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[16] X-ray structure analysis: Enraf-Nonius CAD-4 diffractometer. Unit cell parameters were determined from automatic centering of 25 reflections ($4 < 2\theta < 60^\circ$) and refined by least-squares methods. Data were collected with graphite-monochromatized $Mo_{K\alpha}$ radiation ($\lambda = 0.71069$ Å) by using the ω scan technique. Lorentzian, polarization, and extinction corrections were made. The structure was solved by direct methods and successive Fourier difference syntheses with the SHELXL-93 computing program. Crystal data for **1**: $C_{23}H_{24}ClN_7Ni_2O_{10}$ ($M_r = 711.36$), monoclinic, space group $P2_1/c$, $a = 13.456(3)$, $b = 10.906(2)$, $c = 19.990(7)$ Å, $\beta = 91.59(3)$, $V = 2932.4(13)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.611$ g cm^{-3}, $\mu = 14.40$ cm^{-1}, $F(000) = 1456$. The refinements by full-matrix least-squares methods gave final $R1(F_o) = 0.074$, $wR2(F_o^2) = 0.184$ and $S = 0.999$ for 7643 reflections with $I \geq 2\sigma(I)$ and 389 variables. All non-hydrogen atoms were refined anisotropically. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-102206. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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5-(β-Cyclodextrinylamino)-5-Deoxy-α-D-Riboses as Models for Nuclease, Ligase, Phosphatase, and Phosphorylase**

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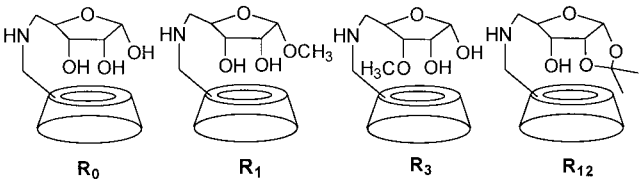
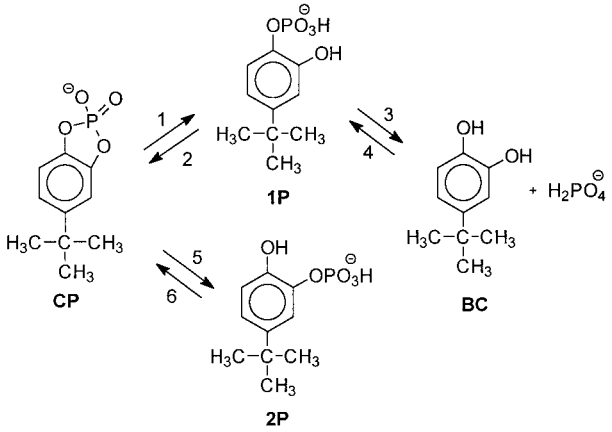
The synthesis and activity testing of enzyme models have drawn attention to the elucidation of enzyme structures and mechanisms.[1] Several models have been synthesized and their activities have been tested for nuclease, ligase, phosphatase, and phosphorylase.[2] We recently reported that ribose-containing polymers show catalytic activity for the cleavage of DNA.[3] The study of various polymer structures strongly suggested that the *vic-cis*-diol groups of the ribose rings play a key role in the catalysis, which is surprising because there are plenty of biopolymers containing ribose rings. This prompted us to further study the mechanism of action by synthesizing enzyme models containing riboses capping β-cyclodextrin (CD) and then performing model reactions with phosphate substrates. The models showed catalytic activity for the hydrolysis of phosphodiester (nuclease activity), the hydrolysis of phosphomonoester (phosphatase activity), the esterification of phosphomonoester to phosphate diester (ligase activity), and the phosphorylation of alcohols with phosphate ions (phosphorylase activity).

