Photo-responsive catalysis by thymine–cyclodextrin conjugates†

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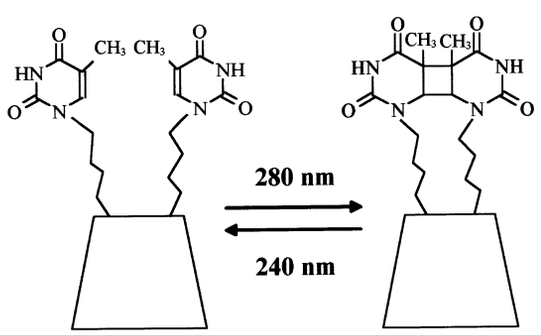
Primary hydroxy groups of β-cyclodextrin (β-CD) have been substituted with thymine (Thy) groups. By photo-irradiation with UV light at 280 nm, the introduced thymine groups adjacent to the CD cavity underwent reversible dimerization and the catalytic efficiency (k_{cat}/K_{diss}) of the modified CD in the hydrolyses of *p*-nitrophenyl acetate and *m*-nitrophenyl acetate increased. By further irradiation with light at 240 nm, the catalytic efficiency decreased to that of the CD–Thy conjugate due to the photo-cleavage of the thymine dimer. This phenomenon implies that the binding of guest molecules by the CD–Thy conjugate and subsequent change in the catalytic efficiency of the conjugate occurred photo-responsively. The steric effect on the acceleration or deceleration of the hydrolyses of phenyl esters by CD–Thy and its derivatives is also discussed.

Introduction

The photochemical transformation of nucleic acids by UV light has been extensively studied due to its lethal effect on biological systems.[1] Of the four nucleic acid bases in DNA, pyrimidine bases (thymine and cytosine) undergo photodimerization on exposure to UV light at 280 nm and revert back to their monomers with UV light at 240 nm (Scheme 1), whereas purine bases (adenine and guanine) do not. Such a reversible photosensitivity has been applied to photoresists, *etc.*[2–5]



Scheme 1 Photodimerization of 1-(2-carboxyethyl)thymine–CD conjugate (CD–Thy)

Enzymatic processes are highly selective and efficient due to the accompanying formation of non-covalent enzyme-substrate complexes before the onset of the reactions. In this regard, study of enzyme-mimetic catalytic systems, which begin to act after the formation of the host–guest complex, has received much attention. One molecule which acts as a catalyst in such a way is cyclodextrin (CD), a cyclic α-1,4-oligoglucopyranoside having a cylindrical shape. Catalytic effects of CD and its derivatives on many chemical reactions such as hydrolyses, decarboxylations and substitutions have been extensively investigated.[6–11]

In this report, in order to obtain a catalyst having a photo-responsiveness, we attempted to introduce thymine groups into the CD molecule. The effect of photo-irradiation on the catalysis of the hydrolyses of phenyl esters by the modified CD has been examined.

Experimental

Materials

β-Cyclodextrin was from Nacalai Tesque, Kyoto, Japan. Thymine was from Wako Pure Chemicals, Osaka, Japan. *p*-Nitrophenyl acetate (PNPA), from Wako Pure Chemicals, was recrystallized from benzene. *m*-Nitrophenyl acetate (MNPA) and 3-nitro-4-acetoxybenzoic acid (NABA) were prepared by the reaction of acetic anhydride with *m*-nitrophenol and 3-nitro-4-hydroxybenzoic acid (Fluka, Buchs, Switzerland), respectively. Other reagents were commercially available. Deionized water was distilled before preparation of sample solutions.

