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REGIOSELECTIVE CLEAVAGE OF DI- AND POLY-RIBONUCLEOTIDES INDUCED BY CYCLODEXTRINS (CYCLOMALTO-OLIGOSACCHARIDES)*,[1]

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ABSTRACT

Cyclodextrins (cyclomalto-oligosaccharides, CDs) induce regioselective cleavage of the 3′,5′-phosphodiester linkages in di- and poly-ribonucleotides at pH 11.08 and 50°. 0.15M Cyclomaltohexaose (α-CD) mediates the cleavage of CpA, CpC, CpG, and CpU to give 96–97% of cytidine 3′-phosphate together with adenosine, cytidine, guanosine, and uridine, respectively. In the absence of α-CD, 50–52% of cytidine 2′-phosphate is formed as by-product. α-CD also promotes the formation of adenosine 3′-phosphate from ApA and ApC, guanosine 3′-phosphate from GpG, and uridine 3′-phosphate from UpU. In contrast, β-CD and γ-CD enhance the formation of the corresponding 2′-phosphates. Regioselective cleavages of poly[A], poly[C], poly[G], and poly[U] are achieved also.

INTRODUCTION

Ribonuclease cleaves[2,3] ribonucleic acids (RNAs; **1**) exclusively to the 3′-phosphorylated fragments **3**. The specificity is ascribed to regioselective cleavage of the P–O-2′ bonds of the 2′,3′-cyclic phosphates (**2**), formed by attack of HO-2′ in **1** on phosphorus. Alkaline hydrolysis of RNAs, however, provides ~1:1 mixtures of **3** and 2′-phosphorylated fragments (**4**).

Several attempts[3] to mimic the function of this enzyme have been made, but the regioselective cleavage of RNAs has not been reported. Breslow *et al.*[4] reported the regioselective cleavage of the cyclic phosphate of 4-*tert*-butylcatechol, using CDs carrying two imidazolyl groups.

In previous papers[5–7], the regioselective cleavage of **2** by ribonuclease was mimicked by cyclodextrins[8] (CDs, cyclomalto-oligosaccharides). α-CD (cyclomaltohexaose) promotes cleavage of the P–O-2′ bonds of the 2′,3′-cyclic phosphates of ribonucleosides, whereas β-CD (cyclomaltoheptaose) and γ-CD (cyclomalto-octaose) enhance the cleavage of the P–O-3′ bonds.

*Dedicated to the memory of Professor Myron L. Bender.

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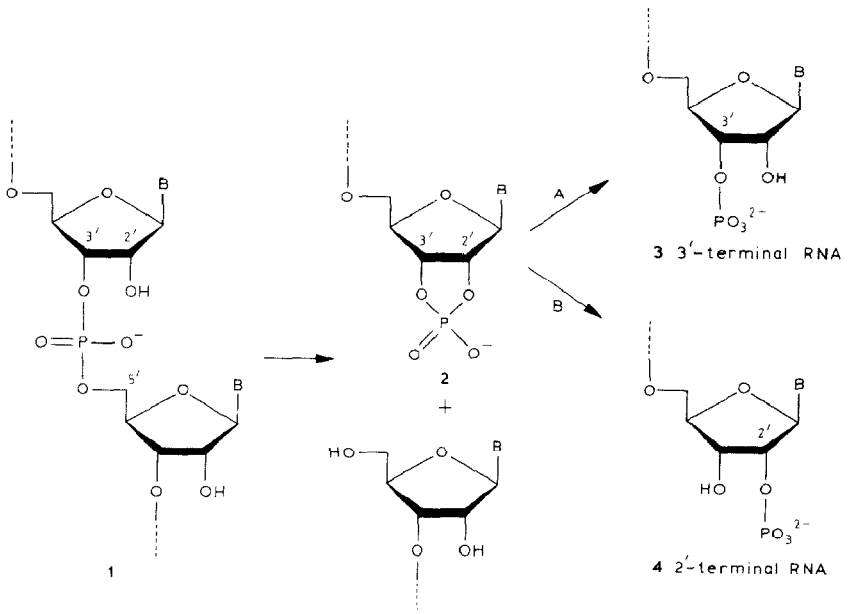
The CD-catalyzed regioselective cleavage of 3′,5′-linked di- and poly-ribonucleotides is now reported, as the first regioselective non-enzymic cleavage.



EXPERIMENTAL

Materials. — The 3′,5′-linked di- and poly-ribonucleotides were purchased from Sigma. CDs were purified by repeated recrystallization from water.

Kinetics. — Cleavage of the di- and poly-ribonucleotides was studied at pH 11.08 and 50°. The initial concentrations were ~0.1mM with respect to the monomeric residues.