6-Monotosyl-β-cyclodextrin was prepared as described in the literature.[4] It was treated with 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribose to yield 5-(β-cyclodextrinylamino)-5-deoxy-1,2-*O*-isopropylidene-α-D-ribose (**R_{12}**), which was hydrolyzed to form 5-(β-cyclodextrinylamino)-5-deoxy-α-D-ribose (**R_0**) (Scheme 1). The derivatives **R_1** and **R_3**, in which 1-OH and 3-OH of the ribose ring are blocked by methyl groups, were synthesized by similar methods.[5] The *N*-methylpyridinium salt of the cyclic phosphate (**CP**)[5] and the 1-phosphate (**1P**) and 2-phosphate (**2P**) of 4-*tert*-butylcatechol (**BC**) were also synthesized as substrates (Scheme 1). Compounds **BC**[6] and **CP** are well known to form inclusion complexes with CD, and CD capped with imidazole has been investigated as a nuclease model using **CP** as substrate.[2]





Scheme 1. β-Cyclodextrinyl compounds and reactions of phosphate esters.

Reactions 1 (and 5), 2, 3, and 4 (Scheme 1) are the model reactions for nuclease, ligase, phosphatase, and phosphorylase, respectively. The rates of the reactions were measured at pH 7.4 (Tris buffer), ionic strength (μ) of 0.2 (KCl), and 25 °C by HPLC in the presence of the cyclodextrinyl compounds (4 mM) and the substrates (0.4 mM). This concentration ratio was chosen for the activity measurements since no significant rate acceleration was observed by further increasing the concentration of the former compound.

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The hydrolysis rates of **CP** were measured in the buffer alone and also in the presence of ribose, CD, $\mathbf{R_0}$, $\mathbf{R_1}$, $\mathbf{R_3}$, or $\mathbf{R_{12}}$. The time-dependent concentration changes of **CP** are shown in Figure 1 a. The hydrolysis was accelerated by $\mathbf{R_0}$, $\mathbf{R_1}$, and $\mathbf{R_3}$ in the order of $\mathbf{R_0} > \mathbf{R_3} > \mathbf{R_1}$, whereas ribose, CD, and $\mathbf{R_{12}}$ showed no catalytic activity above the activity of the buffer,

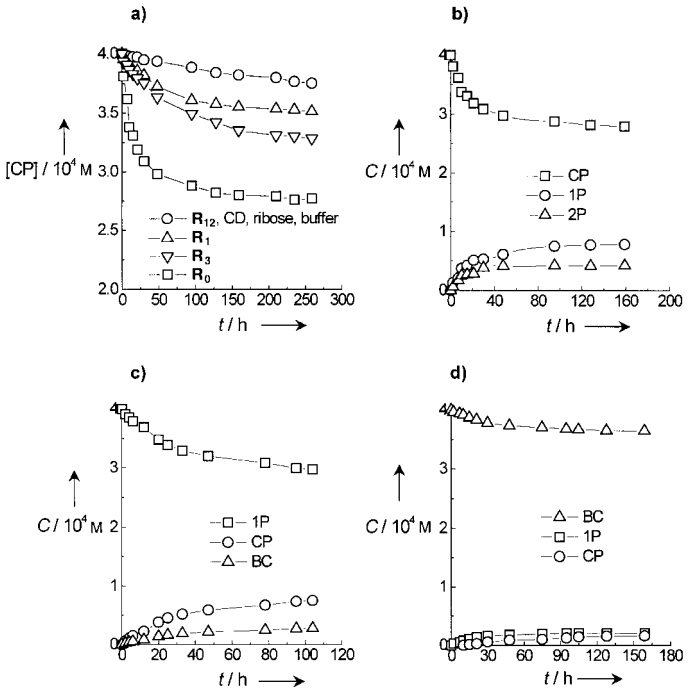


Figure 1. Concentration changes versus time: a) for hydrolysis of cyclic phosphate (**CP**) in Tris buffer solution (○) and in the presence of ribose (○), CD (○), $\mathbf{R_{12}}$ (○), $\mathbf{R_1}$ (△), $\mathbf{R_3}$ (▽), and $\mathbf{R_0}$ (□); b) for the reaction of **CP** (□) → **1P** (○) + **2P** (△) catalyzed by $\mathbf{R_0}$; c) for the reaction of **1P** (□) → **CP** (○) + **BC** (△) catalyzed by $\mathbf{R_1}$; d) for the reaction of **BC** (△) → **1P** (□) → **CP** (○) catalyzed by $\mathbf{R_3}$. (For reaction conditions, see the Experimental Section.)