Preparation of 1-(2-carboxyethyl)thymine[12]

Thymine (10.0 g, 79.3 mmol) was coupled with methyl acrylate (9.11 g, 106 mmol) in the presence of hydroquinone (100 mg, polymerization inhibitor) in EtOH (70 ml) containing NaOH (276 mg) for 50 h at the reflux temperature. After evaporation of the solvent, the methyl ester of 1-(2-carboxyethyl)thymine obtained was washed several times with cold EtOH and dried *in vacuo* (8.90 g, 52.9% yield). The methyl ester (6.49 g, 30.6 mmol) was hydrolysed by 5 M HCl (40 ml) for 3 h at 100 °C [Scheme 2(*a*)]. The pH of the solution was adjusted to 4, the solution mixture was cooled overnight and then filtered and dried *in vacuo* to give a slightly brownish powder (4.67 g, 62.4% yield). δ_H(400 MHz, D_2O) 1.86 (s, 3 H, CH_3), 2.69 (t, 2 H, CH_2COOH), 3.99 (t, 2 H, NCH_2) and 7.51 (s, 1 H, C(6)*H*). ν(KBr)/cm^{-1} 3433, 3304, 3051, 2964, 2927, 1679, 1560, 1419, 1264 and 1130 (Found C, 49.07; H, 5.09; N, 14.07. Calc. for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.09; N, 14.14%).

Modification of CD with 1-(2-carboxyethyl)thymine

The (aminoethyl)aminocyclodextrin[13] [940 mg; degree of substitution (DS) evaluated by ^{1}H NMR, 7] was incubated with 1-(2-carboxyethyl)thymine (1.52 g) in water (20 ml) at pH 5 in the presence of 1-ethyl-3-(dimethylamino)propylcarbodiimide hydrochloride (EDC; 1.60 g) for 24 h [Scheme 2(*b*)]. The β-CD modified with thymine was separated from unreacted reagents by GPC and lyophilized (CD–Thy, 683 mg, 37.4% yield; DS, 7). δ(D_2O) 1.75 (t, 2 H, CH_2NH), 1.81 [s, 3 H, CH_3 (Thy)], 2.61 (br s, 2 H, CH_2CONH), 2.87 (t, 2 H, CH_2NHCO), 3.13, 3.32, 3.45, 3.66 [br s, 5 H, C(2)*H*, C(4)*H*, C(5)*H*, C(6)*H* (CD)], 3.90 [br s, 1 H, C(3)*H* (CD)], 3.99 (t, 2 H, NCH_2), 5.11 [br s, 1 H, C(1)*H* (CD)] and 7.43 [s, 1 H, C(6)*H* (Thy)]. ν(KBr)/cm^{-1} 3392, 3059, 2930, 2823, 1675, 1546, 1468, 1363, 1220, 1082 and 1042.

Instrumentation

^{1}H NMR spectra were recorded on a JEOL JNM-A400 spectrometer. IR spectra were recorded on a Perkin Elmer system

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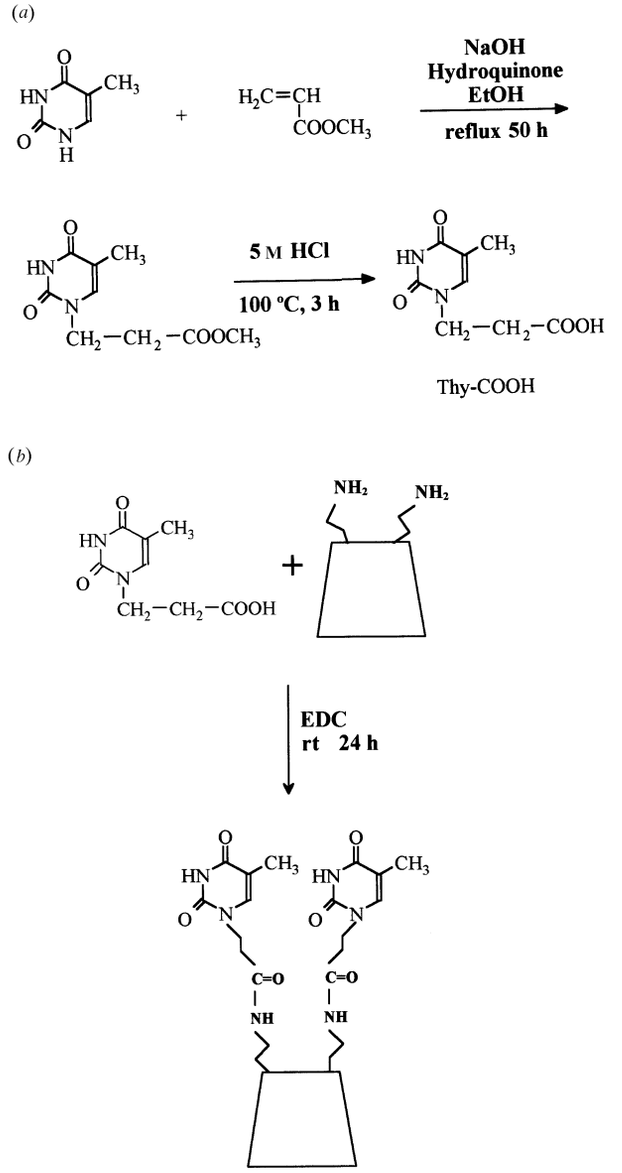

