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SHORT COMMUNICATIONS

Cyclodextrin–Catalyzed Regioselective Cleavage of Adenylyl(3′→5′)adenosine

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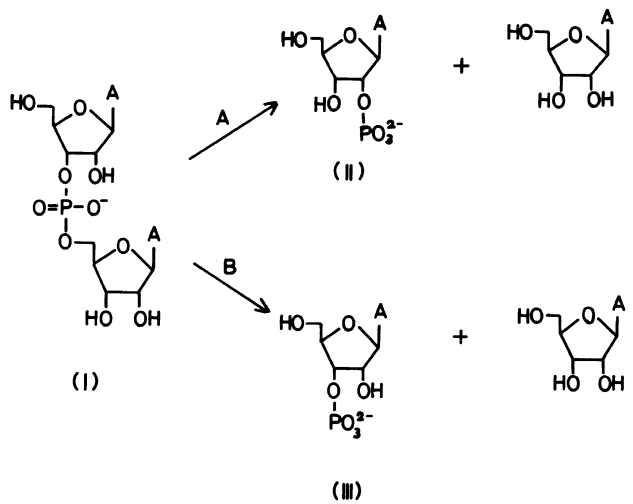
Ribonuclease cleaves ribonucleic acids (RNA) into fragments having phosphate residues at specific positions (usually the 3′-positions) of the terminal nucleotides.[1] Many attempts to mimic this enzyme have been made.[2,3] However, regiospecific cleavage of RNA by artificial system has not been accomplished yet. Non-enzymatic hydrolysis of RNA yields almost equimolar mixtures of RNA fragments having phosphate residues at the 3′- positions and those having residues at the 2′-positions.

This communication reports the regiospecific cleavage of adenylyl (3′→5′)adenosine (I) by use of cyclodextrins (CyDs) as catalysts,[4] which exactly mimics the specific function of ribonuclease. Either adenosine 2′-monophosphate (II) or adenosine 3′-monophosphate (III) is selectively formed, together with adenosine, depending on the CyD used (see Scheme 1).

EXPERIMENTAL

The cleavage of I was achieved at 50°C in pH 11.08 bicarbonate buffer (I=0.1 M). The



Scheme 1.

initial concentration of I was 1×10^{-4} M. The reaction was followed by periodical analysis with HPLC (JASCO $C_{18}S$ column, 20 cm; eluent 94 : 6 water–acetonitrile mixture).

RESULTS AND DISCUSSION

Table I shows the selectivity (II/(II+III)) for the formation of II in the presence and absence of CyDs.

In the presence of β-CyD, the formation of II by process (A) in Scheme 1 is predominant, giving a selectivity of 85% at a concentration of 2×10^{-2} M. γ-CyD also promotes the formation of II.

In contrast with the enhancement of the formation of II by β- and γ-CyDs, α-CyD promotes the formation of III by process (B). The selectivity for the formation of III is 77% at a concentration 10^{-1} M of α-CyD. Thus, the regiospecificity of the catalysis is remarkably dependent on the CyD used.

In the absence of CyDs, the formation of II and III takes place at almost the same rate, and the selectivity for the formation of II is 49%.

The present specific catalyses by CyDs are ascribed to the regiospecific cleavage of the adenosine 2′,3′-cyclic monophosphate (IV), which is formed as an intermediate by the intramolecular attack of the 2′-hydroxyl residue in the first adenosine residue of I to the phosphorus atom. In fact, the cleavage of IV at 20°C in the presence of 10^{-2} M of α-, β-, and γ-CyDs gave the selectivity (for the formation of II) of 32, 86, and 63%, respectively. The regiospecificity in the hydrolysis of RNA by ribonuclease is also due to the specific cleavage of the 2′,3′-cyclic monophosphate of the terminal nucleotide.[1,2] CyDs provide a regiospecific reaction field as also does the enzyme.

The regiospecific reactions proceed *via* complexes between CyDs and IV. β- and γ-CyDs include the adenine residue of IV into their cavities,[5] and thus the O(2′) oxygen atom of IV is located in the vicinity of the secondary hydroxy groups of CyDs. The hydrogen bonding interaction between them makes the O(2′) more electropositive, resulting in the more feasible cleavage of the O(3′)–P bond than that of the O(2′)–P bond. According to the preference rule,[2] the more electronegative ligand (O(3′)) leaves from the pentacoordinate intermediate, formed by the attack of water or hydroxide ions to the phosphorus atom, more efficiently than the more electropositive ligand (O(2′)).

Table I. Selectivity (II/(II+III)) for the formation of II in the cleavage of I in the presence and absence of CyDs

CyDs	Concentration	Selectivity	Rate constant
	10^{-1} M	%	10^{-4} min^{-1}
α	1.0	23	1.1
β	0.2	85	2.1
γ	1.0	72	1.1
None	—	49	1.7

α-CyD forms a hydrogen-bonding complex with IV rather than an inclusion complex, since the adenine residue is too bulky to be accommodated in its cavity. There, the secondary hydroxyl groups of a glucose residue interact with the phosphate residues of IV. In addition, the secondary hydroxyl groups of the glucose residue, which is the farthest from the first one, probably interact with the N-7 or N-3 atoms of IV. A molecular model study indicates that under these circumstances the attack of water or hydroxide ions at the phosphorus atom can occur only from the back side of the P–O(3′) bond, forming III by "in-line" mechanism.[2]

In conclusion, regiospecific cleavage of a ribonucleotide dimer, adenylyl(3′→5′)adenosine, has been successfully achieved by use of CyDs as catalysts. A detailed study on the reaction mechanism is currently under way.

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