$(CH_2)_{10}CH_2CH_2CO$), 4.34 (d, 2 H, J = 4.5 Hz, H-$5_{a,b}$), 5.22 (m, 1 H, H-4), 6.20 (dd, 1 H, J = 5.7, 2.0 Hz, H-3), 7.40 (dd, J = 5.7, 1.5 Hz, H-2); ^{13}C NMR δ 14.08 (q, -, CH_3), 22.65, 24.25, 29.03, 29.16, 29.31, 29.38, 29.59, 31.88, 33.08, and 33.89 (t, +, $-CH_2-$), 62.24 (t, +, C-5), 80.83 (d, -, C-4), 123.25 (d, -, C-3), 152.32 (d, -, C-2), 172.16 (s, +, myristoyl C=O), 173.33 (s, +, C-1). Anal. Calcd for $C_{19}H_{32}O_4$: C, 70.37; H, 9.88. Found: C, 70.32; H, 10.14.

Acknowledgment. The authors thank Dr. John S. Driscoll, Chief of the Laboratory of Medicinal Chemistry, for his constant support and advice. The secretarial help of Mrs. Yetta Buckberg is also appreciated.

Cooperation of Cyclodextrin and Alkali-Metal Halide for Regioselective Cleavage of Ribonucleoside 2',3'-Cyclic Phosphates[1]

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Abstract: Regioselective catalysis of β- and γ-cyclodextrins (β- and γ-CyDs) for the P–O(3') cleavage of adenosine 2',3'-cyclic phosphate (A>p) to adenosine 2'-phosphate is significantly promoted, with respect to both selectivity and reaction rate, by cooperation with NaCl, KCl, RbCl, CsCl, KBr, and KF. A selectivity of 94% is achieved at pH 9.5 and 30 °C by the combination of β-CyD and KCl (3.0 M); the values with β-CyD alone and with KCl alone are 79% and 41%, respectively. By contrast, LiCl and KI reduce the regioselectivity. The logarithm of the 2'-phosphate/3'-phosphate ratio decreases linearly with an increase in the logarithm of the mean ionic activity coefficient of the medium. The α-CyD-induced regioselective P–O(2') cleavage of A>p and U>p to the 3'-phosphates is also enhanced by KCl. The difference in chemical environment of the P–O(2') and P–O(3') bonds provided by CyDs on complex formation with the cyclic phosphates is amplified by the metal salts, resulting in the increase in regioselectivity.

Introduction

Preparation of artificial nucleases has been a most challenging topic. Site-selective fission of deoxyribonucleic acids by oxidative processes was successfully accomplished.[2] Furthermore, artificial systems for efficient hydrolysis of ribonucleic acids (RNAs) were reported.[3-7] However, none of them (except, it is assumed, for the conjugates[7] using natural enzymes as catalytic sites) could mimic the regioselective catalysis of ribonuclease. The enzyme selectively cleaves the P–O(2') bond of 2',3'-cyclic phosphate of ribonucleotide as an intermediate, providing an RNA fragment having the terminal phosphate at the 3'-position.[8]

Breslow reported modified cyclodextrins bearing two imidazolyl residues as elegant models of ribonuclease.[9] Cyclic phosphate of 1,2-dihydroxybenzene, a model compound of the intermediate in RNA hydrolysis, is regioselectively cleaved. Furthermore, the manner of cooperation of the two imidazole residues was investigated in detail by use of precisely modified cyclodextrins.[10]

Recently,[11] we succeeded in regioselective cleavage of 2',3'-cyclic phosphates of ribonucleosides and ribonucleotides by use of cyclodextrins (CyDs) as catalysts.[12] The direction of regioselective catalysis depends largely on the kind of CyD: P–O(2') bonds are selectively cleaved by α-CyD, whereas β- and γ-CyDs enhance P–O(3') cleavage. The regioselective catalysis is ascribed to the formation of a complex between CyD and the cyclic phosphates, in which the P–O(2') and the P–O(3') bonds are differentiated.

The present work reveals that both the selectivity and the reaction rate for the CyD-induced regioselective cleavage of ribonucleoside 2',3'-cyclic phosphates are further increased by cooperation with alkali-metal halides. The cooperative catalysis is kinetically and spectroscopically investigated, and a reaction mechanism is proposed.