Scheme 2 Scheme of preparation of 1-(2-carboxyethyl)thymine–cyclodextrin conjugate

2000 FTIR spectrometer. GPC was carried out using a Sephadex G-10 column (id 2 × 40 cm) with water as eluent. Products were detected at 270 nm using a Soma Optics Model S-310A UV monitor.

Photodimerization of CD–Thy

Photodimerization of the thymine moiety was carried out in a 10 mm quartz cell filled with 3 ml of aqueous solution of CD–Thy at room temperature. A UV lamp (HB-251A, Ushio, Tokyo, Japan) and a diffraction grating (Model 356, Hitachi, Tokyo, Japan) were used to obtain UV lines at 240 and 280 nm. Aqueous CD–Thy (1 mM) was photo-irradiated for 2 h at room temperature.

Measurements of photodimerization

The absorbance of solution of CD derivatives from 220 to 300 nm at 20 ± 0.5 °C was followed with a UV–VIS spectrophotometer (Ubest–35, Japan Spectroscopic Co., Tokyo, Japan). The observation cell was thermostatted by a Peltier device (EHC-441, Japan Spectroscopic Co.). The quantitative estimation of the photodimerization of thymine groups was carried out by ^{1}H NMR spectroscopy (400 MHz, D_2O). The GPC analysis of CD–Thy before and after the photo-irradiation were performed using a Waters Model 440 (column, Wakobeads-F G-30; mobile phase, water).

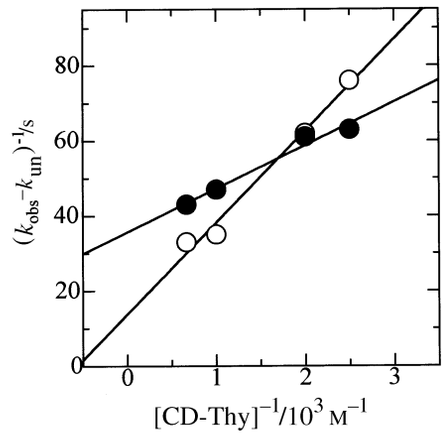

Fig. 1 Double reciprocal plot of the concentration of CD–Thy *vs.* reaction rate constant for the hydrolysis of PNPA before (●) and after (○) UV irradiation at 280 nm and 20 °C

Kinetic measurements

All reactions were followed with a UV–VIS spectrophotometer. The pH of the reaction solution was determined by using a pH meter (Model DS-12, Horiba Kyoto, Japan). In all measurements, the pseudo-first-order conditions were maintained by using an excess amount of cyclodextrin ([CD] = 0.4–1.5 mM). The hydrolyses of PNPA, MNPA and NABA were followed using changes in the absorbance at 400, 390 and 408 nm, respectively.

Determination of dissociation constants, K_{diss}, and rate constants, k_{cat}, of the cyclodextrin–ester complex from the kinetic data

The hydrolytic reaction of the phenyl esters (S) catalysed by cyclodextrin (CD) proceeds according to reaction (1), where

$$CD + S \underset{K_{diss}}{\rightleftharpoons} CD{\cdot}S \xrightarrow{k_{cat}} CD + P \qquad (1)$$

CD·S and P are the cyclodextrin–substrate complex and the product, respectively. The observed first-order rate constants for hydrolyses of phenyl esters in the absence (k_{un}) and in the presence (k_{obs}) of cyclodextrin were determined. By plotting $1/(k_{obs} - k_{un})$ *vs.* 1/[CD] (Fig. 1), a straight line was obtained having an x intercept of $-1/K_{diss}$, where K_{diss} is the dissociation constant of the cyclodextrin–ester complex with a 1 : 1 stoichiometry; k_{cat}, the rate constant for the reaction of the complexed ester (= $k_{obs} - k_{un}$ at infinite cyclodextrin concentration), was estimated from the reciprocal of the y intercept in the plot.

