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Note

Regioselective cleavage of ribonucleoside 2′,3′-cyclic monophosphates induced by 6-*O*-α-D-glucopyranosyl- and 6-*O*-α-maltosyl-cyclodextrins (cyclomalto-oligosaccharides)[1]

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Regioselective cleavage of the 2′,3′-cyclic phosphates of adenosine (**1a**), guanosine (**1b**), cytidine (**1c**), and uridine (**1d**) are carried out by use of α- and β-cyclodextrins (cyclomaltohexaose and cyclomaltoheptaose: CyDs) having D-glucopyranosyl or maltosyl branches. 6-*O*-α-D-Glucopyranosyl-β-CyD (G_1-β-CyD) and 6-*O*-α-maltosyl-β-CyD (G_2-β-CyD) promote with high selectivity the cleavage of the P–O–3′ bonds, giving the corresponding 2′-monophosphates. The selectivity of 10.7:1 (ratio 2′-monophosphate/3′-monophosphate) achieved by 0.1M of G_1-β-CyD in the cleavage of **1a** at pH 11.0 and 30° is significantly larger than the largest value (4.6:1) obtained from a saturated solution of β-CyD without the branch. In contrast with the promotion of P–O–3′ bond cleavage by G_1-β-CyD and G_2-β-CyD, 6-*O*-α-D-glucopyranosyl-α-CyD enhances P–O–2′ cleavage. Hydroxypropyl CyD and methyl CyDs show either no catalytic activity or catalytic activity that is much diminished. The introduction of D-glucopyranosyl and maltosyl residues to CyDs is definitely required for effective catalysis.

INTRODUCTION

Ribonuclease cleaves ribonucleic acids to fragments having the terminal phosphates at the 3′-position. Alkaline hydrolyses, on the other hand, provide ~1:1 mixtures of the 3′-terminal fragments and the 2′-terminal fragments. Enzymatic regioselective cleavage is generally ascribed to the selective fission of the P–O–2′ bonds of 2′,3′-cyclic monophosphates of terminal ribonucleotides formed as intermediates[2,3]. While a considerable number of models for this enzyme have been proposed[3], nonenzymatic regioselective cleavage of 2′,3′-cyclic phosphates of ribonucleosides has not yet been achieved[4].

In previous papers[5–7], the regioselective catalysis of ribonuclease has been

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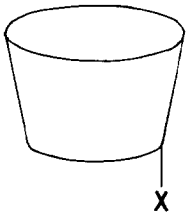
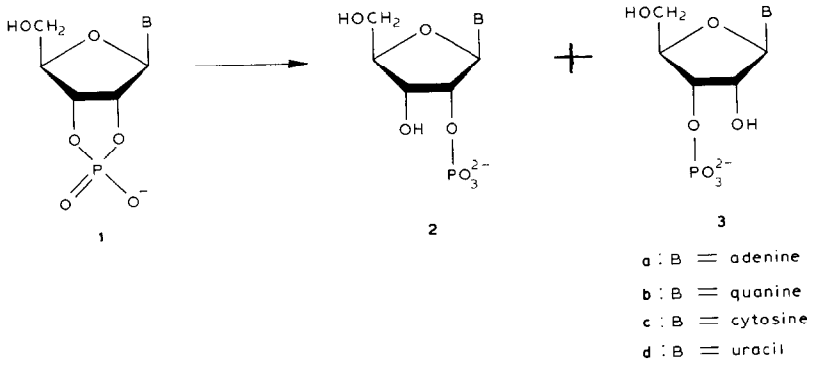


Fig. 1. Structure of the catalysts: for G_1-CyD, X = D-glucopyranosyl; for G_2-CyD, X = maltosyl.

shown to be successfully mimicked by cyclodextrins (cyclomalto-oligosaccharides, CyDs). The P–O–2′ bonds of 2′,3′-cyclic phosphates of ribonucleosides were selectively cleaved by use of α-CyD (cyclomaltohexaose) as catalyst[5], whereas the P–O–3′ bonds were selectively cleaved by β- and γ-CyDs (cyclomaltoheptaose and cyclomalto-octaose)[6]. In addition, the phosphodiester linkages of di- and poly-ribonucleotides were regioselectively cleaved by CyDs[7].