Experimental Section

Kinetics. Cleavage of 2',3'-cyclic phosphates of adenosine, guanosine, cytidine, and uridine (A>p, G>p, C>p, and U>p) was carried out at 30 °C and pH 9.5 unless otherwise noted. Reaction mixtures were prepared by addition of metal salts to aqueous carbonate buffer solutions (ionic strength 0.01 M), followed by adjustment of pH when necessary. The rate constant and regioselectivity in the absence of the metal salts were evaluated by extrapolation to zero buffer concentration.

All the reaction vessels and the water were sterilized immediately before use, and special caution was taken throughout the experiments to avoid contamination of ribonuclease and other nucleases.[13] Rate constants of the cleavage as well as ratios (2'/3') of the 2'-phosphate of ribonucleoside to the 3'-phosphate in the products were determined by periodic analysis with HPLC, as described previously.[11] All the reactions

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(13) Absence of contamination of ribonuclease is further confirmed by the preferable formation of adenosine 2'-phosphate in the cleavage of A>p catalyzed by a combination of β- or γ-CyD and the metal halide. The enzyme if any should produce the 3'-phosphate in 100% selectivity.[8]

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showed first-order kinetics, and the 2′/3′ ratios were constant irrespective of reaction time.

Spectroscopy of the Reaction Mixture. ^{1}H NMR spectra were measured in D_2O at 30 °C on a Bruker AM-500 spectrometer with 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid (Na salt) as internal standard. Absorption spectroscopy on a Jasco Ubest-50 spectrometer and circular dichroism spectroscopy on a Jasco J-600 spectrometer were carried out, also at 30 °C.

Equilibrium constant K_T for the dissociation of ternary complex [β-CyD-Cl⁻-A>p], formed in the β-CyD-KCl-A>p or the β-CyD-LiCl-A>p system, was determined by use of the change in absorbance at 260 nm in terms of the following equilibria.

$$\beta\text{-CyD} + Cl^- \underset{K_{Cl}}{\rightleftharpoons} [\beta\text{-CyD-}Cl^-] \quad (1)$$

$$\beta\text{-CyD} + A{>}p \underset{K_B}{\rightleftharpoons} [\beta\text{-CyD-A>p}] \quad (2)$$

$$[\beta\text{-CyD-}Cl^-] + A{>}p \underset{K_T}{\rightleftharpoons} [\beta\text{-CyD-}Cl^-\text{-A>p}] \quad (3)$$

K_{Cl} and K_B are the equilibrium constants for dissociation of the complex between β-CyD and Cl⁻ ion and of the binary complex between β-CyD and A>p, respectively.

The value of K_{Cl} was independently determined by absorption spectroscopy, taking advantage of the competitive inhibition by NaCl of the complex formation between β-CyD and 1-(4′-nitrophenylazo)-2-hydroxy-6-naphthalenesulfonic acid according to the literature.[14] The dye was synthesized by the usual azo coupling technique. The K_B value was obtained previously (0.026 M at 30 °C).[11b]

Under conditions where $[\beta\text{-CyD}]_0 \gg [A{>}p]_0$ and $[KCl \text{ or } LiCl]_0 \gg [A{>}p]_0$, eq 4 holds and, thus, eq 5 is obtained. The subscript 0 refers

$$[\beta\text{-CyD-}Cl^-\text{-A>p}] = [A{>}p]_0\{1/K_T(K_{Cl} + [Cl^-]_0)/[Cl^-]_0 \times 1/[\beta\text{-CyD}]_0 + 1 + K_TK_{Cl}/K_B[Cl^-]_0\} \quad (4)$$

$$[A{>}p]_0/\Delta d = 1/(\epsilon_T - \epsilon_A)\{K_T(K_{Cl} + [Cl^-]_0)/[Cl^-]_0 \times 1/[\beta\text{-CyD}]_0 + 1 + K_TK_{Cl}/K_B[Cl^-]_0\} \quad (5)$$

to the charged concentration, and Δd is the observed change in absorbance. ϵ_T and ϵ_A are the molar absorption coefficients of the ternary complex and A>p, respectively. Concentration of the binary complex [β-CyD-A>p] is sufficiently small under the conditions employed here.[15] The value of K_T was determined by best fitting of the experimental results ($1/\Delta d$ vs $1/[\beta\text{-CyD}]_0$) to eq 5.