The rate constants for the cleavage of the dimers were determined both from the rates of disappearance of the dimers and from the rates of appearance of the products. Both rate constants were similar. All the reactions showed pseudo-first-order kinetics. The disappearance of the dimers as well as the appearance of ribonucleosides was monitored by h.p.l.c. (JASCO $C_{18}S$ column, 25 cm; flow rate, 1.3 mL/min; 100:6 water–acetonitrile acidified with acetic acid for ApA and ApG, and 100:4 water–acetonitrile for the other dimers). The retention times for ApA and adenosine were 33 and 14 min, respectively, whereas the values for adenosine 3′-phosphate and adenosine 2′-phosphate were 6.0 and 7.1 min, respectively.

The appearance of the 2′- and 3′-phosphates of ribonucleosides was followed with higher resolution by h.p.l.c. analyses under the following conditions: for the phosphates of adenosine and guanosine, JASCO $C_{18}S$ column, 25 cm; flow rate, 0.7 mL/min; 100:2 water–acetonitrile (pH 4.2); and for the phosphates of cytosine and uridine, JASCO $C_{18}S$ columns, 50 cm; flow rate, 0.6 mL/min; water (pH 4.2).

Adenosine 2′- and 3′-phosphates showed retention times of 19.4 and 9.9 min, respectively. The regioselectivities of the cleavage of the dimers were determined by these analyses.

In the cleavage of the poly-ribonucleotides, the selectivities [**4**/(**3** + **4**) or **3**/(**3** + **4**)] were evaluated from the ratios of the final products of the cleavages, the ribonucleoside 2′- and 3′-phosphates. The ratios were determined by h.p.l.c. as described above. Control experiments with ribonucleoside 2′- and 3′-phosphates showed that isomerization between the 2′- and the 3′-positions did not take place to a measurable extent. The selectivities were constant irrespective of the extent of the cleavage. Direct determination of the ratio of the 2′- and 3′-phosphorylated fragments by h.p.l.c. was not successful. The distribution of monomer to dodecamer in the cleavage of poly[A] was assessed also by h.p.l.c. according to a literature procedure[9].

RESULTS AND DISCUSSION

CD-Induced regioselective cleavages of ribonucleotide dimers. — The results are shown in Table I. The selectivities refer to the formation of the 3′-phosphates, *i.e.*, adenosine 3′-phosphate from ApC and cytidine 3′-phosphate from CpA.

In the cleavages of CpA, CpC, CpG, and CpU in the presence of 0.15M α-CD, the formation of cytidine 3′-phosphate preponderated over that of the 2′-phosphate. The selectivity was 96–97%, independent of the ribonucleoside on the 5′-side of the bond cleaved. This finding confirms that the present α-CD-catalyzed cleavages are two-step reactions (**1**→**2**→**3**), with the 2′,3′-cyclic phosphate **2** as the intermediate. Regioselective cleavage of the P–O-2′ bond in **2** then gives the 3′-phosphate **3**.

In the absence of α-CD, cleavage of the P–O-3′ bond of cytidine 2′,3′-cyclic phosphate also occurred and 50–52% of the 2′-phosphate **4** was formed.

TABLE I

SELECTIVITIES AND RATE CONSTANTS FOR THE CLEAVAGE OF RIBONUCLEOTIDE DIMERS AT pH 11.08 AND 50° IN THE PRESENCE AND THE ABSENCE OF 0.15M α-CD[a]

Dimer[b]	*Selectivity for the 3′-phosphate (%)*	*Rate constant (10^{-4} min^{-1})*
ApA	80 (51)	1.1 (1.7)
ApC	79 (48)	1.1 (1.4)
CpA	96 (48)	2.5 (4.4)
CpC	96 (49)	1.7 (3.1)
CpG	97 (48)	1.9 (2.5)
CpU	97 (50)	2.2 (3.3)
GpG	71 (50)	1.1 (1.6)
UpU	94 (49)	1.6 (2.8)

[a]The numbers in parentheses refer to the results in the absence of α-CD. [b]See formula **1**.

TABLE II

SELECTIVITIES AND RATE CONSTANTS FOR THE CLEAVAGE OF RIBONUCLEOTIDE DIMERS AT pH 11.08 AND 50° IN THE PRESENCE AND THE ABSENCE OF 0.05M β-CD[a]

Dimer[b]	*Selectivity for the 2′-phosphate (%)*	*Rate constant (10^{-4} min^{-1})*
ApA	89 (49)	2.3 (1.7)
	72[c]	1.1
ApC	88 (52)	1.6 (1.4)
	70[c]	1.2
ApG	89 (50)	1.5 (1.5)
ApU	90 (49)	1.7 (1.4)
CpA	52 (52)	5.1 (4.4)
CpC	51 (51)	2.8 (3.1)
GpG	67 (50)	1.8 (1.6)
UpU	51 (51)	2.6 (2.8)

[a]The numbers in parentheses refer to the results in the absence of β-CD. [b]See formula **1**. [c]0.10M γ-CD used in place of β-CD.

