recooled to −78 °C, and a solution of **20** (135 mg, 0.20 mmol) in CH_2Cl_2 (8 mL) was added. The reaction mixture was allowed to warm to ~0 °C, quenched by addition of H_2O, diluted with CH_2Cl_2 (150 mL), and extracted 2× with 1 M biethylammonium bicarbonate solution, pH 7.5. The organic layer was dried ($MgSO_4$) and concentrated, the solid residue treated with ethyl acetate, the insoluble part [tris(triethylammonium)-phosphonate] removed by filtration, the filtrate concentrated, and the crude product purified by preparative TLC (CH_2Cl_2)/methanol/triethylamine 100:10:1). The fluorescent band was collected and eluted, yielding **22** as a white foam (141 mg, 84%): ^{1}H NMR δ 10.78 (s, NH), 8.89, 8.24 (2 s, 2 H), 6.67–8.17 (m, ~19 H), 5.10 (m, 1 H), 4.37 (m, 1 H), 3.77 (s, 2 H_3CO), 3.40 (m, 2 H), 3.09 (qua, J = 7.3, ~6 H), 2.50–2.80 (m, 2 H), 1.82 (d, J ~ 1, H_3C-C5), 1.39 (t, J = 7.3, ~9 H).

5-*O*-(Dimethoxytrityl)-2-deoxy-1-β-[5-methyl-2-oxo-(3*H*)-pyrimido-[1,6-*a*]perimidin-3-yl]-3-*O*-ribofuranosylphosphonate Triethylammonium Salt (21). To CH_2Cl_2 (10 mL) were sequentially added at −78 °C *N*-methylmorpholine (1 mL), PCl_3 (150 μL), and triazole (720 mg). The mixture was stirred at ambient temperature for 1 h, and a solution of **19** (105 mg, 0.157 mmol) in CH_2Cl_2 (3 mL) was added at −78 °C. After warming to ~0 °C, the reaction was quenched by addition of H_2O, diluted with CH_2Cl_2 (100 mL), and extracted 2× with 1 M ammonium bicarbonate solution, pH 7.5. The organic layer was dried and concentrated and the residue dispersed in ethyl acetate. The insoluble material [tris(triethylammonium)phosphonate] was removed by filtration, the filtrate concentrated, and the residue purified by preparative TLC (CH_2Cl_2/methanol/triethylamine 100:10:1), yielding **21** as a yellow foam (83 mg, 63%): ^{1}H NMR δ 8.16 (dd, J_1 = 6.8, J_2 = 1.9, H-C12), 6.68–7.56 (m, ~19 H), 6.50 (dd, J_1 = 7.9, J_2 = 5.9, anomeric H), 4.83–5.16 (m, br, 1 H), 4.17–4.38 (m, 1 H), 3.78 (s, 2 H_3CO), 3.36–3.55 (m, 2 H), 3.06 (qua, J = 7.1, 6 H), 2.41–2.64 (m, 2 H), 1.51 (s, br, H_3C-C5), 1.33 (t, J = 7.1 ~9 H).

2-(Isobutyrylamino)-6-[(2-aminophenyl)amino]-9-(3,5-di-*O*-isobutyryl-2-deoxy-1-β-D-ribofuranosyl)purine (18). A solution of *N*2,*O*3′,*O*5′-triisobutyryl-2′-deoxy-6-*O*-[(2,4,6-triisopropylphenyl)-sulfonyl]guanosine[21] (175 mg, 0.23 mmol) and *o*-phenylenediamine (100 mg, 0.92 mmol) in THF (3 mL) was heated to reflux for 24 h. The crude product was purified by chromatography (ethylacetate)/CH_2Cl_2 1:5–1:0), yielding **22** as a foam (103 mg, 76%): ^{1}H NMR δ 7.95 (s, br, 1 H), 7.77 (s, H-C8), 6.61–7.37 (m, ~4 H), 6.25 (dd, J_1 = 8.1, J_2 = 6.1, anomeric H), 5.25–5.45 (m, 1 H), 4.13–4.43 (m, ~3 H), 2.30–3.14 (m, ~5 H), 0.88–1.24 (m, ~18 H); UV (2.73 mg in 100 mL) λ_{max} 230 nm (ε 21 500), 292 (12 100), end absorption to 360; λ_{min} 262 (10 800); MS *m*/*z* 568 (MH^+, 4), 312 (100), 242 (35), 225 (52).