Kinetic Analysis of the Regioselective Cleavage of A>p by β-CyD–KCl Combination. Partial rate constants $k_2(c)$ and $k_3(c)$ for the formation of 2′- and 3′-phosphates of adenosine from A>p (P–O(3′) and P–O(2′) cleavage, respectively) in the ternary complex [β-CyD-Cl⁻-A>p] were evaluated by use of eq 6, which was first used by Breslow for the analysis

$$[k_3(f) - k_2(f)/R]/F = -k_3(c) + k_2(c)/R \quad (6)$$

of α-CyD-catalyzed para-oriented chlorination of anisole.[16] F is equilibrium ratio of A>p in the complexing state to that in the free state. R is the ratio of 2′/3′ in the product. $k_2(f)$ and $k_3(f)$ are rate constants for the formation of 2′- and 3′-phosphates of adenosine from free A>p.

Results

Regioselective Cleavage of Ribonucleoside 2′,3′-Cyclic Phosphates by Combination of β- or γ-CyD and Alkali-Metal Halide. The 2′/3′ ratio in the β-CyD-induced cleavage of A>p increases sharply with increasing concentration of KCl when the concentration of β-CyD is kept constant at 0.015 M (the open circles in Figure 1). The 2′/3′ ratio is 7.8 at $[KCl]_0 = 3.0$ M, whereas the ratio without KCl is only 2.2.[17] In the absence of both β-CyD and KCl the cleavage is nonselective (the 2′/3′ ratio is 0.8). The observed rate constant for the cleavage k_{obsd} increases almost linearly with $[KCl]_0$ (closed circles). Simultaneous increase of the regioselectivity (for the P–O(3′) cleavage) and the reaction rate is also achieved by NaCl, RbCl, and CsCl (Table I). The selectivity-increasing activity is in the following order: KCl > RbCl > CsCl > NaCl.

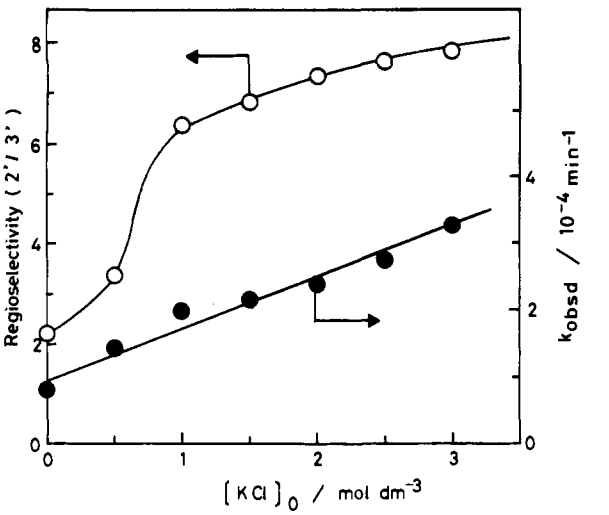

Figure 1. Plots of the 2′/3′ ratio (○) and observed rate constant k_{obsd} (●) vs the concentration of KCl for β-CyD-induced regioselective cleavage of A>p at pH 9.5, 30 °C. The concentration of β-CyD is kept constant at 0.015 M.

Table I. Regioselective Cleavage of A>p by the Combination of β-CyD and Alkali-Metal Halide[a]

metal halide	rate constant,[b] 10^{-4} min^{-1}	selectivity[b] (2′/3′)
KCl	3.3 (0.92)	7.8 (0.7)
	2.7[c]	6.9[c]
NaCl	13.3 (3.2)	4.6 (0.7)
RbCl	2.2 (0.81)	5.8 (0.8)
CsCl	2.2 (0.52)	5.5 (0.9)
LiCl	23.6 (13.8)	1.8 (0.9)
none	0.91 (0.28)[d]	2.2 (0.8)[d]
KBr[c]	11.0 (0.91)	4.3 (0.7)
KI[c]	10.0 (0.64)	1.4 (0.8)

[a] pH 9.5, 30 °C; $[\beta\text{-CyD}]_0 = 0.015$ M; $[\text{metal halide}]_0 = 3.0$ M. [b] Numbers in parentheses refer to the result for reaction with metal halide alone, in the absence of β-CyD. [c] $[\beta\text{-CyD}]_0 = 0.10$ M. [d] In the absence of both β-CyD and metal halide.

The increase in regioselectivity is ascribed to cooperation of the metal halide and β-CyD, since the metal halide alone in the absence of β-CyD hardly increases the selectivity (the numbers in parentheses in Table I). In addition, the rate of cleavage by β-CyD–metal halide combination is larger than the sum of the rate catalyzed by β-CyD alone and that by the metal halide alone.

