

POTASSIUM FLUORIDE FOR THE PROMOTION OF β -CYCLODEXTRIN-INDUCED REGIOSELECTIVE P—O(3') CLEAVAGE OF ADENOSINE 2',3'-CYCLIC PHOSPHATE

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Both the regioselectivity and the reaction rate for the β -cyclodextrin (β -CyD)-induced P—O(3') cleavage of adenosine 2',3'-cyclic phosphate (A > p) to adenosine 2'-phosphate are greatly promoted by KF. The promoting activity of KF is much larger than that of KCl. KF amplifies the difference in the chemical circumstance between the P—O(2') and the P—O(3') bonds of A > p, induced by β -CyD on complex formation with A > p. The higher activity of KF is ascribed to absence of complex formation of F[−] ion with β -CyD, which results in deeper penetration of A > p into the cavity of β -CyD.

INTRODUCTION

Mimicking of the function of ribonuclease has been widely attempted, and efficient catalysts for the hydrolysis of RNAs have been reported.^{1–4} However, another feature of ribonuclease, regioselective cleavage of 2',3'-cyclic phosphates as intermediates, has not yet been sufficiently accomplished.

In previous work,⁵ 2',3'-cyclic phosphates of ribonucleosides were regioselectively cleaved by the use of cyclodextrins (CyDs) as catalysts. Further, both the selectivity and the reaction rate for the CyD-catalysed cleavage were increased by alkali metal chlorides, especially KCl.⁶ There the cyclic phosphate was shallowly included in the cavity of CyD, which was also accommodating Cl[−] ion as well [see Fig. 3(b)]. This finding indicated that the CyD–KF combination can exhibit an even higher catalytic activity, since F[−] ion is hardly included in the cavity and hence the penetration of the cyclic phosphate can be deeper.

This paper reports that KF greatly promotes both the selectivity and the reaction rate for the regioselective cleavage of adenosine 2',3'-cyclic phosphate induced by β -CyD and α -glycosyl- β -CyD (G₁- β -CyD). The magnitude of promotion is much larger than that given by

KCl. The reactions were studied kinetically and spectroscopically and a reaction mechanism is proposed.

EXPERIMENTAL

Materials. 2',3'-Cyclic phosphates of adenosine, guanosine, cytidine and uridine (A > p, G > p, C > p, and U > p) were purchased from Sigma. G₁- β -CyD was kindly supplied by Ensuiko (the molecular structure of G₁- β -CyD is described in the legend to Fig. 4; the specimen was free from impurities according to HPLC analysis). All the reaction vessels and the water were sterilized immediately before use, in order to prevent contamination of ribonuclease. Absence of contamination was confirmed by the preferred formation of adenosine 2'-phosphate (see below). The enzyme if any should produce the 3'-phosphate with 100% selectivity.

Kinetics. Cleavage of the 2',3'-cyclic phosphates (10^{−4} M) at 30 °C and pH 9.5 was followed by high-performance liquid chromatography (HPLC), as described previously.^{5,6} KF was added to carbonate buffer solutions (ionic strength 0.01 M) and then the pH was adjusted to 9.5.

Partial rate constants $k_2(c)$ and $k_3(c)$ for the formation of 2'- and 3'-phosphates of adenosine from the A > p, in the [G₁- β -CyD–A > p] complex were

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evaluated by use of the equation⁷

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

where R is the ratio of the 2'-phosphate to the 3'-phosphate in the product, F is the equilibrium ratio of $A > p$, complexing state to that in the free state and $k_2(f)$ and $k_3(f)$ are the rate constants for the formation of 2'- and 3'-phosphates of adenosine, respectively, from free $A > p$.

Spectroscopy of the reaction mixture. ^1H NMR spectra (in D_2O) and absorption spectra were measured at 30°C on a Bruker AM-500 spectrometer and on a JASCO Ubest-50 spectrophotometer, respectively.

RESULTS

Regioselective cleavage of $A > p$ by combination of β -CyD and KF

Figure 1 shows the plot of the 2'/3' ratio vs the concentration of β -CyD for the cleavage of $A > p$ in the presence of KF. The corresponding plot either in the presence of KCl or in the absence of any metal halide is also presented. With KF and KCl (3.0 M), the 2'/3' ratio increases monotonically with increasing concentration of β -CyD (\circ and \bullet). The magnitudes of the increase are much larger than those observed in their absence (\circ). Cooperation of β -CyD with KF or KCl is clearly evidenced. The slight decrease in the 2'/3' ratio with increasing $[\beta\text{-CyD}]_0$ for the β -CyD-KF system, observed only when $[\beta\text{-CyD}]_0 \geq 0.01\text{ M}$, is associated with precipitation of β -CyD, as described below.