Results and discussion

Dimerization of thymine groups in CD–Thy

It was previously reported that the photodimer of thymine is not readily produced by UV irradiation of a thymine monomer model with only one thymine residue, whereas for models which contain two thymine residues the photodimerized thymine is produced reversibly.[14] Kita *et al.* observed a gradual decrease in the absorption at 275 nm accompanying the formation of photodimers upon photo-irradiation of polymer compounds which had many thymine residues as side chains.[15]

By irradiation of a solution of CD–Thy with UV light at 280 nm, the absorption at 271 nm corresponding to the thymine moiety decreased, but the absorbance was increased again by the further irradiation at 240 nm (Fig. 2). However, reversible photodimerization of CD–Thy by repeated exposure to UV light at 240 and 280 nm was incomplete, probably because of the unavoidable photodecomposition of the thymine rings or the formation of stable photodimers.

^{1}H NMR measurements were also made before and after UV irradiation at 280 nm in order to confirm the relationship

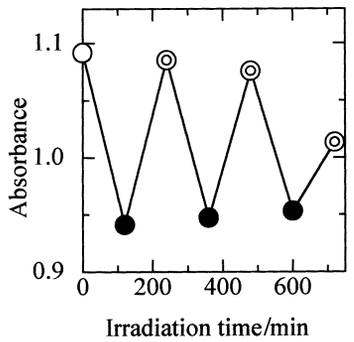

Fig. 2 Absorption change of CD–Thy at 271 nm by exposure to UV light at 280 and 240 nm. The photo-irradiation was performed in a quartz cell containing aqueous CD–Thy solution (0.02 mM) at pH 7: ○, before photo-irradiation; ●, after UV irradiation at 280 nm; ◎, after UV irradiation at 240 nm.

Table 1 Hydrolysis of PNPA in the presence of CD derivatives at 20 °C[a]

Host	$k_{cat}/10^2$ s^{-1}	$K_{diss}/10^4$ M	(k_{cat}/K_{diss})/M^{-1} s^{-1}
β-CD	18.5 (±0.8)	60.2 (±3.1)	31 (±3)
CD–Thy	6.6 (±0.4)	15.7 (±0.2)	42 (±12)
CD–Thy–D[b]	5.1 (±0.05)	3.1 (±0.1)	162 (±6)
CD–Thy–M[c]	6.4 (±0.9)	11.6 (±3.2)	55 (±18)

[a] Hydrolysis was carried out at pH 11.6 (±0.1) using a sodium carbonate buffer (0.2 M). [PNPA] = 0.1 mM. The first-order rate constants for the hydrolysis of PNPA in the absence of cyclodextrin (k_{un}) was 2.96×10^{-2} s^{-1}. [b] After the exposure of CD–Thy to UV light at 280 nm for 120 min. [c] After the further exposure of CD–Thy–D to UV light at 240 nm for 120 min.

between the actual percentage of photodimerized thymine groups and the absorption change observed. By irradiation at 280 nm, the absorption at 271 nm corresponding to the thymine moiety decreased by 14%, whereas the peak area corresponding to the proton at the C(6) position (7.43 ppm)[16] and the methyl proton (1.81 ppm) of the thymine ring decreased by 22%. This result shows that the observed absorption change was smaller than the decrease in the actual concentration of monomeric thymine groups, which might be partly due to the overlap of the absorption of dimeric species with that of monomeric ones, and partly due to the extremely large local concentration of thymine groups (Beer's law was not obeyed). Therefore, by irradiation at 280 nm for 120 min, 1.5 thymine groups per CD molecule would make photodimerization on average (three photodimers per four CD molecules).