This paper reports the regioselective cleavage of 2′,3′-cyclic phosphates of adenosine (**1a**), guanosine (**1b**), cytidine (**1c**), and uridine (**1d**) induced by branched CyDs having D-glucopyranosyl or maltosyl residues at the O-6 positions[8] (shown diagrammatically in Fig. 1). The catalytic activities of the branched CyDs are compared with those of CyDs without the branches.

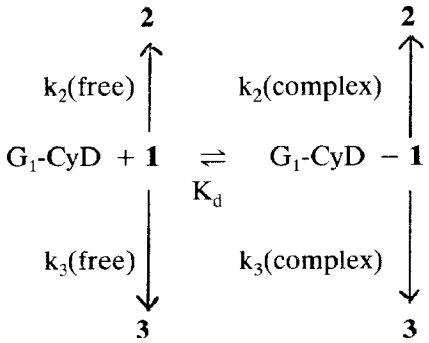


EXPERIMENTAL

Materials. — 6-*O*-α-D-Glucopyranosyl-α-CyD (G_1-α-CyD), 6-*O*-α-D-glucopyranosyl-β-CyD (G_1-β-CyD), and 6-*O*-α-maltosyl-β-CyD (G_2-β-CyD) were kindly supplied by Ensuiko Sugar Refining Co. 6-*O*-(2-Hydroxypropyl)-β-CyD (average number of 2-hydroxypropyl groups per one β-CyD molecule = 2.0) was synthesized by the literature method[9], with an initial molar ratio 3.5:1 of propylene oxide to β-CyD.

Kinetics. — The cleavage of **1a–d** was carried out at pH 11.0 [0.05M hydrogen carbonate buffer, I = 0.1M (KCl)], 30°. The initial concentrations of **1a–d** were around 10^{-4}M. The reaction mixtures were directly subjected to periodic analyses by h.p.l.c. (Merck Lichrospher RP-18 columns). For analysis the cleavage products of **1a** and **1b**, the column length was 25 cm, and the eluent was 97:3 water–acetonitrile (pH 3.8); for analysis of products from the cleavage of **1c** and **1d**, the column length was 50 cm, and the eluent was water (pH 3.8). The reactions satisfactorily demonstrated pseudo-first-order kinetics, and the ratios of the 2′-phosphates (**2**) to the 3′-phosphates (**3**) were constant irrespective of conversion.

The reactions induced by G_1-CyD were analyzed according the processes shown in Scheme 1. Partial rate constants [k_3(complex) and k_2(complex)] for the formation of each of **2** and **3** from the G_1-CyD-**1** complex were determined by use of Eq. *1* (ref. 10),



Scheme 1.

$$[k_3(\text{free}) - k_2(\text{free})/R]/F = -k_3(\text{complex}) + k_2(\text{complex})/R \qquad (1)$$

where k_2(free) and k_3(free) are the rate constants for the formation of **2** and **3** from free **1**. F is the molar ratio of the G_1-CyD-**1** complex to free **1**, and R is the ratio of **3** to **2** in the product mixture.

The equilibrium constant (K_d) for the dissociation of the G_1-CyD-**1** complex was evaluated from the dependence of the observed rate constant of the cleavage on the initial concentration of G_1-CyD by the standard method[11].

RESULTS AND DISCUSSION

Regioselective cleavage of **1** *by* G_1*-CyD and* G_2*-CyD.* — The open circles in Fig. 2 show the dependence of the ratio of the 2′-monophosphate to the 3′-monophosphate (**2a/3a**) on the initial concentration of G_1-β-CyD for the cleavage of **1a**. In the absence of G_1-β-CyD, the formation of **3a** by the cleavage of the P–O–2′ bond is dominant over that of **2a**, and the **2a/3a** ratio is only 0.79. The **2a/3a** ratio increases with increasing concentration of G_1-β-CyD, reaching a value