Significantly, the regioselectivity with KF is considerably greater than that with KCl. When $[\beta\text{-CyD}]_0$ is 0.01 M, for example, the 2'/3' ratio with KF is 12, whereas the ratio with KCl is 8. In addition, the cleavage with KF is faster than that with KCl at any β -CyD concentration investigated. The pseudo-first-order rate constant ($6.0 \times 10^{-4}\text{ min}^{-1}$) with KF in the presence of β -CyD (0.005 M) was 3.3 times larger than that ($1.8 \times 10^{-4}\text{ min}^{-1}$) with KCl.

Kinetic analysis of the regioselective cleavage

Remarkable cooperation of β -CyD and KF for the cleavage of $A > p$ has been now confirmed. However, the reaction mixtures with this combination involve some precipitation of β -CyD (probably due to the salting-out effect of KF), which makes a more detailed kinetic study complicated. Therefore, the hydrolysis of $A > p$ by the $\text{G}_1\text{-}\beta\text{-CyD}$ -KF combination was examined kinetically in place of the β -CyD-KF combination. The reactions with the branched β -CyD proceed totally homogeneously throughout the reaction time. The selectivities and the reaction rates are almost identical with the values with the β -CyD-KF system, as

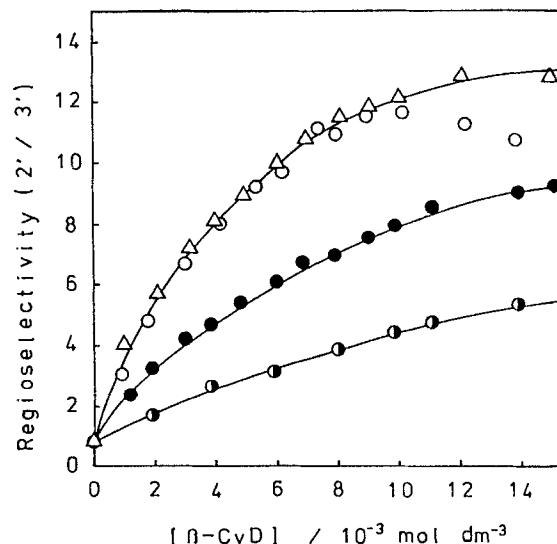


Figure 1. Plots of the 2'/3' ratio vs the concentration of β -CyD for the cleavage of $A > p$ in the presence or absence of alkali metal halide (3.0 M) at pH 9.5 and 30°C . (\circ) With KF; (\bullet) with KCl; (\circ) without alkali metal halide; (Δ) results for the reactions catalysed by the combination of $\text{G}_1\text{-}\beta\text{-CyD}$ and KF

depicted in Figure 1 (compare curve Δ with \circ). Deviations of the results with β -CyD from those with $\text{G}_1\text{-}\beta\text{-CyD}$ are observed only at large $[\beta\text{-CyD}]_0$ ($> 0.01\text{ M}$), where the amount of β -CyD precipitates is significant. Apparently the glucose branch at the primary hydroxyl side of the cavity in $\text{G}_1\text{-}\beta\text{-CyD}$ has no important effect on the catalysis, apart from increasing the solubility.

The observed rate constant k_{obsd} in the presence of KF increased with increasing $[\text{G}_1\text{-}\beta\text{-CyD}]_0$, but gradually saturated at high concentrations of $\text{G}_1\text{-}\beta\text{-CyD}$. A fairly linear relationship was obtained between $1/(k_{\text{obsd}} - k_{\text{un}})$ and $1/[\text{G}_1\text{-}\beta\text{-CyD}]_0$, where k_{un} is the rate constant in the absence of $\text{G}_1\text{-}\beta\text{-CyD}$. Participation of the $[\text{G}_1\text{-}\beta\text{-CyD}-A > p]$ complex in the catalysis is conclusive.⁸ The dissociation constant K_d of the complex, derived from the linear plot, is $3.0 \times 10^{-3}\text{ M}$.

The plot of $[k_3(f) - k_2(f)/R]/F$ vs $1/R$ according to equation (1) showed fair linearity (Figure 2). The F values were estimated from the K_d value determined above kinetically. Partial rate constants, determined from the slope and the intercept, are $k_2(c) = 5.2$, $k_3(c) = 0.30$, $k_2(f) = 0.39$ and $k_3(f) = 0.52$ ($\times 10^{-4}\text{ min}^{-1}$), where (c) and (f) denote complexing and free $A > p$, respectively. Complex formation of $A > p$ with $\text{G}_1\text{-}\beta\text{-CyD}$ accelerates the production of the 2'-phosphate 13-fold [$k_2(c)/k_2(f) = 13$], whereas the production of the 3'-phosphate is suppressed by the complex formation [$k_3(c)/k_3(f) = 0.6$].