5-Methyl-2′-deoxy-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-4-*N*-(2-amino-3-pyridyl)cytidine (17). A solution of 3′,5′-*O*-(*tert*-butyldimethylsilyl)-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]thymidine (**7**; 115 mg, 0.15 mmol) and 2,3-diaminopyridine (100 mg, 0.92 mmol) in THF (2 mL) was heated to reflux for 18 h. The crude reaction mixture was purified by preparative TLC (ether/hexane 4:1), and the yellow band was collected and eluted with ether, yielding **17** as a foam (40 mg, 47%): ^{1}H NMR δ 7.74 (dd, J_1 = 4.8, J_2 = 0.9, H-C6″), 7.23 (m, H-C6) 6.99 (dd, J_1 = 8.0, J_2 = 0.9, H-C4″), 6.80 (dd, J_1 = 8.0, J_2 = 4.8, H-C5″), 6.40 (dd, J_1 = 6.5, J_2 = 6.0, anomeric H), 4.30–4.54 (m, 1 H), 3.74–4.00 (m, 3 H), 2.02 (s, H_3C), 1.92–2.45 (m, H_2C2'), 0.78–1.05 (m, ~18 H), 0.05–0.22 (m, ~12 H); UV (1.03 mg in 25 mL); λ_{max} 286 nm (ε 12 500), 370 (16 400), end absorption to 440; λ_{min} 252 (8500), 315 (4200); MS *m*/*z* 562 (MH^+, 2), 218 (100).

Oligonucleoide Synthesis. Polymer-bound nucleotide H-phosphonates were prepared as previously described[12] on control-pore glass by using the DBU salts of the protected nucleoside H-phosphonates.[22] For introducing the polycyclic nucleosides, a solution of ~25 mg of the triethylammonium salts **21** and **22**, respectively, in 1 mL of pyridine/acetonitrile (1:1) was used in the automated synthesis. For efficient coupling the wait time in the programmed coupling step for **21** and **22** was increased from 6 × 8 to 26 × 8 s. After oxidation and deblocking, the fragments were purified by polyacrylamide gel electrophoresis, the fluorescent (in the case of **22**) or yellow (in the case of **21**) bands were eluted and the eluate was desalted by loading onto a reversed-phase C_{18} Sep-Pak column (Waters Associates), washing with H_2O, and finally eluting the fragments with 25% aqueous acetonitrile.

Hypochromicity Measurements. Hypochromicities of the duplexes were measured at 260 nm with a Kontron Uvikon 810 spectrophotometer in a 1-cm masked cuvette. The samples were 100 mM NaCl, 10 mM Na_2HPO_4, and 1 mM EDTA at pH 7.2 and contained the oligomers at a concentration of 2 μM in a total volume of 1 mL. The extinction coefficients (ε) of the oligomers were calculated,[23] and the ε of the oligomers containing the extra tetracyclic bases were approximated by adding the values of the ε of the monomers of 260 nm (for **10**, ε(260) = 15 mL/μmol; for **11**, ε(260) = 6 mL/μmol) to the ε of the oligomers devoid of the tetracyclic bases. The samples were degassed with He, heated to ~55 °C for 4 h, allowed to cool, and maintained at 0 °C overnight. The absorbance of the samples was monitored from 15 to ~80 °C, increasing the temperature at a rate of ~0.2 °C/min. Each sample was measured at least twice, and the data were separately normalized to percent denaturation (% denaturation = $100[A_0 - A_i]/[A_f - A_i]$, where A_0 is the observed, A_i is the initial, and A_f is the final absorbance) and combined to obtain a melting curve. A linear least-squares analysis of this data gave a slope of transition and *y* intercept from which the T_m values were calculated.

Acknowledgment. We thank Mark Vasser, Parkash Jurani, and Peter Ng for their help with the oligodeoxynucleotide synthesis, John Chakel for the MS data, and Mike Mulkerrin for his assistance with the fluorescence spectroscopy and with the T_m data.

α-Cyclodextrin-Catalyzed Regioselective P–O(2′) Cleavages of 2′,3′-Cyclic Monophosphates of Ribonucleosides[1]

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Abstract: Regioselective P–O(2′) cleavages of 2′,3′-cyclic monophosphates of cytidine (Ia), uridine (Ib), adenosine (Ic), and guanosine (Id) to the corresponding 3′-monophosphates (IIIa–d) are achieved at pH 11.08, 20 °C by α-cyclodextrin (α-CyD) as catalyst. The selectivities asymptotically increase with increasing concentration of α-CyD, attaining 98, 94, 76, and 67% for the cleavages of Ia–d, respectively, at the concentration of 0.05 M α-CyD. In the absence of α-CyD, however, significant concurrent cleavages of the P–O(3′) bonds take place and the selectivities for IIIa–d are only 47, 50, 54, and 52%. The rate of the P–O(2′) cleavage of Ia in the α-CyD–Ia complex is 14 times as large as that of free Ia, and the rate of the P–O(3′) cleavage in the complex is virtually zero. β- and γ-CyDs show no regioselective catalyses. ^{1}H NMR spectroscopy indicates that the α-CyD–Ia and –Ib complexes are formed by hydrogen bondings and I's are located on the secondary hydroxyl side of the cavity of α-CyD. The selective cleavages of ribonucleotide dimers CpC, CpU, CpA, and CpG to IIIa are also successfully carried out by α-CyD as catalyst.