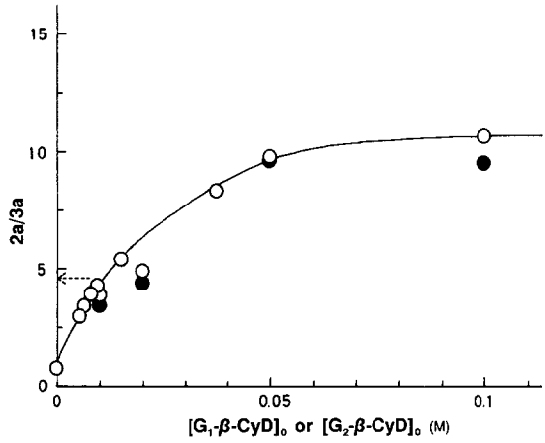


Fig. 2. Dependence of the **2a/3a** ratio (○) on the concentration of G_1-β-CyD for the G_1-β-CyD-induced regioselective cleavage of **1a** at pH 11.0 and 30°. The closed circles (●) show the results for the G_2-β-CyD-induced reactions, and the dotted arrow (----→) refers to the largest **2a/3a** ratio achieved by β-CyD without the branches (see text for details).

of 10.7 at 0.1M. At this point the cleavage is 7.4 times as fast as that observed in the absence of G_1-β-CyD.

G_2-β-CyD was also shown to promote the formation of **2a** by P–O–3′ cleavage (the closed circles in Fig. 2). The **2a/3a** ratio was 9.4 at the concentration 0.1M.

Quite significantly, these regioselectivities achieved by use of G_1-β-CyD and G_2-β-CyD are much larger than the largest value attained by use of β-CyD without the branches. The largest **2a/3a** ratio by β-CyD, which was obtained in the saturated aqueous solution of β-CyD ($[\beta\text{-CyD}]_0$ = 0.016M), was only 4.6, as shown by the dotted arrow in Fig. 2. The P–O–3′ bonds of **1b–d** are also regioselectively cleaved by G_1-β-CyD (Table I).

In contrast, G_1-α-CyD enhanced the cleavage of the P–O–2′ bonds. The ratios of **3/2** in the cleavage of **1a–d** at the concentration 0.1M of G_1-α-CyD were

TABLE I

REGIOSELECTIVE CLEAVAGE OF **1b–d** INDUCED BY G_1-β-CyD AT pH 11.0 AND 30°

Substrate	*Additive*[a]	*Rate constant* ($10^{-4}.min^{-1}$)	**2/3**
1b	G_1-β-CyD	14.6 (9.8)[b]	1.1 (1.0)[b]
	None	7.9	0.85
1c	G_1-β-CyD	8.4	0.85
	None	5.7	0.65
1d	G_1-β-CyD	11.7	0.86
	None	8.0	0.72

[a]$[G_1\text{-}\beta\text{-CyD}]_0$ = 0.1M. [b]The values in parentheses show the results for the reaction catalyzed by 0.015M β-CyD.

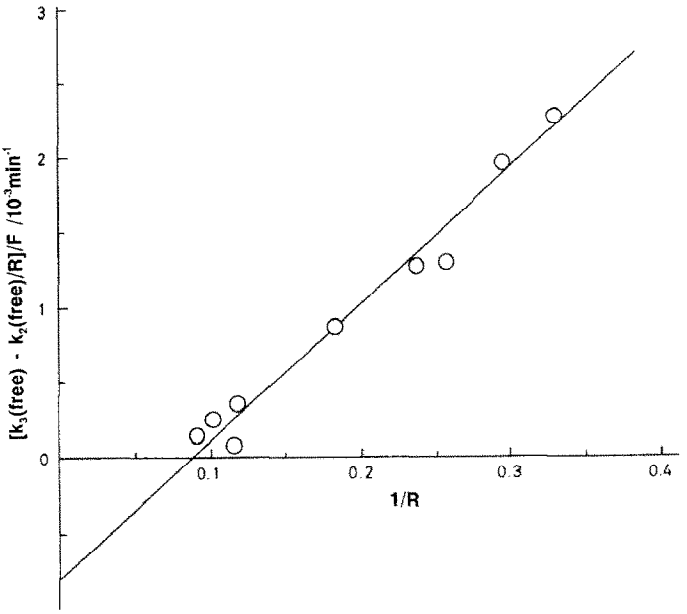


Fig. 3. Plot of $[k_3(free) - k_2(free))/R]/F$ *vs.* 1/R according to Eq. *1* for the G_1-β-CyD-induced regioselective cleavage of **1a** at pH 11.0 and 30°.

2.3, 1.7, 4.0, and 6.7, respectively. The regioselective P–O–2′ cleavage is parallel to the regioselective catalysis shown by α-CyD[5].