The GPC measurements showed that the molecular size of CD–Thy did not change before or after photo-irradiation, which shows that intramolecular dimerization of thymine groups occurred. These phenomena suggest that thymine groups binding to CD molecules made the intramolecular photodimerization and photodissociation partly reversible by UV irradiation.

Hydrolysis of PNPA in the presence of cyclodextrin derivatives

Hydrolysis of *p*-nitrophenyl acetate (PNPA) in the presence of cyclodextrin derivatives were carried out. The pseudo-first-order rate constants (k_{un}, k_{cat}) and dissociation constants (K_{diss}) were estimated from measurements of absorbance of the product, the *p*-nitrophenolate (PNP) anion, and compiled in Table 1. These results show the remarkable effects of the modification of cyclodextrin with thymine residues and the dimerization of thymine residues on the rate of the hydrolysis.

By modifying CD with thymine, the catalytic efficiency (k_{cat}/K_{diss}, the apparent second-order rate constant that describes the rate in terms of the concentration of free catalyst and free substrate)[17] increased due to the decrease in the dissociation constant (K_{diss}). There is another possibility that nucleophilic attack of the secondary amino group of CD–Thy derived from ethylenediamine partly participates in the hydrolysis of the phenyl ester. Since dependence of the catalytic efficiency of CD–Thy on pH was similar to that of unmodified CD and the efficiency drastically reduced below pH 10 (pK_a of the secondary hydroxy group of CD is 12.3[18]), the catalytic effect of the secondary amine might be small. The rate constant (k_{cat}) for the CD modified with thymine before photo-irradiation (CD–Thy) was about *ca*. 40% of that for unmodified CD, whereas K_{diss} was about *ca*. 25%. These phenomena might be induced by the increase in hydrophobic microenvironment around the CD cavity due to a high local concentration of thymine, which would enhance the hydrophobic interaction between PNPA and CD–Thy. To be effectively hydrolysed, the carbonyl group of the substrate has to approach one of the dissociated secondary hydroxy groups located on the rim of the CD cavity. The nucleophilic attack of the ester by the dissociated hydroxy group of CD–Thy was, however, sterically hindered by the too deep penetration of the substrate into the cavity (which decreases the k_{cat} value), giving a slight increase in k_{cat}/K_{diss}.

Furthermore, by exposure to UV light at 280 nm, which induced the photodimerization of the thymine moiety (CD–Thy–D), the catalytic efficiency was about four times larger than that of CD–Thy. The k_{cat} of CD–Thy–D was *ca*. 77% of that for CD–Thy, whereas K_{diss} was *ca*. 20%. The large decrease in K_{diss} would imply that the capping effect was induced by the photodimerization of thymine residues on the rim of the cyclodextrin. Since the new hydrophobic field would be afforded adjacent to the CD cavity with the photodimerization of thymine groups (substituted to the primary OH groups at the 6 position), the capacity of hydrophobic cavity of CD–Thy–D would be larger than those of unmodified CD and CD–Thy (Scheme 1). The substrate PNPA having a substituent group at the *para* position would be included more deeply and tightly into the cavity of the CD–Thy–D than unmodified CD and CD–Thy, which results in the decrease in both k_{cat} and K_{diss}.

By exposure to UV light at 240 nm, which causes the photodimerized thymine to revert to monomeric thymine, both k_{cat} and K_{diss} increased, but the values did not reach those of the starting CD–Thy. These results show that some photodimerized thymine groups did not revert to the monomeric form (CD–Thy–M) by photo-irradiation, which is in agreement with the result of absorption measurements (Fig. 2).

Hydrolyses of MNPA and NABA in the presence of CD derivatives

In the hydrolysis of NABA, CD accelerated the reaction ($k_{cat} = 0.12$ s^{-1} and $K_{diss} = 6.6$ mM), whereas CD–Thy showed an inhibitory effect (Fig. 3). This unexpected result is probably due to the steric hindrance of substituents (both nitro and carboxy groups) restricting the inclusion of NABA in the CD cavity to make the ester group approach to the secondary hydroxy groups in the modified CD: it seemed to be sterically very difficult for the substrate to penetrate into the cavity of CD–Thy through the inlet at the C(6) side. For catalytic hydrolysis, therefore, the phenyl group of the substrate has to penetrate into the cavity through the inlet at the secondary hydroxy group side, which would be largely hindered by the bulky substituents at the *meta* and *para* positions.