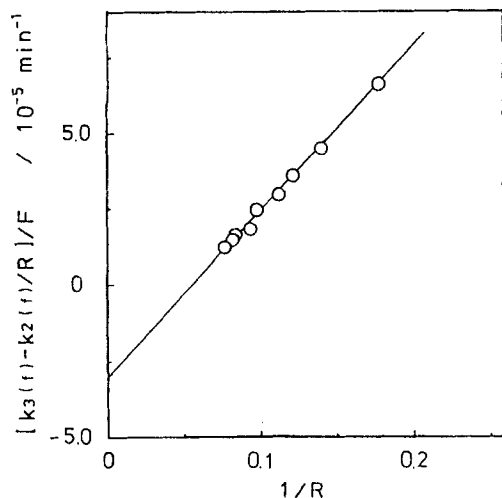


Figure 2. Plot of $[k_3(f) - k_2(f)/R]/F$ vs $1/R$ for the cleavage of A > p by the combination of G_1 - β -CyD and KF (3.0 M) at pH 9.5 and 30°C

Complex formation in the reaction mixture

When A > p was added to aqueous solutions of G_1 - β -CyD in the presence of KF (3.0 M), the NMR resonances of the H-3 and H-5 protons of G_1 - β -CyD shifted toward higher magnetic field; the limiting shifts were 0.08 and 0.07 ppm, respectively (the limiting shifts were evaluated by use of the kinetically determined K_d value). Shifts for the other protons of G_1 - β -CyD were marginal. The adenine residue of A > p is included in the cavity and its anisotropic shielding effect⁹ operates effectively on the protons located in the interior of the cavity.

Absence of complex formation between F^- ion and G_1 - β -CyD was confirmed by absorption spectroscopy. Addition of 0.1 M KF to aqueous solutions of 1-(4'-nitrophenylazo)-2-hydroxy-6-naphthalenesulphonic acid containing 0.1 M G_1 - β -CyD showed no change in the 450–550 nm region. If the $[G_1\text{-}\beta\text{-CyD-F}^-]$ complex were formed to a considerable extent, the competitive inhibition by F^- on the complex formation between the dye and G_1 - β -CyD should cause appreciable spectral changes. The competitive inhibition was clearly observed when Cl^- was used in place of F^- .^{6,10}

Catalytic activities of CyD-KF combinations for the cleavage of other ribonucleoside 2',3'-cyclic phosphates

The increase in the regioselectivity by KF for the G_1 - β -CyD-induced cleavage of G > p, C > p and U > p was marginal. With KF (3.0 M), the 2'/3' ratios were 1.2, 0.8 and 1.1, respectively (the corresponding values

in the absence of KF were 1.2, 0.6, and 0.8). KF did not show any measurable effects on the α -CyD-induced regioselective P—O(2') cleavage of A > p, G > p, U > p and C > p.⁵

DISCUSSION

The regioselective catalysis of β -CyD (and of G_1 - β -CyD) for the P—O(3') cleavage of A > p is considerably amplified by KF. Although KCl also promotes the catalysis, KF greatly exceeds KCl in activity. This is ascribed to marginal complex formation of F^- with β -CyD, as clearly evidenced by absorption spectroscopy. The binding activity of halide ions to β -CyD decreases rapidly with decrease in the atomic number: $I^- > Br^- > Cl^-$.¹⁰ The catalysis by the β -CyD-KF combination proceeds via the $[\beta\text{-CyD-A} > p]$ complex, in which the adenine residue of the A > p is included fairly deeply in the cavity [Figure 3(a)]^{5b} and hence the regioselective catalysis by β -CyD is more efficient. The catalysis by the β -CyD-KCl combination, however, involves a ternary complex composed of β -CyD, A > p and Cl^- [Figure 3(b)].⁶ As a result, penetration of the A > p is shallower, since the cavity is largely occupied by Cl^- ion.

These arguments are totally consistent with the fact that K_d for the G_1 - β -CyD-KF-A > p system (3.0×10^{-3} M) is about four times smaller than that (11×10^{-3} M) for the β -CyD-KCl-A > p system.⁶ The deeper penetration is also supported by 1H NMR spectroscopy: the limiting shifts for the H-3 protons on the formation of the G_1 - β -CyD-KF-A > p system (0.10 ppm) are larger than the corresponding value (0.05 ppm) for the β -CyD-KCl-A > p system.

In the $[\beta\text{-CyD-A} > p]$ complex, the P—O(2') bond of the A > p is located in the vicinity of the cavity (apolar medium), whereas the P—O(3') bond is far away from the cavity (more polar medium).^{5b} KF exists more preferentially in the polar medium [near the P—O(3') bond], and decreases the mean ionic activity coefficient therein more effectively. Hence the cleavage of the P—O(3') bond, located in the more polar medium, is effectively facilitated by the metal halide. In a medium of mean ionic activity coefficient γ_{\pm} , a transition state involving total charge separation is stabilized (with respect to the state formed in ideal solution) by the amount $-2RT \ln(\gamma_{\pm})$. In contrast, the cleavage of the P—O(2') bond is hardly affected by KF.

