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Regioselective P-O(2') Cleavage of Cytidine 2',3'-Cyclic Monophosphate Catalyzed by α-Cyclodextrin

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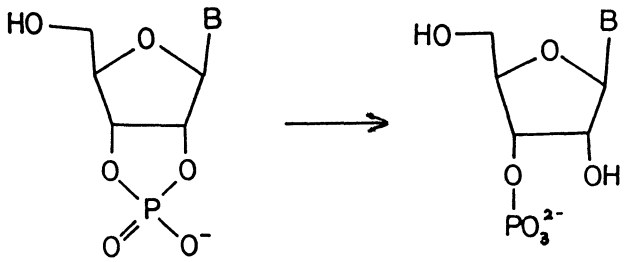
Regioselective cleavage (selectivity 98%) of the P-O(2') bond of cytidine 2',3'-cyclic monophosphate is carried out at pH 11.08, 20 °C by use of α-cyclodextrin as catalyst. Without α-cyclodextrin, the selectivity is only 47% due to concurrent cleavage of the P-O(3') bond. The regioselective catalysis is ascribed to the formation of a complex between α-cyclodextrin and the monophosphate.

Mimicing the functions of ribonucleases, which proceed via 2',3'-cyclic monophosphate of polynucleotides as intermediates, has been widely attempted.[1-4] However, regioselective cleavage of the P-O(2') bonds of the intermediates, one of the most important specificities of this enzyme, has not been sufficiently accomplished by any of the artificial systems.[5]

Quite recently,[6] the present author showed that cyclodextrins (CyDs), cyclic oligomers of glucose,[7] exhibit regioselective catalyses in the cleavage of 2',3'-cyclic monophosphate of adenosine.

This paper reports the regioselective P-O(2') cleavage of cytidine 2',3'-cyclic monophosphate (1) to cytidine 3'-monophosphate (2) (Eq. 1), catalyzed by α-CyD. Quite a high regioselectivity (98%) is achieved. Furthermore, a marked dependence of the regiospecific catalysis on the kind of CyD is shown.

The cleavage of 1 was achieved at pH 11.08 (bicarbonate buffer, 1 = 0.1 mol dm^{-3}), 20 °C. The reaction mixture was periodically analyzed by HPLC (JASCO $C_{18}S$ column, 30 cm; eluent, water).



(1)

1 2 B: cytosine

Table 1 shows the ratio of 2 to cytidine 2'-monophosphate (3) as a byproduct in the presence and the absence of α-CyD, as well as the first-order rate constants for their formation. In the absence of α-CyD, the formation of 3 by the cleavage of the P-O(3') bond takes place more efficiently than that of 2 (the cleavage of the P-O(2') bond), giving the 2/3 ratio only 0.89 (the selectivity 47% for the formation of 2).

The rate of the formation of 2 largely increases with increase in the concentration of α-CyD. In contrast, the rate of the formation of 3 gradually decreases. As a result, the 2/3 ratio remarkably increases with increasing concentration of α-CyD, attaining 49 at the concentration 5 x 10^{-2} mol dm^{-3}. This corresponds to the selectivity 98% for the formation of 2. The asymptotical increase of the 2/3 ratio and the rate of formation of 2 with the concentration of α-CyD shows that the reactions proceed via complex between 1 and α-CyD.

Table 1. Selectivity and rate constants for the cleavage of 1 in the presence and the absence of α-CyD[a)]

$[\alpha\text{-CyD}]_0/10^{-2}$ mol dm^{-3}	Rate constant[b)]$/10^{-4}$ min^{-1}		2/3
	2	3	
0.0	0.82	0.92	0.89
1.0	3.0	0.38	7.9
2.0	4.8	0.25	19
5.0	6.9	0.14	49

a) At pH 11.08 (bicarbonate buffer), 20 °C.

b) The rate constants for the formation of 2 and 3.