The significant effect of halide ion on the cooperative catalysis is evidenced by the higher activity of KCl than that of KBr. KF exhibited still higher activity than KCl. With KF (3.0 M) and β-CyD (0.001 M), the 2′/3′ ratio was 3.0 and the rate constant 2.6×10^{-4} min^{-1}. The corresponding values for KCl were 1.5 and 5.0×10^{-5} min^{-1}. A quite large 2′/3′ ratio (12) was obtained when 0.01 mmol of β-CyD was added to 3.0 M aqueous KF solution (1.0 cm^3). Some β-CyD precipitated here.[18]

In contrast with the promotion of regioselective catalysis by all the metal halides previously described, LiCl and KI decrease the regioselectivity. Only accelerating effects are perceived (Table I). The cooperation with β-CyD is highly dependent on the kinds of both metal ion and halide ion.

Regioselective P–O(3′) cleavage of A>p by γ-CyD was also enhanced by KCl. The 2′/3′ ratios in the presence and the absence of 3.0 M KCl were 2.1 and 1.5, respectively: $[\gamma\text{-CyD}]_0 = 0.015$ M. Acceleration by KCl was 1.3-fold.

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Table II. Partial Rate Constants (10^{-4} min^{-1}) for β-CyD-Induced Regioselective Cleavage of A>p in the Presence and the Absence of KCl (3.0 M) at pH 9.5, 30 °C

kinetic parameter	value[a] with KCl	value[a] without KCl
k_2(c)	6.5	1.6
k_3(c)	0.45	0.42
k_2(f)	0.39	0.12
k_3(f)	0.52	0.16

[a] Experimental error in k_2(c) and k_3(c) values is estimated to be around 5%.

In the β-CyD-induced cleavage of G>p, C>p, and U>p, KCl (3.0 M) showed only acceleration (5.7-, 3.3-, and 3.9-fold at $[\beta\text{-CyD}]_0 = 0.01$ M). The 2′/3′ ratios (1.3, 1.0, and 0.7) were almost identical with the values for β-CyD alone, without KCl.

Effects of Alkali-Earth Metal Chlorides on the β-CyD-Induced Regioselective Cleavage of A>p. $MgCl_2$ and $CaCl_2$ remarkably accelerated cleavage of A>p in the presence of β-CyD (0.015 M): the rate constants at pH 7.0 with the metal salts (0.5 M) were 1.7×10^{-4} and 1.8×10^{-4} min^{-1} (about 2000-fold acceleration). However, regioselective catalysis of β-CyD for P–O(3′) cleavage was totally inhibited by these alkali-earth metal chlorides: The 2′/3′ ratios were 0.50 for $MgCl_2$ and 0.51 for $CaCl_2$ either with or without β-CyD. Only the alkali-metal salts exhibited successful cooperation with β- and γ-CyDs.

Kinetic Parameters in Regioselective Cleavage of A>p by Combination of β-CyD and KCl. The plot of k_{obsd} vs $[\beta\text{-CyD}]_0$ in the presence of KCl shows gradual saturation at large concentration of β-CyD. Clearly, a complex between β-CyD and A>p (the ternary complex [β-CyD-Cl^--A>p] as shown below) is involved in the catalysis.

The plot of $[k_3(f) - k_2(f)/R]/F$ vs $1/R$ according to eq 6 gave a fairly straight line. Partial rate constants for the regioselective cleavage, determined from the slope and the intercept, are shown in Table II (the middle column). The rate constant k_2(c) for the formation of adenosine 2′-phosphate (P–O(3′) cleavage) from the ternary complex is 16.7 times the k_2(f) from free A>p. By contrast, formation of the 3′-phosphate is slightly slowed by the ternary complex formation (k_3(c)/k_3(f) = 0.87). As a result, formation of the 2′-phosphate from the ternary complex is 14.4 times as fast as formation of the 3′-phosphate. This corresponds to 94% selectivity for the 2′-phosphate.

For comparison, the corresponding partial rate constants for the β-CyD-induced regioselective cleavage in the absence of KCl are also presented in Table II. KCl accelerates the P–O(3′) cleavage of the A>p, which is complexing with β-CyD, by 4.1-fold (6.5/1.6), whereas the rate constant for the P–O(2′) cleavage of the complexing A>p is virtually unchanged by KCl. The increase of regioselectivity by KCl originates from the large acceleration of P–O(3′) cleavage of the complexing A>p, with minimal effect on P–O(2′) cleavage.

