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Stereospecific Cleavage of Adenosine 2',3'-Cyclic Monophosphate Catalyzed by Cyclodextrins

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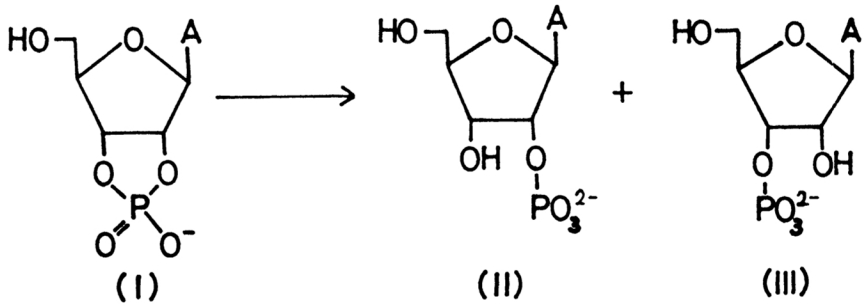
Stereospecific cleavage of adenosine 2',3'-cyclic monophosphate to the 2'-monophosphate is achieved at pH 11.08, 20 °C by use of β- and γ-cyclodextrins as catalysts. The 2'/3' ratio for the product is 7.7 at the concentration 1.5×10^{-2} mol dm^{-3} of β-cyclodextrin. In contrast, α-cyclodextrin promotes the formation of the 3'-monophosphate, giving the product 2'/3' ratio 0.49 at the concentration 1.0×10^{-2} mol dm^{-3}.

Ribonucleases cleavage ribonucleic acids, producing the polynucleotide fragments having phosphate residues at the specific positions (usually at the 3'-positions) of the terminal nucleotides.[1,2] The reactions proceed via the formation of intermediates, 2',3'-cyclic monophosphates, by the intramolecular attack of 2'-hydroxyl residues, followed by the stereospecific cleavage of the intermediates.

Many studies to mimic the functions of ribonucleases were made.[2] However, stereocontrol of the cleavages of 2',3'-cyclic monophosphates of nucleosides has not been successful yet.[3]

This paper reports stereoselective cleavage of adenosine 2',3'-cyclic monophosphate (I) catalyzed by cyclodextrins, cyclic oligomers of glucose, to the 2'-monophosphate (II) or the 3'-monophosphate (III) of adenosine (Eq. 1). Marked dependence of the stereospecificity on the kind of cyclodextrin is described.

Cleavage of adenosine 2',3'-cyclic monophosphate was carried out at pH 11.08 (bicarbonate buffer, I = 0.1 mol dm^{-3}), 20 °C, with the initial concentration of



(1)

the substrate 10^{-4} mol dm^{-3}. The reaction mixture was periodically analyzed by HPLC (JASCO $C_{18}S$ column, 10 cm; eluent, 96:4 water-acetonitrile mixture). All the reactions satisfactorily followed the first-order kinetics, and the ratios of the 2'-monophosphate to the 3'-monophosphate for the products (the product 2'/3' ratios) were constant irrespective of the reaction time.

Table 1 shows the pseudo-first order rate constants and the product 2'/3' ratios. In the presence of β-cyclodextrin, the formation of the 2'-monophosphate is predominant, and the product 2'/3' ratio is 7.7 at the concentration 1.5×10^{-2} mol dm^{-3} of β-cyclodextrin. This corresponds to the selectivity 89% for the formation of the 2'-monophosphate. The formation of the 2'-monophosphate is also dominant in the presence of γ-cyclodextrin.

In the absence of the cyclodextrins, however, the formations of the 2'-monophosphate and the 3'-monophosphate take place at almost the same rates, giving the product 2'/3' ratio 0.84.

In contrast with the enhancement of the formation of the 2'-monophosphate by β- and γ-cyclodextrins, α-cyclodextrin promotes the formation of the 3'-monophosphate. At the concentration 10^{-2} mol dm^{-3}, the product 2'/3' ratio is 0.50 (the selectivity 67% for the formation of the 3'-monophosphate). Thus the size of the cavity of cyclodextrin exhibits an overwhelming effect on the stereochemistry of the cleavage of the cyclic phosphate.

The selective catalyses by cyclodextrins are ascribed to the acceleration of the formation of II or III, together with the slight suppression of the formation of the counterpart. For all the cyclodextrins, the plot of either the product 2'/3' ratio or the rate of cleavage vs. the concentration of cyclodextrin shows saturation at large concentrations, indicating the involvement of the complexes between adenosine 2',3'-cyclic monophosphate and cyclodextrins in the catalyses.

Table 1. Cleavages of adenosine 2',3'-cyclic monophosphate in the presence and the absence of cyclodextrins[a)]

Additive	Rate constant[b)]/10^{-4} min^{-1}		2'/3' Ratio
	2'	3'	
α-Cyclodextrin	1.4	2.8	0.50
β-Cyclodextrin	11.3	1.8	6.3
	13.1[c)]	1.7[c)]	7.7[c)]
γ-Cyclodextrin	2.9	1.7	1.7
Hexa-2,6-dimethyl-α-cyclodextrin	0.9	1.1	0.82
Hepta-2,6-dimethyl-β-cyclodextrin	0.6	0.7	0.86
Hepta-2,3,6-trimethyl-β-cyclodextrin	1.3	1.5	0.87
None	1.6	1.9	0.84

a) At pH 11.08, 20 °C. The concentrations of the additives are 10^{-2} mol dm^{-3} unless otherwise noted.

b) The rate constants for the formation of II and III.

c) The concentration of β-cyclodextrin is 1.5 x 10^{-2} mol dm^{-3}.

In the complexes of adenosine 2',3'-cyclic monophosphate with β- and γ-cyclodextrins, the adenine moiety penetrates in the cavity.[4)] A CPK molecular model study indicates that the secondary hydroxyl groups of the cyclodextrins are located in the vicinity of the 2'-oxygen atom of adenosine 2',3'-cyclic monophosphate so that hydrogen bonds are formed between them. These interactions decrease the electron density at the 2'-oxygen atom, 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" in the pseudo rotation,[5)] more electropositive ligands occupy the equatorial position of the pentacoordinate phosphorus intermediate, and

α-Cyclodextrin probably includes the 5'-hydroxymethyl residue of adenosine 2',3'-cyclic monophosphate, since its cavity is too small to accommodate the adenine residue. Here, hydrogen bonds are formed between the secondary hydroxyl residues of α-cyclodextrin and the 2'-oxygen atom of the substrate, promoting the cleavage of the O(2')-P bond with respect to the cleavage of the O(3')-P bond.

These arguments are supported by the fact that hexa-2,6-dimethyl-α-cyclodextrin, hepta-2,6-dimethyl-β-cyclodextrin, and hepta-2,3,6-trimethyl-β-cyclodextrin show virtually no effects on the product 2'/3' ratio (see Table 1). The secondary hydroxyl groups having rather low pK_a (around 12)[6] are absolutely required for the stereospecific catalysis.

In conclusion, stereospecific cleavages of adenosine 2',3'-cyclic monophosphate are successfully effected by use of cyclodextrins as catalysts. Detailed analysis of the reaction mechanism is currently under way.

References

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5) Ref. 2, p. 113.

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