Ribonuclease cleaves ribonucleic acids to fragments having the terminal phosphates at specific positions (mostly the 3′-positions).[2,3] This specificity is attributed to regiospecific cleavage of the P–O(2′) bond of the 2′,3′-cyclic monophosphate of the terminal

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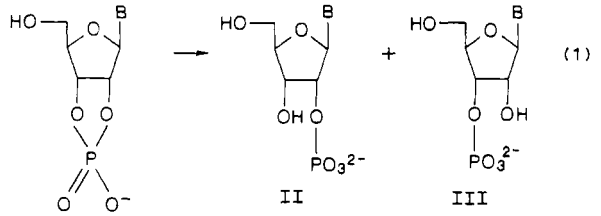
ribonucleotide, formed as the intermediate by the intramolecular attack of the 2′-hydroxyl group of the ribonucleotide in the 3′-side of the cleaved phosphodiester linkage. Mimicking the function of this enzyme has been attempted.[3-8] However, regioselective cleavages of the P–O(2′) bonds of 2′,3′-cyclic monophosphates of ribonucleotides by artificial systems have not been successful yet.

Breslow reported that chemically modified cyclodextrins, in which two imidazolyl residues are introduced, exhibit regiospecific cleavage of cyclic monophosphate of 4-*tert*-butylcatechol.[6,7] The interactions between nucleotides and cyclodextrins (CyDs) were also investigated by several workers.[8-11]

This paper reports the first nonenzymatic regioselective P–O(2′) cleavage of 2′,3′-cyclic monophosphates of ribonucleosides. The 2′,3′-cyclic monophosphates of cytidine, uridine, adenosine, and guanosine (Ia–d) are selectively cleaved to the corresponding 3′-monophosphates (IIIa–d) by use of α-CyD, the cyclic oligomer of six glucoses,[12] as catalyst (eq 1). Marked dependence of the



Ia, B = cytosine
b, B = uracil
c, B = adenine
d, B = guanine

regiospecific catalysis on the kind of CyD is shown. The results of the ^{1}H NMR spectroscopy on the α-CyD–I complexes are presented, and the reaction mechanism of the regiospecific catalysis is proposed. Furthermore, the regiospecific catalysis by α-CyD in the cleavages of dimers of ribonucleotides, which contain cytidine residues at the 3′-sides, is shown.

Experimental Section

Materials. CyDs were purified by repeated recrystallization from water. Ia–d, cytidylyl(3′-5′)cytidine (CpC), cytidylyl(3′-5′)uridine (CpU), cytidylyl(3′-5′)adenosine (CpA), and cytidylyl(3′-5′)guanosine (CpG) were purchased from Sigma Chemical Co. All other chemicals were purified by the usual methods.

Kinetics. Cleavages of I's were carried out at pH 11.08 (hydrogen bicarbonate buffer, I = 0.1 M), 20 °C. The initial concentrations of I's were ~10^{-4} M. The reaction mixtures were periodically analyzed by HPLC (Jasco C_{18}S column, 30 cm; eluent, water for Ia and Ib and water–acetonitrile (98:2) mixture for Ic and Id). All the reactions satisfactorily followed first-order kinetics, and the selectivities for the formation of III with respect to the 2′-monophosphates (II) were constant irrespective of the conversion. The cleavages of CpC, CpU, CpA, and CpG were achieved at pH 11.08, 50 °C.

The rate constants (k_c) of the cleavages of I's in the α-CyD–I complexes and the equilibrium constants (K_d) for the dissociation of the complexes were determined by plotting $1/k_{obsd} - k_{un})$ vs $1/[\alpha\text{-CyD}]_0$.[12] Here, k_{obsd} and k_{un} are the rate constants of the cleavages in the presence and the absence of α-CyD.

The rate constants k_{OH} and k_{H_2O} for the hydroxide ion catalyzed and the water-catalyzed cleavages of I were determined from the pH–rate constant profiles by use of eq 2. All the k_{obsd}'s were extrapolated to zero buffer concentration.

$$k_{obsd} = k_{H_2O} + k_{OH}[OH^-] \quad (2)$$

Spectroscopy. Absorption spectra were measured at pH 11.08, 20 °C on a Shimadzu UV-180 spectrophotometer. The K_d value of the α-CyD–I complex was evaluated by the method in the literature.[13]

^{1}H NMR spectroscopy was carried out in D_2O (pD 9) at 30 °C on a Bruker AM-500 or a JEOL GX-400 spectrometer.