For the P–O(3′) cleavage, multiplication of the magnitude of acceleration of KCl alone in the absence of β-CyD (0.39/0.12 = 3.3-fold) by the value of β-CyD alone (1.6/0.12 = 13-fold) gives a value of 43-fold acceleration. This value, estimated for cooperative acceleration by β-CyD and KCl, is close to the observed acceleration (6.5/0.12 = 54-fold) by the combination. For the P–O(2′) cleavage, however, cooperation is not observed.

Complex Formation in Regioselective Cleavage of A>p by β-CyD–KCl Combination. The K_{Cl} value for complex formation between β-CyD and Cl^- ion was determined to be 0.5 ± 0.1 M by absorption spectroscopy. This value is in reasonable accord with the literature value (0.39 M at 25 °C).[14]

When A>p was added to aqueous solutions of β-CyD in the presence of KCl (3.0 M), the NMR signals for both H-3 and H-5 protons of β-CyD shifted toward higher magnetic field; the limiting shifts were 0.046 and 0.091 ppm, respectively. None of the other protons of β-CyD showed an appreciable shift. The upfield shifts for the H-3 and H-5 protons, located in the interior of the cavity of β-CyD,[12] are ascribed to anisotropic shielding effects of the adenine residue[19] in the cavity.

Under the present conditions, about 86% of the β-CyD forms complex with Cl^- ion, as estimated from the K_{Cl} value. Thus, the adenine residue is efficiently included in the cavity of β-CyD, which already accommodates Cl^- ion, forming a ternary complex [β-CyD-Cl^--A>p].

Formation of the ternary complex was further confirmed by the fact that the absorption of A>p in the 240–270-nm region gradually weakened with increasing concentration of β-CyD, even in the presence of KCl (3.0 M). By applying the absorbance change at 260 nm to eq 5, the value of K_T was determined to be 0.011 M. When LiCl (3.0 M) was used in place of KCl, a similar change in absorption spectrum was observed and the K_T value was 0.010 M.

The circular dichroism spectrum of A>p in the 200–300-nm region was unchanged by KCl either in the presence of β-CyD or in its absence. The possibility that KCl alters the distribution of syn/anti conformers (with respect to the C–N glycosidic bond) of A>p,[20] rather than directly assisting the regioselective catalysis of β-CyD, is unlikely.

Cooperation of KCl with α-CyD for Regioselective P–O(2′) Cleavage of Ribonucleoside Cyclic Phosphates. The regioselective catalysis of α-CyD for the P–O(2′) cleavage[11a] was also promoted by KCl, although the magnitude was not remarkable. The ratio of the 3′-phosphate to the 2′-phosphate for the cleavage of A>p in the presence of KCl (3.0 M) was 2.4, whereas the ratio was 1.9 in its absence. Note that KCl increased the regioselectivity for the formation of the 3′-phosphate here; the effect of KCl is entirely the reverse of that in the β-CyD-catalyzed cleavage of A>p (promotion of the formation of the 2′-phosphate). Cooperation was also successful for the cleavage of U>p (the 3′/2′ ratios with and without KCl were 3.1 and 2.6, respectively). In the cleavages of G>p and C>p, however, no increase in regioselectivity was perceived. All the α-CyD-induced cleavages were accelerated by KCl (1.6–3.0-fold).

Discussion

Formation of Ternary Complex from β-CyD, A>p, and Chloride Ion for Regioselective Cleavage. Both ^1H NMR and absorption spectroscopy have confirmed that the adenine residue of A>p is accommodated in the cavity of β-CyD, which is complexing with Cl^- ion. This is the first finding of ternary complexing of CyD involving halide ion as one of the guest compounds.[21] The ternary complex is responsible for regioselective catalysis by the β-CyD–metal chloride combination.

Quite significantly, the K_T value (0.011 M with KCl and 0.010 M with LiCl) for the dissociation of the ternary complex is smaller than the K_B value (0.026 M)[11b] of the binary complex [β-CyD-A>p]. Interaction between A>p and β-CyD is *promoted* by KCl.

These results are in great contrast to previous findings that complex formation between β-CyD and 1-(4′-nitrophenylazo)-2-hydroxy-6-naphthalenesulfonic acid[14] or phenolphthalein[22] is competitively *inhibited* by complex formation between β-CyD and Cl^- ion. The interpretation that inclusion of A>p into the β-CyD cavity is stabilized by KCl simply through enhancement of apolar binding is unacceptable.

