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Water-Soluble Calixarene as the First Man-Made Catalyst for Regioselective Cleavage of Ribonucleoside 2',3'-Cyclic Phosphate

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The P-O(2') bond of cytidine 2',3'-cyclic phosphate, an intermediate in RNA hydrolysis, is regioselectively cleaved at pH 2 by use of water-soluble calix[4]arene-5,11,17,23-tetrasulfonate. This is the first totally man-made mimic for the regioselective catalysis of ribonuclease.

Preparation of artificial ribonucleases has been attracting much interest. Large acceleration of RNA hydrolysis was achieved by elegantly designed catalysts.[1-4] However, another feature of the enzyme, regioselective P-O(2') cleavage of ribonucleotide 2',3'-cyclic phosphate as intermediate, has not been mimicked by non-enzymatic systems.[5,6] Recently,[7] the authors succeeded in the first non-enzymatic regioselective cleavage of 2',3'-cyclic phosphates of ribonucleosides and ribonucleotides by use of cyclodextrins, naturally occurring cyclic oligosaccharides, as catalysts. This paper reports regioselective cleavage of the 2',3'-cyclic phosphates by totally synthetic cyclic host compounds, water-soluble calixarenes (see Fig. 1).[8]

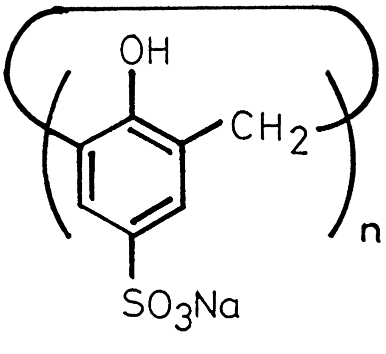

Fig. 1. Structures of water-soluble calix[n]arenes.

Hydrolysis of 2',3'-cyclic phosphates of cytidine, uridine, adenosine, and guanosine (C>p, U>p, A>p, and G>p) in the presence of water-soluble calixarenes, calix[n]arene-4-sulfonates,[9] was followed by HPLC.[7] The initial concentration of the cyclic phosphate was 10^{-4} mol dm^{-3}. All the reactions showed pseudo first-order kinetics, and regioselectivity (the ratio of ribonucleoside 3'-phosphate to the 2'-phosphate) was constant irrespective of conversion.

Table 1 shows the results of hydrolysis of ribonucleoside 2',3'-cyclic phosphates at pH 2. Regioselective P-O(2') cleavage of C>p to cytidine 3'-phosphate was successfully achieved by use of calix[4]arene as catalyst. The 3'/2' ratio is 2.7 at 30 °C, and is 3.5 at 4 °C. Acceleration by the calixarenes is also significant. Both the regioselectivity and the reaction rate monotonously increased with increasing concentration of calix[4]arene, exhibiting gradual saturation at large concentration of the calixarene. Apparently, the catalysis involves complex formation between the calixarene and C>p prior to chemical transformation. Lineweaver-Burk plot of $1/(k_{obsd} - k_{un})$ vs. $1/[\text{calix[4]arene}]_0$ gave a fairly straight line, from which the equilibrium constant K_d for dissociation of the calix[4]-arene-C>p complex was determined to be 7.0×10^{-3} dm^3 mol^{-1}; k_{obsd} and k_{un} are the rate constants in the presence and absence of the calixarene.

Definite requirement of the cyclic structure of calix[4]arene for the regioselective catalysis has been confirmed by the fact that 4-hydroxybenzenesulfonic acid, a monomeric analog, showed no increase in the regioselectivity. Accelerating effect of the analog (0.04 mol dm^{-3}) was only

Table 1. Regioselectivity and reaction rate for the hydrolysis of ribonucleoside 2',3'-cyclic phosphates by use of water-soluble calixarenes [a)]

Substrate	Water-soluble calixarene	Reaction rate 10^{-3} min^{-1}	Regioselectivity (3'/2')
C>p	Calix[4]arene	16	2.7
		2.2 [b)]	3.5 [b)]
	Calix[6]arene	8.9	1.7
	Calix[8]arene	9.3	1.6
	None	2.9	1.5
		0.14 [b)]	1.8 [b)]
A>p	Calix[4]arene	8.9	2.0
	None	3.3	1.7
G>p	Calix[4]arene	6.4	2.0
	None	3.0	1.9
U>p	Calix[4]arene	5.4	1.6
	None	5.3	1.6

a) pH 2, 30 °C; $[\text{calixarene}]_0 = 0.01$ mol dm^{-3}.
b) pH 2, 4 °C.