Table I. Selectivities and Rate Constants for the Cleavage of Ia in the Presence and the Absence of CyDs[a]

CyD	concn, 10^{-2} M	rate const, 10^{-4} min^{-1}	selectiv,[b] %
α-CyD	1.0	3.4	81
	5.0	7.0	98
β-CyD	1.0	1.9	45
γ-CyD	1.0	1.4	47
hexa-2,6-dimethyl-α-CyD	1.0	2.0	47
hepta-2,6-dimethyl-β-CyD	1.0	1.7	47
none		1.7	47

[a] At pH 11.08, 20 °C. [b] IIIa/(IIa + IIIa).

Table II. Selectivities and Rate Constants for the Cleavage of Ib–d in the Presence and the Absence of CyDs[a]

substr	CyD	concn, 10^{-2} M	rate const, 10^{-4} min^{-1}	selectiv,[b] %
Ib	α-CyD	1.0	1.7	70
Ib	α-CyD	5.0	1.9	94
Ib	β-CyD	1.0	1.3	43
Ib	γ-CyD	1.0	1.3	50
Ib	none		1.3	50
Ic	α-CyD	1.0	4.2	67
Ic	α-CyD	5.0	4.7	76
Ic	none		3.5	54
Id	α-CyD	1.0	4.8	62
Id	α-CyD	5.0	6.2	67
Id	none		2.6	52

[a] At pH 11.08 (bicarbonate buffer), 20 °C. [b] III/(II + III).

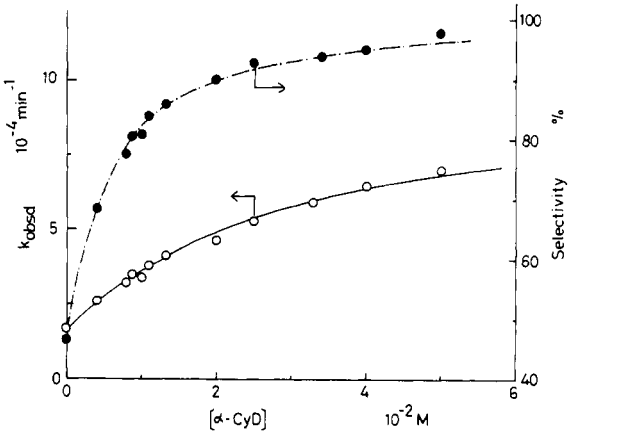


Figure 1. Dependence of the rate constant (○) and the selectivity (IIIa/(IIa + IIIa) (●) on the concentration of α-CyD for the cleavage of Ia at pH 11.08, 20 °C. The solid and the broken lines are the theoretical lines calculated by use of the parameters in Table V.

Results

Regioselective P–O(2′) Cleavage of Ia Catalyzed by α-CyD. Table I shows the selectivity of the formation of IIIa by the cleavage of the P–O(2′) bond of Ia with respect to the formation

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Table III. Values of k_{OH} and k_{H_2O} for the Cleavage of Ia in the α-CyD Complex and in the Free State

rate const	value
k_{OH}(complex)[a]	5.3×10^{-1}
k_{OH}(free)[a]	3.0×10^{-2}
k_{H_2O}(complex)[b]	3.4×10^{-4}
k_{H_2O}(free)[b]	1.1×10^{-4}

[a] In M^{-1} min^{-1}. [b] In min^{-1}.

of IIa by the P–O(3′) cleavage. In the presence of α-CyD, the cleavage of the P–O(2′) bond is predominant, giving IIIa in high selectivity. The selectivity as well as the rate of cleavage asymptotically increase with increase in the concentration of α-CyD, as depicted in Figure 1. The selectivity attains 98% at the concentration of 0.05 M α-CyD. The k_c value is 5.9 times as large as k_{un} (see Table V).

In the absence of α-CyD, however, the cleavage of the P–O(3′) bond takes place more efficiently than that of the P–O(2′) bond, and the selectivity for IIIa is only 47%.

In contrast with the remarkable regioselective catalysis by α-CyD, β-CyD and γ-CyD show no measurable effects on the selectivity. The catalyses by hexa-2,6-dimethyl-α-CyD and hepta-2,6-dimethyl-β-CyD are negligible.

Regioselective P–O(2′) Cleavage of Ib–d Catalyzed by α-CyD. As shown in Table II, regioselective cleavage of the P–O(2′) bond of Ib to IIIb is successfully achieved by α-CyD. α-CyD also enhances the formation of IIIc or IIId from Ic or Id, although the selectivities are smaller than the values for Ia or Ib.