UpU was also cleaved regioselectively by α-CD to give uridine 3′-phosphate, ApA and ApC to give adenosine 3′-phosphate, and GpG to give guanosine 3′-phosphate.

In contrast to the α-CD-mediated formation of the 3′-phosphates, β-CD promoted the formation of the 2′-phosphates (Table II). The selectivity was 88–90% for the cleavages of ApA, ApC, ApG, and ApU. γ-CD also promoted the formation of the 2′-phosphates.

The proposed schemes for the regioselective cleavages are supported by previous results[5–7] that cleavage of the 2′,3′-cyclic phosphates of cytidine and uridine at 20° in the presence of 0.05M α-CD was 99 and 94% selective, respectively, for the 3′-phosphates, whereas the selectivities for the 2′-phosphates for the cleavage of the 2′,3′-cyclic phosphates of adenosine and guanosine were 89 and 66%, respectively, in the presence of 15mM β-CD.

β-CD slightly accelerated the cleavages of the dimers, whereas α-CD and γ-CD showed deceleration. The rate effects are those on the formation of the 2′,3′-cyclic phosphate by intramolecular attack of HO-2′ on phosphorus, which is rate-determining. The regioselectivities, however, are determined by the cleavage of the cyclic phosphates.

CD-Induced regioselective cleavages of polyribonucleotides. —The results are shown in Table III. In the presence of 0.15M α-CD, poly[C] yielded 97% of the 3′-terminal poly[C] fragments **3**. Likewise, poly[U], poly[A], and poly[G] were cleaved to the corresponding 3′-terminal fragments **3**. In contrast, the 2′-terminal fragments **4** were formed from poly[A] and poly[G] by use of β-CD as catalyst. These regioselectivities are virtually identical with those for the dimers (Tables I and II).

The regioselective reactions catalyzed by α-CD, to give the 3′-terminal

TABLE III

SELECTIVITIES FOR THE CD-CATALYZED CLEAVAGES OF POLYRIBONUCLEOTIDES AT pH 11.08 AND 50°

Polymer[a]	*Selectivity (%)*[b]	
	3'-Terminal fragment by α-CD (0.15M)	*2'-Terminal fragment by β-CD (0.05M)*
Poly[A]	77 (51)	86 (49)
Poly[C]	97 (48)	52 (52)
Poly[G]	68 (50)	61 (50)
Poly[U]	97 (49)	49 (51)

[a]See formula **1**. [b]The numbers in parentheses show the selectivities in the absence of CDs.

fragments **3**, parallel the action of ribonuclease. In the CD-catalyzed cleavages of the polyribonucleotides, the phosphoric diester bonds cleaved are distributed randomly along the polymer chain, as assessed by h.p.l.c. which can distinguish fragments in the range from monomer to dodecamer.

Proposed mechanism of CD-induced regioselective cleavages. — The remarkable dependence of the regiospecificity on the number of glucose residues in the CD is ascribed to the differences in the structures of the complexes between the 2',3'-cyclic phosphates of the terminal ribonucleotides **2** and the CDs.

In the complex between α-CD and **2**[7], the cyclic phosphate moiety is hydrogen-bonded to the secondary hydroxyl groups of the α-CD; in addition, the O-2 of C or U of the terminal ribonucleoside of **2** interacts with the secondary hydroxyl groups of the farthest glucose residue. The five-membered ring of the cyclic phosphate is located at the top of the cavity with the plane nearly parallel to its longitudinal axis, and with the P–O-3' bond closer to the cavity than the P–O-2' bond, so that the latter bond is cleaved selectively.

For β-CD[6,10], the base of the terminal ribonucleoside of **2** is included in the cavity of β-CD, and the cyclic phosphate residue interacts with its secondary hydroxyl groups. The five-membered ring of the cyclic phosphate locates with the P–O-2' bond closer to the cavity, so that the P–O-3' bond is cleaved preferentially. The cavity of β-CD is sufficiently large and apolar to accommodate adenine and guanine residues.

The proposed mechanisms are based mainly on previous results[6,7,10] obtained using ^{1}H-n.m.r. spectroscopy and competitive inhibition of the CD-induced regioselective cleavages of the 2',3'-cyclic phosphates of ribonucleosides. The regioselectivities in these cleavages are almost identical with those of the CD-induced cleavages of the polyribonucleotides (shown in Table III).

The hydroxyl groups of the CDs are essential for the regioselective catalysis. Hexakis(2,6-di-*O*-methyl)-α-CD and heptakis(2,6-di-*O*-methyl)-β-CD, and heptakis(-2,3,6-tri-*O*-methyl)-β-CD have virtually no catalytic activity. The pK_a of the secondary hydroxyl groups is rather low (~12)[8], and thus the hydrogen bonds with **2** are formed efficiently even in aqueous solutions.

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