1 shows the rate constants of the cleavages of IIa-d in the presence and the absence of the I-Zn^{2+} complex. The cleavages of all the 2',3'-cyclic phosphates are largely accelerated by the complex. Significantly, the accelerating effects of the I-Zn^{2+} complex on the cleavages of IIa and b (23 and 28 fold) are much larger than those on the cleavages of IIc and d (3.5 and 9.6 fold). This result indicates that the catalyses involve the inclusion of the nucleic bases of II's in the cavity of the β-CyD residue, prior to the bond cleavages. The purine residues in IIa and b form inclusion complexes with β-CyD more favorably than the pyrimidine residues in IIc and d.[8]

The present catalyses are ascribed to the complex between the diethylenetriamine residue of I and the Zn^{2+} ion. In the absence of the Zn^{2+} ion, the cleavages were decelerated by I (2-4 fold).

The β-CyD residue of I is definitely required for the efficient catalyses. When diethylenetriamine, in place of I, was added to zinc hydroxide at the molar ratio 1.0 and pH 9.5, significant amount of precipitate (the amine-Zn^{2+} complex and/or zinc hydroxide) was formed. Homogeneous solutions were obtained, only when

Table 1. Rate constants for the cleavages of IIa-d in the presence and the absence of the I-Zn^{2+} complex [a]

Ribonucleoside cyclic phosphate	I-Zn^{2+} (10^{-2} mol dm^{-3})	Rate constant [b] 10^{-4} min^{-1}
IIa	Presence	6.5 (23)
	Absence	0.28
IIb	Presence	4.8 (28)
	Absence	0.17
IIc	Presence	2.1 (3.5)
	Absence	0.60
IId	Presence	5.2 (9.6)
	Absence	0.54

a) At pH 9.5, 20 °C. b) The numbers in parentheses show the ratios of the rate constants in the presence of the I-Zn^{2+} complex to those in its absence.

the molar ratio of diethylenetriamine to zinc hydroxide was 5.0 or larger. The ratios of the rate constants in the presence of the 5:1 mixture to those in its absence were 3.6, 8.2, 1.7, and 1.5 for the cleavages of IIa-d, respectively, and thus were considerably smaller than the values for the I-Zn^{2+} complex.

The selectivities (56 and 59%) for the formation of the corresponding 3'-monophosphates of ribonucleosides, with respect to the formation of the 2'-phosphates as byproduct, in the I-Zn^{2+} complex-catalyzed cleavages of IIa and b were almost identical with those (54 and 55%) for the cleavages in the absence of the complex.

In addition to the cleavages of II's, the cleavages of the ribonucleotide dimers were successfully achieved by use of the I-Zn^{2+} complex as catalyst. The accelerating effects of the complex on the cleavage of ApA were 7.1 and 3.4 fold at pH 10.0 and 11.0, respectively. The complex also accelerated the cleavages of ApC, CpA, and UpA (4.0, 1.4, and 1.3 fold at pH 11.0).

In contrast with the effective catalyses by the I-Zn^{2+} complex, the diethylenetriamine-Zn^{2+} (5:1) mixture showed slight deceleration of the cleavages of all the dimers. Thus, the requirement of the β-CyD residue is still more remarkable than that described above for the cleavages of IIa-d.

The complexes of I with Cu^{2+} ion and with Mg^{2+} ion showed small acceleration (1.4 fold and 1.2 fold, respectively) of the cleavage of ApA.

In conclusion, the complex between the modified β-CyD (I) and zinc(II) ion has large catalytic activity for the cleavages of ribonucleoside 2',3'-cyclic phosphates and ribonucleotide dimers. The magnitude of the accelerating effect is highly dependent on the kind of the nucleic base adjacent to the phosphodiester bond cleaved.

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