In contrast with the effective regioselective catalysis by α-CyD, γ-CyD does not increase the selectivity for IIIb to a measurable extent. β-CyD decreases the selectivity.

NMR Spectroscopy on the α-CyD–I Complexes. On the complex formation between α-CyD and Ia, the resonances for the H-2 and H-3 protons of α-CyD shifted considerably toward higher magnetic field (0.075 and 0.055 ppm when the charged concentrations of α-CyD and Ia were 10^{-2} and 2×10^{-2} M). The H-1 and H-4 protons also showed upfield shifts (0.036 and 0.028 ppm). On the other hand, the H-5 and H-6 protons of the cytosine residue of Ia experienced downfield shifts on complex formation (0.014 and 0.018 ppm when $[\alpha\text{-CyD}]_0 = 2 \times 10^{-2}$ M and $[Ia]_0 = 10^{-2}$ M). The H-3′ proton of the ribose residue of Ia also showed a downfield shift (0.018 ppm). The changes for all the other protons were marginal. Formation of the complex between α-CyD and Ib exhibited similar changes in the chemical shifts.

In the β-CyD–Ia and –Ib systems, however, there were virtually no chemical shift changes.

On the formation of the complex between α-CyD and Ic, the H-2 and H-3 protons of α-CyD showed small but definite upfield shifts (0.011 and 0.007 ppm).

Absorption Spectroscopy. On the addition of α-CyD to aqueous solutions of Ia or Ib, the absorbance in the 250–290-nm region increased. By use of these changes, the K_d values of the α-CyD–Ia and –Ib complexes were determined to be 3.6×10^{-2} and 5.8×10^{-2} M. The value for the α-CyD–Ia complex is in fair agreement with the value (3.2×10^{-2} M) determined by the kinetic analysis.[14]

In the case of the β-CyD–Ia and –Ib systems, no measurable changes in absorption spectra were perceived at the β-CyD concentration of $(0.0–1.5) \times 10^{-2}$ M. No changes were observed either on the addition of α-CyD to aqueous solutions of Ic or Id.

pH–Rate Constant Profiles for the Cleavages of Ia in the Presence and the Absence of α-CyD. In the pH–rate constant profile for the α-CyD-catalyzed cleavage of Ia, the slope in the pH 10–12 region is considerably smaller than 1.0, showing significant contribution of the water-catalyzed reaction in addition to alkaline hydrolysis. At pH greater than 12, the slope is 1.0. In the absence of α-CyD, the slope is smaller than 1.0 throughout the pH region investigated.

Table III lists the values of k_{OH} and k_{H_2O}, determined from these profiles. The hydroxide ion catalyzed cleavage of Ia in the α-CyD–Ia complex is 18-fold faster than that of free Ia. The water-catalyzed cleavage of Ia is also accelerated (3.1-fold) by the complex formation with α-CyD.

(14) Kinetic determination of the K_d value of the α-CyD–Ib complex was not successful due to the small magnitude of the accelerating effect by α-CyD.

Table IV. Effects of Additives on the α-CyD-Catalyzed Regioselective Cleavage of Ia[a]

additive	concn, 10^{-2} M	rate const,[b] 10^{-4} min^{-1}	selectiv,[b,c] %
4-nitrophenolate	0.5	2.8 (2.3)	69 (65)
	1.2	2.5 (2.0)	62 (57)
tetramethylammonium chloride	2.0	2.8 (2.4)	76 (68)
	5.0	2.6 (2.1)	72 (60)
none		3.4	81

[a] [α-CyD] = 10^{-2} M; pH 11.08, 20 °C. [b] The numbers in parentheses refer to the values evaluated under the assumption that the α-CyD–additive complexes show no regioselective catalyses (see text in detail). [c] IIIa/(IIa + IIIa).

Table V. Rate Constants for the α-CyD-Catalyzed Regioselective Cleavage of Ia[a,b]

parameter	value, 10^{-4} min^{-1}
k_c	10.1
k_{un}	1.7
k_{II}(complex)	0.0 (0.0)
k_{III}(complex)	11.0 (13.8)
k_{II}(free)	0.9 (1.1)
k_{III}(free)	0.8 (1.0)

[a] At pH 11.08, 20 °C. [b] The numers in parentheses show the ratios with respect to k_{III}(free).