The larger regioselectivity with KF than with KCl is ascribed to the deeper penetration of A > p into the cavity. Thus, the chemical nature of the two P—O bonds is differentiated more explicitly. Consistently, the ratio $k_3(c)/k_3(f)$ given by KF (0.6) is considerably smaller than that given by KCl (1.2).⁶

These interpretations are further supported by the log-log plot of the regioselectivity (2'/3') and the mean ionic activity coefficient γ_{\pm} of the medium in Figure 4.

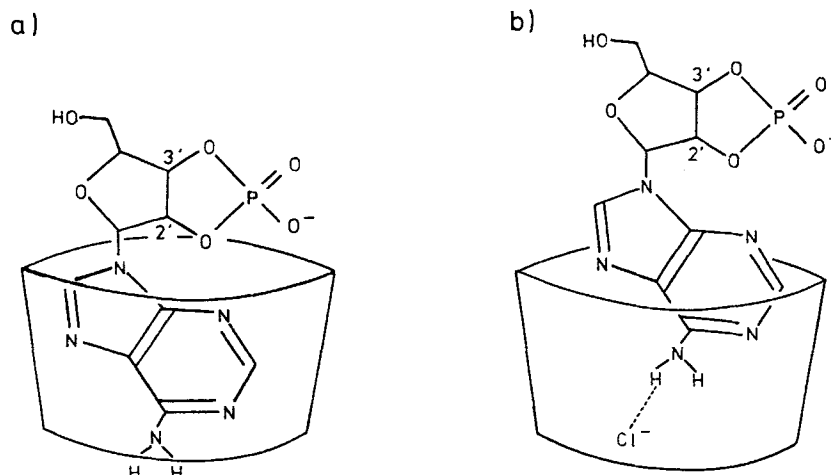


Figure 3. Schematic diagrams of the β -CyD-A > p complexes in the presence of (a) KF and (b) KCl. G_1 - β -CyD has a glucose branch at the primary hydroxyl side (the bottom side) of the cavity

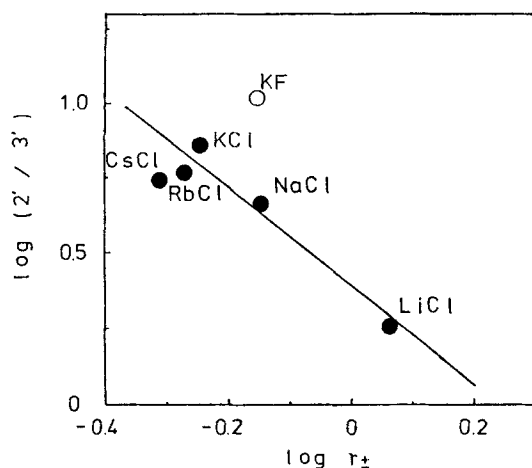


Figure 4. Log-log plot of the regioselectivity ($2'/3'$) and the mean ionic activity coefficient (γ_{\pm}) of the medium for the β -CyD-induced regioselective cleavage of A > p at pH 9.5 and 30 °C. The open circle refers to the result for the cleavage by the G_1 - β -CyD-KF combination obtained in the present study; all other data (closed circles) are taken from Ref. 6. $[\beta\text{-CyD or } G_1\text{-}\beta\text{-CyD}]_0 = 0.015$ and $[\text{alkali metal halide}]_0 = 3.0 \text{ M}$

The straight line is for the cleavage of A > p by the combination of β -CyD and various alkali metal chlorides reported in a previous paper.⁶ The point for the G_1 - β -CyD-KF system deviate considerably in a positive direction from the straight line (the mean ionic activity coefficient of 3.0 M aqueous KF solution is 0.705¹¹). Apparently the superior promoting activity of KF over KCl cannot be interpreted in terms of the difference in

the ionic activity coefficient of the medium. Rather, the structural difference in the complexes formed in the reaction mixtures is responsible for the superiority of KF, as proposed above.

The absence of cooperative catalysis of the α -CyD-KF and β -CyD-KF combinations for the cleavages of other ribonucleoside 2',3'-cyclic phosphates is associated with poor binding of the cyclic phosphates to CyDs.⁶

In conclusion, A > p is regioselectively and promptly cleaved to adenosine 2'-phosphate by the cooperation of β -CyD and KF. The simultaneous improvement of selectivity and reaction rate should be important for the molecular design of advanced artificial ribonucleases.

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