The additional stability found only in the present case should originate from some interaction between the adenine residue and the Cl^- ion in the cavity. The amino residue at the 6-position of the adenine probably forms a hydrogen bond with the Cl^- ion, as schematically depicted in Figure 2. The amino residue serves as proton donor, as in the usual Watson–Crick type base pairs.[8]

Consistent with this interpretation, the K_T value (0.011 M) for the β-CyD-KCl-A>p system is identical with that (0.010 M) for the β-CyD-LiCl-A>p system within experimental error. Alka-

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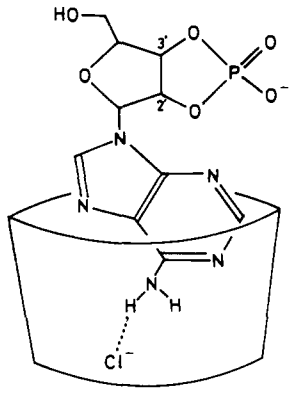

Figure 2. Proposed structure of the [β-CyD-Cl⁻-A>p] ternary complex.

li-metal cation has no significant effect on the stability of the ternary complex.

The argument is further supported by the fact that cooperative catalysis by the β-CyD–KCl combination is operative only in the cleavage of A>p. G>p has a proton-accepting oxygen atom at the 6-position of the nucleic base and is unable to interact with the Cl^- ion in the cavity. C>p and U>p are too polar to be accommodated in the cavity of β-CyD, as shown previously.[11]

The limiting upfield shifts for H-3 and H-5 protons of β-CyD on the formation of the ternary complex (0.046 and 0.091 ppm, respectively) are smaller than the values (0.118 and 0.184 ppm)[11b] on the formation of the binary complex [β-CyD-A>p]. Penetration of the adenine residue into the cavity of β-CyD in the ternary complex is shallower than that in the binary complex. This is consistent with the proposed structure of the ternary complex.

The poorer cooperation of KBr with β-CyD is associated with the smaller electronegativity of Br^- ion, which is less favorable for proton acceptance from the 6-NH_2 group of the adenine residue. I^- ion, with a still smaller electronegativity and a larger ionic radius, competitively inhibits formation of the [β-CyD-A>p] complex required for the regioselective catalysis (see Table I).

Total suppression by $MgCl_2$ and $CaCl_2$ of the regioselective catalysis of β-CyD is ascribed to inhibition of formation of the β-CyD-A>p complex, as clearly evidenced by absorption spectroscopy (data not shown). The divalent alkali-earth metal ions strongly bind to the phosphate residue,[23,24] decreasing the apolar character of A>p. The complexes between A>p and the divalent ions, which are free from β-CyD, undergo rapid but nonselective hydrolysis.

Reaction Mechanism of Regioselective Cleavage by CyD–Alkali-Metal Halide Combination. Cooperative acceleration by the β-CyD–metal halide combination is ascribed to increase in rate constant k_f for the rate-determining formation of pentacoordinate phosphorus intermediate. The intermediate, formed from the ternary complex [β-CyD-Cl^--A>p] by nucleophilic attack of hydroxide ion or water,[25] is decomposed either to the 2′-phosphate (by P–O(3′) fission) or to the 3′-phosphate (by P–O(2′) fission).

Figure 3 shows a log–log plot between the regioselectivity (2′/3′) and the mean ionic activity coefficient $\gamma_\pm$[26] of the reaction medium (aqueous solution of metal chloride). A fairly linear relationship (slope −1.4) is obtained. The increase in regioselectivity by NaCl, KCl, CsCl, and RbCl as well as its decrease by LiCl is explainable in terms of stabilization of the transition states for the P–O(2′) and the P–O(3′) fission by the media.

The P–O(3′) fission to the 2′-phosphate (rate constant $k_{d2'}$) takes place far away from the β-CyD, and thus almost in the bulk reaction medium itself (see Figure 2). The fission is facilitated in aqueous solutions of NaCl, KCl, CsCl, and RbCl (3.0 M), since partial charge separation (−P^+···O(3′)−) in the transition state is stabilized by media having $\gamma_\pm$ values smaller than 1.0.[27] In