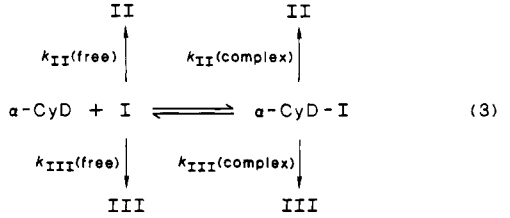
Effects of Competitive Inhibitors on the α-CyD-Catalyzed Regioselective Cleavage of Ia. Both 4-nitrophenolate and tetramethylammonium chloride decrease the rate constant and the selectivity of the α-CyD-catalyzed cleavage of Ia (Table IV), due to competitive inhibition. Quite importantly, the magnitudes of the decrease both in the rate constant and in the selectivity are considerably smaller than the values evaluated by use of the rate constants in Table V under the assumption that the complexes between α-CyD and these competitive inhibitors exhibit no regioselective catalyses.[15] Thus, the α-CyD residue in the α-CyD–competitive inhibitor complex is still active as catalyst, although its cavity is occupied by the inhibitor. This shows that the α-CyD–Ia complex for the present regioselective catalysis is not an "inclusion" complex but rather a hydrogen-bonding one, as described later in the Discussion.

α-CyD-Catalyzed Regioselective Cleavages of Ribonucleotide Dimers. When CpC, CpU, CpA, and CpG were cleaved at pH 11.08, 50 °C in the presence of 0.05 M α-CyD, IIIa was predominantly formed (the selectivities 89, 91, 91, and 91%), together with cytidine, uridine, adenosine, and guanosine, respectively. In its absence, however, the ratio of IIIa to IIa is almost 1:1.

The cleavages were slightly (0.2–0.5-fold) decelerated by α-CyD.

Discussion

Kinetic Analysis of the α-CyD-Catalyzed Regioselective Cleavage of I. The present regioselective cleavages of I proceed via the α-CyD–I complex with concurrent reaction of free I (eq 3). Thus,



(3)

(15) The K_d value of the α-CyD–tetramethylammonium chloride complex was independently determined to be 8.6×10^{-2} M by the competitive inhibition of the α-CyD-catalyzed cleavage of 3-nitrophenyl acetate by the method in the literature (ref 13). For the α-CyD–4-nitrophenolate complex, the value (4.0×10^{-4} M) in the literature was used: Bergeron, R. J.; Channing, M. A.; Gibeily, G. J.; Pillor, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 5146–5151.

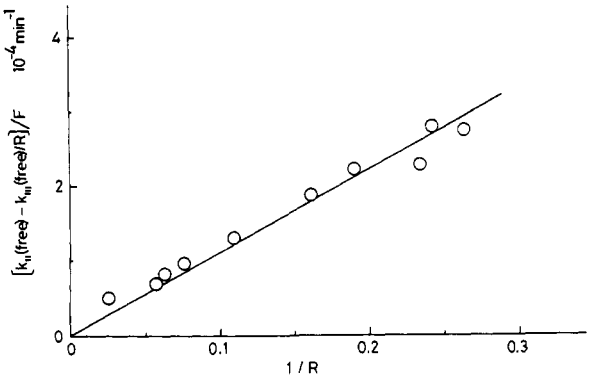


Figure 2. Plot of the relationship of eq 4 for the α-CyD-catalyzed cleavage of Ia at pH 11.08, 20 °C.

the dependence of the ratio (R) of IIIa to IIa in the product on the charged amount of α-CyD for the α-CyD-catalyzed cleavage of Ia has been analyzed by use of eq 4. This equation was

$$[k_{II}(\text{free}) - k_{III}(\text{free})/R]/F = -k_{II}(\text{complex}) + k_{III}(\text{complex})/R \quad (4)$$

previously used by Breslow for the CyD-catalyzed para-selective chlorination of anisole.[16] Here, F is the ratio of the equilibrium concentration of the α-CyD–Ia complex to that of free Ia and is evaluated by use of the kinetically determined K_d value (3.2×10^{-2} M). The k's are the rate constants for the four reactions in eq 3.

As depicted in Figure 2, the plot of the data in Figure 1 according to eq 4 gives a fairly straight line. The rate constants determined are listed in Table V. The rate constant k_{III}(complex) for the P–O(2′) cleavage of Ia in the α-CyD–Ia complex is 13.8 times as large as that (k_{III}(free)) of free Ia. In contrast, the P–O(3′) cleavage of Ia is totally suppressed in the α-CyD complex (the k_{II}(complex) is zero).

The fair agreement of the sum (11.0×10^{-4} min^{-1}) of k_{II}(complex) and k_{III}(complex) with k_c (10.1×10^{-4} min^{-1}), each of which is determined by independent plot, confirms the validity of the present kinetic analysis.

The selective cleavages of the ribonucleotide dimers CpC, CpU, CpA, and CpG to IIIa are attributable to regiospecific cleavage of Ia, formed as intermediate by the intramolecular attack of the 2′-hydroxyl group of the cytidine residue at the phosphorus atom in the ribonucleotide dimers. Here, the rate-determining steps are the formation of Ia, for which α-CyD shows deceleration.