Kinetics analysis of regioselective P–O–3′ cleavage of **1a** *by* G_1-β-*CyD.* — The plot of the data in Fig. 2 according to Eq. *1* gave a fairly straight line, as depicted in Fig. 3. From the slope and the intercept of the line, the partial rate constants, k_2(complex) and k_3(complex), for the formation of each of **2a** and **3a** from the G_1-β-CyD-**1a** complex were evaluated (Table II). Formation of **2a** is accelerated 13.1-fold by the complex formation with G_1-β-CyD [k_2(complex)/k_2 (free) = 13.1]. In contrast, the formation of **3a** is decelerated by 7%. As a result, the maximal **2a/3a** ratio for the complex is 11.2.

TABLE II

KINETIC PARAMETERS FOR THE G_1-β-CyD-INDUCED AND THE β-CyD-INDUCED REGIOSLECTIVE CLEAVAGE OF **1a**[a]

Parameter[b]	*Catalyst*	
	G_1-β-CyD	*β-CyD*
k_2(complex)	9.1	7.9
k_3(complex)	0.81	0.45
k_2(free)	0.69	
k_3(free)	0.87	
K_d[c]	1.7	2.6

[a]At pH 11.0 and 30°. [b]In 10^{-3}.min^{-1} unless otherwise noted. [c]In 10^{-2}M.

Functions of the branches in the regioselective catalyses. — The k_2(complex) and k_3(complex) values for the G_1-β-CyD-induced regioselective cleavage of **1a** are rather close to the values for the β-CyD-induced reaction, evaluated also by use of Eq. *1* (Table II). Thus, the larger regioselectivity achieved by G_1-β-CyD is mostly ascribed to its greater solubility. The possibility that the D-glucopyranosyl residue directly participates in the regioselective catalysis is unlikely.

The introduction of the D-glucopyranosyl or maltosyl residues is definitely necessary for the promotion of the regioselective catalysis. As shown in Table III, the catalytic activity of the 6-*O*-(2-hydroxypropyl)-β-CyD was much smaller than that of G_1-β-CyD. Neither heptakis-2,6-di-*O*-methyl-β-CyD nor heptakis-2,3,6-tri-*O*-methyl-β-CyD showed any regioselective catalysis.

Regioselective cleavage by the branched CyDs proceeds via complexes between **1a–d** and the CyD derivatives. The cyclic phosphate residues are apparently located near the secondary hydroxyl groups of the CyD and are regioselectively cleaved due to the interactions with these groups. These arguments are supported by the previous results on the CyD-**1** complexes[5,12]. Thus, the D-glucopyranosyl and the maltosyl residues on the O-6 atoms show no suppression of the regioselective catalysis, since they are highly hydrophilic and do not effectively interact with the apolar cavity.

In the 6-*O*-(2-hydroxypropyl)-β-CyD, however, the methyl groups of the hydroxypropyl residues are believed to competitively suppress the inclusion of the heterocyclic bases of **1**, resulting in the observed decrease in the catalytic activity. Heptakis-2,6-di-*O*-methyl-β-CyD and heptakis-2,3,6-tri-*O*-methyl-β-CyD demonstrate no catalytic activity since the secondary hydroxyl residues of β-CyD are definitely required for the catalysis.

The K_d value for the G_1-β-CyD-**1a** complex is smaller than the value for the β-CyD-**1a** complex (Table II). The guest-binding activity of β-CyD is increased by the introduction of the D-glucose residue.

In conclusion, the regioselective cleavage of the 2′,3′-cyclic phosphates of ribonucleosides is successfully achieved by the D-glucopyranosyl- and maltosyl-derivatized CyDs.

TABLE III

REGIOSELECTIVITIES ACHIEVED BY CyD DERIVATIVES IN THE CLEAVAGE OF **1a** AT pH 11.0 AND 30°[a]

Additive	*Selectivity* (**2a**/**3a**)
6-*O*-(2-Hydroxypropyl)-β-CyD	3.5
Heptakis-2,6-di-*O*-methyl-β-CyD	0.79
Heptakis-2,3,6-tri-*O*-methyl-β-CyD	0.79
G_1-β-CyD	10.7
None	0.79

[a] $[\text{Additive}]_0 = 0.1$M.

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