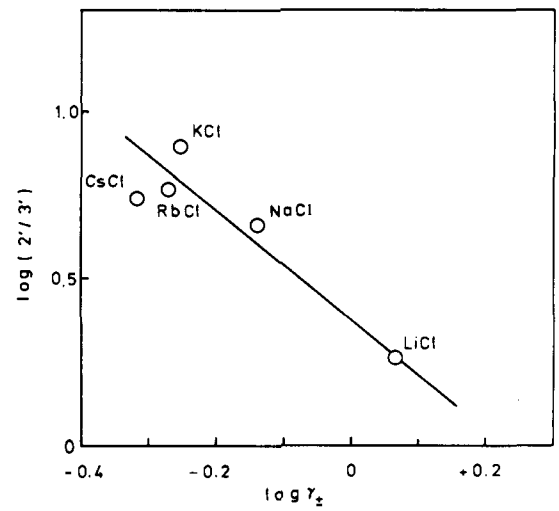

Figure 3. Log–log plot of the 2′/3′ ratio vs mean ionic activity coefficient $\gamma_\pm$ of reaction medium for the regioselective cleavage of A>p induced by the combination of β-CyD (0.015 M) and alkali-metal chloride (3.0 M).

contrast, 3.0 M aqueous solution of LiCl with $\gamma_\pm$ value larger than 1.0 (1.16) renders the transition state unstable.

On the other hand, the P–O(2′) fission (rate constant $k_{d3'}$) near the apolar cavity of β-CyD is affected by the metal salt to a lesser extent. Local concentration of the metal salt in the apolar case is smaller than the value in the bulk medium.

This interpretation is exactly in accord with the kinetic result that the k_2(c) value for the P–O(3′) cleavage of the A>p complexing with β-CyD is selectively increased by KCl with k_3(c) virtually unchanged (Table II). Formation of the 2′-phosphate is greatly accelerated, since $k_2(c) = k_f k_{d2'}/(k_{d2'} + k_{d3'})$ and the term $k_{d2'}/(k_{d2'} + k_{d3'})$ is around 1.0 ($k_{d2'} \gg k_{d3'}$). In the formation of the 3′-phosphate, however, the increase in k_f is mostly compensated by the increase in $k_{d2'}$: $k_3(c) = k_f k_{d3'}/(k_{d2'} + k_{d3'}) \approx k_f k_{d3'}/k_{d2'}$. In the absence of β-CyD, the P–O(2′) and P–O(3′) bonds are in similar chemical circumstances, and KCl accelerates the cleavages of both bonds almost equally.

Regioselective cleavage by the β-CyD–KF combination probably proceeds via [β-CyD-A>p] binary complex, since complex formation between β-CyD and F^- ion is inefficient. Discrimination between the P–O(2′) and the P–O(3′) bonds in the binary complex is enhanced by KF.

The selective P–O(2′) cleavages of A>p and U>p to the 3′-phosphates by the α-CyD–KCl combination are attributable to formation of binary complex between α-CyD and the phosphate. α-CyD does not form a complex with the Cl^- ion to a measurable extent.[14] In the complex, the P–O(3′) bonds are located near the cavity of α-CyD and the P–O(2′) bonds are far away from it.[11a] The orientation of the five-membered ring of the phosphate residue is the reverse of that (P–O(2′) bond near the cavity) in the β-CyD-A>p complex. KCl preferentially enhances fission of the P–O(2′) bond, in the same way as described above for selective P–O(3′) fission by the β-CyD–metal halide combination.

Conclusion

Regioselective catalysis of CyDs for the cleavage of ribonucleoside 2′,3′-cyclic phosphates, the simplest form of the intermediate in RNA hydrolysis, is greatly enhanced by cooperation with certain alkali-metal halides. CyDs differentiate the reaction field for P–O(3′) fission and that for P–O(2′) fission by complex formation with the cyclic phosphates, and the metal salt amplifies the difference still more. The regioselectivity by α-CyD is in the same direction as the specificity (P–O(2′) cleavage) exhibited by natural ribonuclease, whereas that by β- and γ-CyDs is in the

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(27) According to electrolyte theory, a transition state involving total charge separation is stabilized, with respect to the state formed in ideal solution, by the amount $-2RT \ln(\gamma_\pm)$ when formed in a medium of mean ionic activity coefficient $\gamma_\pm$.

reverse direction. This is, to our knowledge, the first report of the remarkable cooperation of CyD catalysis with salt effect for highly selective reactions.

The present success in simultaneous improvement of both regioselectivity and reaction rate should be significant for the further development of artificial ribonuclease.