The catalytic activity of α-CyD was unchanged even after treatment in vacuo at 120 °C for 1 week. Thus, a possibility that the present catalyses are simply due to contaminating ribonuclease is ruled out.

Structure of the α-CyD–I Complex. The significant upfield shifts for the H-2 and H-3 protons of α-CyD on complex formation with Ia indicate that the secondary hydroxyl groups of α-CyD form hydrogen bonds as proton donors and thus electron densities on these hydrogen atoms are increased. Both these protons and the secondary hydroxyl groups are directly attached to the C-2 and C-3 carbon atoms. The upfield shifts for the H-1 and H-4 protons, which are on the adjacent carbon atoms, are ascribed to through-chain transfer of the effects from the C-2 and C-3 carbon atoms.

On the other hand, the cytosine residue of Ia functions as proton acceptor in the complex formation, as shown by the downfield shifts for the H-5 and H-6 protons.[17] Furthermore, the downfield shift for the H-3′ proton of the ribose residue of Ia indicates that the adjacent O(3′) atom also functions as proton acceptor for hydrogen bonding.

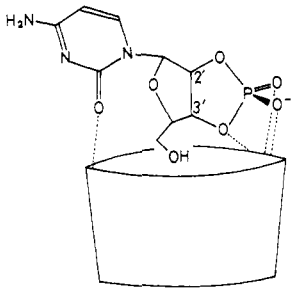


Figure 3. Proposed structure of the α-CyD–Ia complex. The dotted lines show the hydrogen bondings between Ia and the secondary hydroxyl groups of α-CyD (see text in detail).

In aqueous solutions, Ia and Ib overwhelmingly take syn conformations with respect to the rotation of the N-glycosyl bonds, as definitely shown by previous NMR studies.[18]

Thus, the structure of the α-CyD–Ia complex is proposed as depicted in Figure 3. The phosphate residue of Ia (at the O(1) and O(2) atoms) forms hydrogen bonds with the secondary hydroxyl group of α-CyD, and the oxygen atom at the C-2 carbon of the cytosine residue forms another hydrogen bond with the secondary hydroxyl group of the farthest glucose residue. The plane of the cyclic phosphate residue of Ia is almost parallel to the longitudinal axis of the cavity of α-CyD. As a result, the cavity of α-CyD is flexibly capped by Ia at the secondary hydroxyl side.[19] The structure is stabilized by the cooperation of several types of hydrogen bonds.

According to a CPK molecular model study, the proposed complex is formed without steric repulsion. The lack of significant changes of the chemical shifts of the H-5 and H-6 protons of the α-CyD residue on complex formation is consistent with the structure. The α-CyD–Ib complex has a structure similar to the α-CyD–Ia complex.

A possibility that the cytosine or uracil residue of Ia or Ib is deeply included in the cavity is ruled out by the ^{1}H NMR spectroscopy. The chemical shifts of the H-1, H-2, and H-4 protons of α-CyD are hardly affected by the anisotropic shielding effects of the guest compounds included in the cavity, since they are on the exterior of the cavity.[12a]

The above arguments are further confirmed by virtually no chemical shift changes for the β-CyD–Ia system. The cavity of β-CyD is so large and thus the farthest glucose residues are so far away from each other that a complex such as the proposed structure cannot be formed. When a glucose unit other than the farthest one is used, severe steric repulsion occurs.

Still more concrete evidence for the proposed structure is provided by the results of the competitive inhibition experiments (Table IV). The α-CyD residue in the α-CyD–4-nitrophenolate or –tetramethylammonium chloride complex can function as catalyst, since its cavity is not essential for the complex formation with Ia. If the regioselective catalysis involved deep penetration of Ia in the cavity, the catalytic activity would be totally inhibited by the inhibitors.

The increase in the absorbance of Ia or Ib in the 250–290-nm region on complex formation with α-CyD is associated with formation of the hydrogen bond between the C-2 oxygen atom of the cytosine or uracil residue and the secondary hydroxyl groups of α-CyD. In the case of β-CyD, the hydrogen bonding is less efficient due to poor cooperation of the hydrogen bondings, resulting in no measurable change in the absorption spectra.

The α-CyD–Ic and –Id complexes probably involve the hydrogen bondings between the phosphate groups and the secondary

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hydroxyl groups of α-CyD as well as apolar interaction between the purine residue of I and the cavity of α-CyD. The upfield shifts for the H-2 and H-3 protons of α-CyD on complex formation are consistent with this argument.