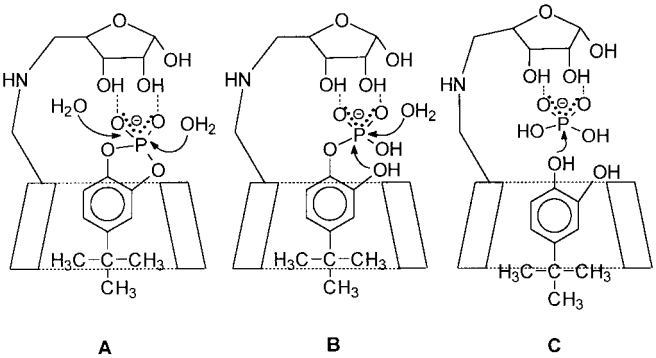
indicating clearly that the *vic-cis*-diol groups of riboses on CDs are essential for the catalysis. As the reactions were pseudo-first-order, the rate constants for the hydrolysis were obtained by plotting logarithmic concentrations of **CP** against time and were found to be 10.7, 1.41, and $2.03 \times 10^{-3}\ h^{-1}$ for $\mathbf{R_0}$, $\mathbf{R_1}$, and $\mathbf{R_3}$. These were, respectively, 33, 4.4, and 6.3 times faster than for the uncatalyzed reaction ($3.2 \times 10^{-4}\ h^{-1}$). The hydrolysis products of **CP** were found to be **1P** and **2P** as previously reported.[2] The reactions in the presence of the cyclodextrinyl compounds ($\mathbf{R_0}$, $\mathbf{R_1}$, and $\mathbf{R_3}$) reached equilibrium after extended times, that is, the reactions are reversible. In the hydrolysis of **CP** in the presence of $\mathbf{R_0}$ (Figure 1 b), the concentration of **CP** decreased while **1P** and **2P** simultaneously formed.[7] After 120 hours the reactions reached equilibrium.

When **1P** was mixed with $\mathbf{R_0}$, $\mathbf{R_1}$, or $\mathbf{R_3}$ in Tris buffer solution at pH 7.4, cyclization to **CP** and hydrolysis to **BC** occurred simultaneously. Compound **1P** was found to be stable in the buffer solution and in the presence of CD or $\mathbf{R_{12}}$ under the same conditions. No significant reaction was found after one month. The concentration changes of the starting material and the products in the reaction of **1P** in the presence of $\mathbf{R_1}$ are shown in Figure 1 c. The reaction reached equilibrium after 100 hours. Similar reactions occurred in the presence of $\mathbf{R_0}$ or $\mathbf{R_3}$.[8]

When **BC**, cyclodextrinyl compounds ($\mathbf{R_0}$, $\mathbf{R_1}$, or $\mathbf{R_3}$), and KH_2PO_4 were mixed in the mole ratio of 1:10:15 at pH 7.4 (Tris buffer), esterification to form **1P** occurred, which was followed by cyclization to yield **CP**. In order to make this reaction first order, phosphate was added in excess. These reactions did not occur in the buffer solution or in the presence of CD or $\mathbf{R_{12}}$. The concentration change in the presence of $\mathbf{R_3}$ versus time of the reaction is plotted in Figure 1 d. Phosphate compound **1P** formed immediately at the beginning of the reaction and then **CP** began to form. The reaction reached equilibrium after 140 hours. Similar reactions occurred in the presence of $\mathbf{R_0}$ and $\mathbf{R_1}$.[9]