The inhibition constant, K_i, estimated for CD–Thy (1.1 mM) was reduced by photo-irradiation at 280 nm (0.53 mM for CD–Thy–D) and slightly increased by the subsequent irradiation at 240 nm (0.69 mM for CD–Thy–M). The increase in the inhibitory effect by the dimerization is probably due to the disadvantageous cooperative effect of (*i*) the closure of the inlet at the C(6) side, (*ii*) the increase in capacity of the hydrophobic cavity of CD–Thy–D (the substrate once included might be too deeply penetrated into the cavity) and (*iii*) the steric hindrance of the substituents for the substrate to penetrate into the CD cavity.

Observations made in the hydrolysis of MNPA were similar

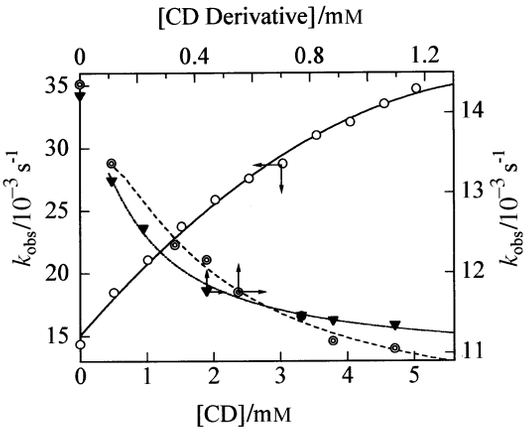

Fig. 3 Effect of cyclodextrin derivatives on k_{obs} for the hydrolysis of NABA at 20 °C and pH 11.6 (0.2 M sodium carbonate buffer): ○, CD; ◎, CD–Thy; ▼, CD–Thy–D

Table 2 Hydrolysis of MNPA in the presence of CD derivatives at 20 °C[a]

Host	$k_{cat}/10^2$ s^{-1}	$K_{diss}/10^4$ M	(k_{cat}/K_{diss})/M^{-1} s^{-1}
β-CD	46.4 (±3.2)	98.3 (±8.6)	49 (±8)
CD–Thy	18.8 (±0.7)	11.5 (±1.1)	164 (±21)
CD–Thy–D[b]	15.8 (±0.7)	6.9 (±0.4)	231 (±20)
CD–Thy–M[c]	16.5 (±0.5)	10.1 (±0.9)	162 (±20)

[a] Hydrolysis was carried out at pH 11.6 (±0.1) using a sodium carbonate buffer (0.2 M). [MNPA] = 0.1 mM. The first-order rate constants for the hydrolysis of MNPA in the absence of cyclodextrin (k_{un}) was 1.18×10^{-2} s^{-1}. [b] After the exposure of CD–Thy to UV light at 280 nm for 120 min. [c] After the further exposure of CD–Thy–D to UV light at 240 nm for 120 min.

to those for the hydrolysis of PNPA, *i.e.* the introduction of the thymine group increased the catalytic efficiency and the photoirradiation at 280 nm further promoted the efficiency, whereas the subsequent irradiation at 240 nm caused a reduction in efficiency (Table 2). The size of the increase caused by photodimerization at 280 nm was, however, smaller than that for PNPA, probably because of the similar, but much smaller steric effect than that in the hydrolysis of NABA. This might be due to the smaller number of substituents than NABA, which resulted in the acceleration effect on the hydrolysis of MNPA.

In conclusion, by photodimerization of the thymine groups in CD–Thy, PNPA and MNPA molecules could be tightly included into the CD cavity and the catalytic efficiency of CD–Thy was promoted. Such cyclodextrin derivatives modified with thymine groups would be useful to obtain information for designing highly functional photoresponsive compounds.

Acknowledgements

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