Effective formation of the hydrogen-bonding complexes between α-CyD and I in water, a hydrogen-bonding solvent, is ascribed to the considerably small pK_a value (~12)[12a] of the secondary hydroxyl groups of α-CyD. The proton-donating ability of these hydroxyl groups is so large that α-CyD forms complexes with I without significant competition with water. Otherwise, use of non-hydrogen-bonding solvent such as chloroform is absolutely required, as is the case for most of the hydrogen-bonding host compounds previously studied.[20]

Methanism of the Regioselective Catalysis by α-CyD. The regioselective catalyses by α-CyD are definitely attributable to the discrimination between the P–O(2′) bonds of I's and the P–O(3′) bonds by the formation of the α-CyD–I complexes. In the complexes, the cleavage of the P–O(3′) bond from the pentacoordinate intermediate is quite unfavorable, since it is accompanied by the increase of the distance between the phosphate residue and the secondary hydroxyl groups of α-CyD in the course of the reaction (see Figure 3), and thus the transition state is destabilized by the suppression of the hydrogen bonding between them. In the cleavage of the P–O(2′) bond, however, the distance decreases with the progress of the reaction, resulting in the efficient reaction. Alternatively, the selective P–O(2′) cleavage is associated with catalysis by the secondary hydroxyl groups of α-CyD, which promotes the attack of hydroxide ion or water at the phosphorus atom of I from the backside of the P–O(2′) bond. As a result, the P–O(2′) bond is selectively cleaved by the "in-line" mechanism, in the same way as the P–O(2′) fission in the ribonuclease-catalyzed cleavages of ribonucleic acids.[3,21]

Covalent catalysis by α-CyD, which involves the formation of a covalent intermediate by the nucleophilic attack of the secondary alkoxide ion of α-CyD at the phosphorus atom of I,[12] is unlikely, since the rate of disappearance of I was exactly identical with the total rate of the appearance of II and III.

No catalytic effect of hexa-2,6-dimethyl-α-CyD definitely supports the proposed mechanism, which involves an essential role of the secondary hydroxyl groups of α-CyD. No regiospecific catalyses by β- and γ-CyDs also agree with the mechanism, since it requires a strict regulation of stereochemistry of the α-CyD–Ia and –Ib complexes for effective catalysis.

In the cleavage of Ic and Id, the regioselectivities are smaller than the values for Ia and Ib, since the C-2 oxygen atoms, which are important for the complex formation of α-CyD with Ia or Ib, are absent here.

The α-CyD-catalyzed regioselective cleavage of Ia at pH 11.08 proceeds via both the attack of hydroxide ion (65%) at the phosphorus atom and the attack of water (35%). The values in parentheses are estimated by use of the k_{OH}(complex) and k_{H_2O}(complex) values in Table III.

The 18-fold larger value of k_{OH}(complex) than of k_{OH}(free) (Table III) is ascribed to the formation of the hydrogen bond between the phosphate residue of Ia and the secondary hydroxyl group of α-CyD. The negative charge on the phosphate residue is reduced by the hydrogen bonding, and thus the electrostatic repulsion between the phosphate residue and the incoming hydroxide ion is decreased. In addition, the electrophilicity of the phosphorus atom is enhanced by the hydrogen bondings. Thus, the water-catalyzed reaction is also promoted by the complex formation with α-CyD (k_{H_2O}(complex)/k_{H_2O}(free) = 3.1). The smaller magnitude of acceleration effect of α-CyD on the cleavage of Ib is attributable to weaker interaction, as clearly shown by the larger K_d value (5.8×10^{-2} M).

Conclusion

Regiospecific cleavages of Ia–d are achieved by α-CyD as catalyst. The regioselectivities for the pyrimidine nucleotides (Ia and Ib) are larger than those for the purine ones (Ic and Id). These regioselective catalyses are ascribed to the formation of hydrogen-bonding complexes between α-CyD and Ia–d. The complexes are effectively formed in aqueous solutions, since the pK_a of the secondary hydroxyl groups of α-CyD is small. As a result, α-CyD provides a regiospecific reaction field, as the enzyme ribonuclease does. The dimers of ribonucleotides having cytosine residues at the 3′-sides of the cleaved phosphodiester linkages are also regioselectively cleaved.

Acknowledgment. I should like to thank Mr. Tokiharu Kawamura at the University of Tokyo and Mr. Yuichi Takeshige in our laboratory for valuable assistance in the ^{1}H NMR spectroscopy.

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(21) One of the reviewers kindly suggested as another possibility that the fission of the P–O(2′) bond can be facilitated, since the more effective solvation of the O(2′) atom by water enhances the leaving group ability of the corresponding alkoxide ion. Solvation of the O(3′) atom, which is closer to the apolar cavity of α-CyD, is less efficient, resulting in the suppression of the fission of the P–O(3′) bond.