In order to investigate whether the *sec*-amino groups on the cyclodextrinyl compounds contribute to the catalysis as a general base or, more likely at the operating pH of 7.4, as a general acid, we synthesized 6-monomethylamino-β-cyclodextrin as a model compound. The reactions with **CP**, **1P**, and **BC** were repeated in the presence of the 6-monomethylamino-β-cyclodextrin (instead of the enzyme models $\mathbf{R_0}$, $\mathbf{R_1}$, and $\mathbf{R_3}$) using the same conditions, and the rates of reaction were measured. The rate constant for the hydrolysis of **CP** was found to be $3.38 \times 10^{-4}\ h^{-1}$, nearly the same as that of the uncatalyzed reaction ($3.23 \times 10^{-4}\ h^{-1}$), and reactions 2, 3, and 4 (Scheme 1) did not occur, indicating that the *sec*-amino groups do not participate in the catalysis.

Based on the experimental results described above, the mechanism of action can be postulated as follows: the *vic-cis*-diols form hydrogen bonds with the two phosphoryl oxygen atoms of the phosphate group when the substrate is in the CD cavity of $\mathbf{R_0}$. This activates the phophorus atom to be attacked by nucleophiles (H_2O or ^-OH) as shown in Scheme 2. In the case of hydrolysis of **CP** (Scheme 2 A), water could attack either side of the activated phosphate to form **1P** or **2P**. The hydrolysis and esterification of **1P** (Scheme 2 B) occur when water or the 2-OH group of **1P** attack the phosphorus atom



Scheme 2. Action mechanisms of β-cyclodextrinyl compounds. The 2,3-*cis*-diol of ribose forms hydrogen bonds with the two phosphoryl oxygen atoms of the phosphate to activate the phosphorus atom for attack by nucleophiles (H_2O or OH of catechol). a) The phosphorus atom of **CP** is attacked by H_2O to form **1P** or **2P**. b) The hydrolysis and esterification of **1P** occur when H_2O attacks the phosphorus atom to break the P–O ester bond or the 2-OH group of **1P** attacks to eliminate the OH group of the phosphate, respectively. c) The phosphorylation of **BC** occurs by nucleophilic attack of the 1-OH group of **BC** to the phosphorus atom to eliminate the OH group from the phosphate.

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and either break the P–O ester bond or cause the OH group of the phosphate to leave, respectively. The phosphorylation of **BC** (Scheme 2C) occurs by nucleophilic attack of the 1-OH group of **BC** at the activated phosphorus atom with the OH group of the phosphate as the leaving group. Since the cleavage of **CP** is occasionally regioselective, depending on the directions of hydrogen bonds and attacking nucleophiles,[2] **2P** is not formed for the reactions shown in Figure 1c and d.

For catalytic activity, two hydrogen bonds should be formed simultaneously between the *vic-cis*-diols of ribose and the two phosphoryl oxygen atoms of the phosphate group. The order of catalytic activity, $\mathbf{R_0} > \mathbf{R_3} > \mathbf{R_1}$, seems to depend on the extent of hydrogen bond formation; in the case of $\mathbf{R_0}$, hydrogen bonds can be formed either by the 1,2- or the 2,3-diol of the ribose so its activity is the highest. As the arm connecting ribose to CD in cyclodextrinyl compounds is rather short and in a *trans* position to the diols, the 1,2-diol is probably more easily accessible to the phosphate than the 2,3-diol and thus, the activity of $\mathbf{R_3}$ was higher than that of $\mathbf{R_1}$. The 1-OH of **BC** (Scheme 2C) protrudes toward the phosphate so that **1P** was formed more favorably than **2P**.