1/28 of calix[4]arene (0.01 mol dm^{-3}).

Calix[4]arene exhibited regioselective catalysis in the hydrolysis of A>p and G>p, although the effect was small (see Table 1). In the hydrolysis of U>p, however, the activity was none. Electrostatic interaction between calix[4]arene and the substrate is essential for the catalysis. The cytosine residue of C>p is positively charged under the present conditions (the pK_a for protonation of the N-3 is around 4),$^{5)}$ whereas calix[4]arene is negatively charged (one of the hydroxyl groups is deprotonated).$^{10)}$ The adenine residue of A>p (pK_a around 4) and the guanine residue of G>p (pK_a 3.3) are also mostly protonated.$^{5)}$

Complex formation between calix[4]arene and C>p has been definitely evidenced by ^{1}H-NMR spectroscopy. Most of the protons of the ribose residue of C>p shifted toward lower magnetic field (negative sign): chemical shift changes in ppm at $[\text{calix[4]arene}]_0 = [\text{C>p}]_0 = 0.02$ mol dm^{-3} are shown in Fig. 2. The shifts are mostly ascribed to anisotropic shielding effects of the aromatic rings of the calixarene. Accordingly, C>p is sitting at the top of the cavity of the calix[4]arene rather than penetrating into the cavity, as estimated from shielding map of benzene ring $^{11)}$ (see Fig. 2). The chemical shift changes of the methylene and the meta-protons of the calixarene are +0.05 and +0.06 ppm, respectively. In addition to the electrostatic attraction, hydrogen bonding is assumedly operative between the phenolic hydroxyl group of the calixarene and the phosphate residue of C>p. The complex formation largely differenciates the chemical circumstances of the P-O(2') bond and the P-O(3') bond, which are otherwise almost identical with each other, resulting in the selective fission of the P-O(2') bond. The proposed mechanism is consistent with the one for the cyclodextrin-catalyzed regioselective cleavage.$^{7)}$ Totally man-made calixarenes are superior to cyclodextrins with respect to freedom in molecular design for the improvement of regioselectivity.

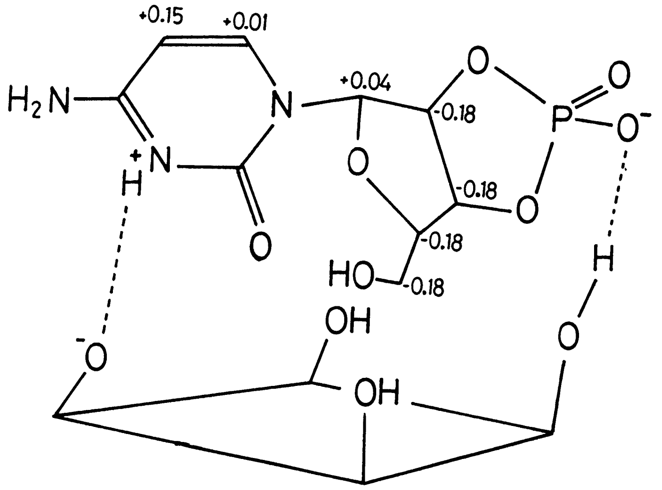

Fig. 2. Proposed structure of calix[4]arene-C>p complex at pH 2: the numbers show the chemical shift changes (- : downfield shift).

The present catalysis by calix[4]arene is the first successful regioselective cleavage of ribonucleoside 2',3'-cyclic phosphate in acidic media by non-enzymatic system: the regioselective catalysis by cyclodextrins was effective only in neutral and alkaline media.7) Marginal regioselective catalysis by calix[6]arene and calix[8]arene is probably associated with rapid exchange between cone, partial-cone, alternate, and other conformations, which suppresses the differentiation between the P-O(2') and the P-O(3') bonds of the cyclic phosphate.8)

In conclusion, ribonucleoside 2',3'-cyclic phosphate as intermediate for RNA hydrolysis is regioselectively cleaved, for the first time, by man-made catalyst calixarene. This finding is significant for the further development of artificial ribonuclease showing both large acceleration and precise regiochemistry. Detailed study on the reaction mechanism as well as promotion of regioselectivity is in progress.

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