In contrast with the remarkable regiospecific catalysis by α-CyD, β- and γ-CyDs show no measurable effects on the 2/3 ratio. The ratios in the presence of 0.01 mol dm^{-3} of β- and γ-CyDs were 0.82 and 0.89.

According to a preliminary ^{1}H-NMR spectroscopy, the signals for the H-1, the H-2, the H-3, and the H-4 protons of α-CyD shift toward higher magnetic field on the complex formation of α-CyD with 1 (the ratio, 0.5:1.0:0.7:0.4). The changes in the shifts for the H-5 and the H-6 protons of α-CyD are minimal. The large upfield shifts for the H-2 and H-3 atoms, which are on the C-2 and the C-3 carbon atoms having the secondary hydroxyl groups, indicate that these secondary hydroxyl groups are interacting with 1 in the complex. Probably, the negatively charged phosphate of 1 forms hydrogen bonds with the secondary hydroxyl groups of α-CyD, whereas the C-2 carbonyl residue of the cytosine group forms another hydrogen bond with the secondary hydroxyl group of the glucose residue at the other side of the cavity (see Fig. 1). The plane of the five-membered ring of the cyclic monophosphate residue lies almost paralell to the longitudinal axis of the cavity. The upfield shifts for the H-1 and the H-4 protons of α-CyD, which are on the carbon atoms adjacent to the C-2 and C-3 carbon atoms, are consistent with this structure.

A possibility that the changes in the chemical shifts for α-CyD are due to anisotropic shielding effect by 1, included in the cavity, is ruled out, since the H-1, the H-2, and the H-4 protons are directing toward the outside of the cavity and hardly experience the anisotropic shielding effect.[7a)]

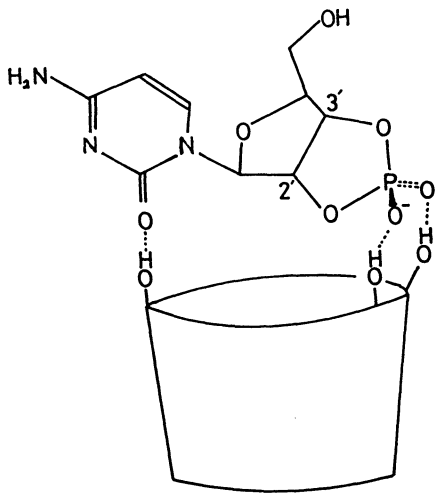


Fig. 1. Proposed structure of the complex between 1 and α-CyD.

A CPK molecular model study has shown that in the α-CyD-1 complex of the proposed structure, the attack by water as well as hydroxide ion at the phosphorus atom of 1 can proceed only from the back side of the O(2') atom. The attack from the back side of the O(3') atom is sterically hindered by the glucose residues of α-CyD. The P-O(2') bond is selectively cleaved by "in-line" mechanism.[1,2] The acceleration effect of α-CyD is associated with the promotion of nucleophilicity of the phosphorus atom by the hydrogen bonding between the phosphate and α-CyD.

The significant role of the hydrogen bond between the C-2 carbonyl residue of 1 and the secondary hydroxyl group of α-CyD for the effective catalysis is confirmed by the fact that the regioselectivity (89%) by 0.01 mol dm^{-3} of α-CyD for the P-O(2') cleavage of 1 is much larger than that (67%)[6] for the cleavage of 2',3'-cyclic monophosphate of adenosine. No catalytic effects of β- and γ-CyDs on the cleavage of 1 are consistent with the proposed mechanism. Here, the farthest secondary groups are so far away from each other that the complex depicted in Fig. 1 can not be formed. More hydrophilic property of cytosine residue than adenine residue prevents effective formation of "inclusion" complex with CyDs.

In conclusion, regioselective cleavage of the P-O(2') bond of cytidine 2',3'-cyclic monophosphate is successfully achieved by α-cyclodextrin as catalyst.

References

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