The formation of strong hydrogen bonds was also born out by a theoretical study of the interactions between 3,4-*cis*-dihydroxytetrahydrofuran (DHTHF) and $H_2PO_4^-$, and DHTHF and HPO_4^{2-}. Quantum-chemical calculations using density functional theory at the B3LYP/6-31 + G** level and also at the MP2/6-31 + G** level of theory[10] showed that: 1) the hydrogen bond distances are rather short (1.76 Å and 1.52 Å for the $H_2PO_4^-$ and HPO_4^{2-} adducts, respectively), 2) the dissociation energies of the hydrogen bonds are quite large (19.3 and 42.5 kcal mol^{-1}), and 3) the red shifts of the OH-stretching frequencies of DHTHF in the adducts are also very large (up to 300 and 650 cm^{-1}, respectively). The reduction of the charge density at the phosphate sites was found to be appreciable (0.057 and 0.191 electrons).

As the synthetic ribose-containing polymers have shown nuclease activity,[3] biopolymers containing riboses may have similar enzyme activities. One of the typical biopolymers containing riboses with *vic-cis*-diols is poly(ADP-ribose) formed from NAD^+ in chromatin. However, its functions are not clear so far; since its discovery in 1966,[11] it has been suggested to be involved in numerous biological reactions.[12] Poly(ADP-ribose) forms during apoptosis[13] and DNA replication, transcription, and repair,[14] when nuclease, ligase, phosphatase and/or phosphorylase actions will be required. Further study on the enzyme functions of naturally occurring biopolymers having riboses with free *vic-cis*-diols is necessary.

Experimental Section

Reaction of 6-monotosyl-β-cyclodextrin (0.5 g, 3.88 mmol) with 5-amino-5-deoxy-1-*O*-methyl-D-ribose (0.15 g, 7.76 mmol) or 5-amino-5-deoxy-3-*O*-methyl-1,2-*O*-isopropylidene-α-D-ribose (0.15 g, 7.51 mmol) in DMF (5 mL) gave 5-(β-cyclodextrinylamino)-5-deoxy-1-*O*-methyl-α-D-ribose ($\mathbf{R_1}$, yield: 72.4%) and 5-(β-cyclodextrinylamino)-5-deoxy-3-*O*-methyl-1,2-*O*-isopropylidene-α-D-ribose ($\mathbf{R_{12}}$, yield: 74.9%), respectively. The latter compound was hydrolyzed in 1N HCl to result in 5-(β-cyclodextrinylamino)-5-deoxy-α-D-ribose ($\mathbf{R_0}$, yield: 79.5%). 4-*tert*-Butylcatechol-1-phosphate (**1P**) and 2-phosphate (**2P**) were obtained by separation of the hydrolysis products of the cyclic phosphate of 4-*tert*-butyl catechol (**CP**) with the aid of preparative liquid chromatography with 0.025M KCl aqueous solution as the eluent. 6-Monomethylamino-β-cyclodextrin was synthesized by a reaction of 6-monotosyl-β-cyclodextrin (1 g, 0.78 mmol) with aqueous 40 wt% of methylamine (0.72 mL, 0.93 mmol) for 48 h at room temperature (yield: 41.5%, m.p.: 219–220 °C).

Cyclodextrinyl compounds (4×10^{-3}M) and phosphates (4×10^{-4}M) were dissolved in Tris-buffer solution (pH 7.4) with an ionic strength of 0.2 (KCl) in a vial, which was immersed in a water bath at 25 ± 0.1 °C. Aliquots (80 µl) of the reaction solution were taken by microsyringe at defined time intervals and injected into a Waters liquid chromatograph with UV detector at 285 nm (eluent: 0.025M KCl, flow rate: 0.8 mL min^{-1}). The concentrations of the reactants and products were evaluated by peak areas, which were corrected by the extinction coefficients of the compounds (ε = 2460 for **CP**, 3500 for **1P**, 3490 for **2P**, 2310 for **BC** at 285 nm and pH 7.4).

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