Acknowledgment. We express our sincere thanks to Professors Jinsai Hidaka and Kenichi Okamoto at the Institute of Chemistry, University of Tsukuba, for their assistance in circular dichroism spectroscopy, and to Professor Edward M. Eyring at the Department of Chemistry, University of Utah, for valuable comments on the spectroscopic determination of the K_{Cl} value of the β-CyD-Cl^- complex. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

Registry No. α-CyD, 10016-20-3; β-CyD, 7585-39-9; γ-CyD, 17465-86-0; A>p, 634-01-5; U>p, 606-02-0; G>p, 634-02-6; C>p, 633-90-9; NaCl, 7647-14-5; KCl, 7447-40-7; RbCl, 7791-11-9; CsCl, 7647-17-8; KBr, 7758-02-3; KF, 7789-23-3; LiCl, 7447-41-8; KI, 7681-11-0; $MgCl_2$, 7786-30-3; $CaCl_2$, 10043-52-4; adenosine 2′-phosphate, 130-49-4; adenosine 3′-phosphate, 84-21-9; uridine 3′-phosphate, 84-53-7.

Formation of Etheno Adducts of Adenosine and Cytidine from 1-Halooxiranes. Evidence for a Mechanism Involving Initial Reaction with the Endocyclic Nitrogen Atoms

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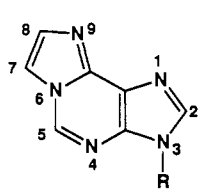
Received June 24, 1991

Abstract: The etheno derivatives of nucleic acid bases contain an additional ring and are of interest because of their useful fluorescence properties and their potential as mutagenic lesions in DNA. The mechanism of formation from 2-haloacetaldehydes is known to involve initial Schiff base formation at an exocyclic nitrogen; however, mechanisms of formation from the more relevant 1-substituted oxiranes have not been established. The reaction of N^6-methyladenosine (**5**) with 1-chlorooxirane yielded the stable carbinolamine 7,8-dihydro-8-hydroxy-9-methyl-3-β-D-ribofuranosylimidazo[2,1-*i*]purinium species (**10**), consistent with initial attack of the N^1 atom of adenine at the methylene of 1-chlorooxirane. No products indicative of initial reaction at the N^6 atom of adenine were found. Reaction of 2,2-dibromoethanol with adenosine or cytidine at pH 9.2 yielded 1,N^6-ethenoadenosine (**1**) or 3,N^4-ethenocytidine (**2**), respectively, presumably via the base-catalyzed formation of 1-bromooxirane from the bromohydrin. When reactions were done with 2,2-dibromo[1-^{13}C]ethanol, **1** contained label only at C-7 and **2** contained label only at C-3. A role for 2-bromoacetaldehyde in these reactions was ruled out by the lack of incorporation of deuterium from ^2H_2O into **1** under conditions where the exchange of the methylene protons of 2-bromoacetaldehyde with the solvent was relatively rapid. The collective results are most consistent with a mechanism in which the basic endocyclic nitrogen (N^1 of adenine or N^3 of cytosine) reacts with the methylene carbon of the 1-halooxirane, and, after ring opening and loss of the leaving group, the resulting aldehyde reacts with the exocyclic nitrogen to form the additional ring.

Introduction

Etheno-substituted nucleosides were discovered as products of the reactions of nucleosides with 2-haloacetaldehydes.[1] The 1,N^6-ethenoadenosine (**1**) and 3,N^4-ethenocytidine (**2**) derivatives have been studied the most extensively, and the fluorescence properties of **1** have been of utility in studying the interactions of enzymes with their cofactors.[2] 1,N^2-Ethenoguanosine (**3**), N^2,3-ethenoguanosine (**4**), and a number of other derivatives of nucleosides containing the additional etheno ring have now been prepared.[2,3] These nucleoside derivatives are also of biological interest because of their formation in nucleic acids after exposure of experimental animals to pro-carcinogens that may be enzymatically activated to reactive electrophiles capable of forming these derivatives (e.g., vinyl chloride, vinyl bromide, acrylonitrile, vinyl carbamate, and ethyl carbamate).[4] These compounds have all been demonstrated to yield **1** in vitro (in the presence of adenosine) after oxidation by cytochrome P-450 enzymes.[5] In general the N^7-(2-oxoethyl)guanosine adducts are formed in considerably greater abundance,[6] but the etheno adducts show



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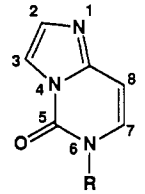

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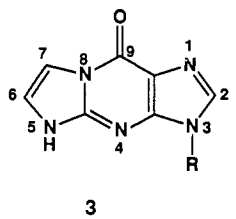

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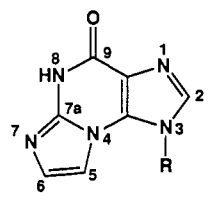

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