Stabilizing and Solubilizing Effects of Sulfobutyl Ether β-Cyclodextrin on Prostaglandin E_1 Analogue

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Purpose. Parent cyclodextrins are known to accelerate the degradations such as dehydration and isomerization of E-type prostaglandins in neutral and alkaline solutions. The objective of this study was to attempt the stabilization and solubilization of E_1-type prostaglandin analogue in aqueous solution by biocompatible cyclodextrin derivatives.

Methods. The interaction of an E_1-type prostaglandin, methyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxy-4-(*m*-methoxymethylphenyl)1-butenyl]-5-oxocyclopentyl]-5-thiaheptanoate (MEester) with cyclodextrins (CyDs) was studied by spectroscopies and the solubility method. The degradation of MEester was monitored by high-performance liquid chromatography.

Results. ^{1}H-nuclear magnetic resonance spectroscopic studies indicated that MEester forms 1:1 inclusion complexes with α-, β-, and γ-CyDs in solutions, where α-CyD interacts with the α-side chain containing methyl ester moiety of the drug, whereas β- and γ-CyDs preferentially include around the five-membered ring and both side chains of the drug. Parent α-CyD and hydrophilic derivatives, such as 2-hydoxypropyl-α- and -β-CyDs, sulfobutyl ether β-CyD (SBE-β-CyD) and maltosyl β-CyD showed higher solubilizing abilities against MEester over parent β- and γ-CyDs. SBE-β-CyD and 2,6-dimethyl-β-CyD (DM-β-CyD) significantly decelerated the degradation of MEester, particularly the base-catalyzed dehydration, in neutral and alkaline solutions, whereas other CyDs accelerated the degradation. The acid-catalyzed degradation of MEester (pH < 3) was decelerated by the addition of CyDs, especially α-CyD.

Conclusions. SBE-β-CyD with low hemolytic activity and low toxicity is useful as a pharmaceutical carrier for the preparation of injectable MEester, because of its higher stabilizing and solubilizing effects on MEester. Furthermore, SBE-β-CyD can be useful as a stabilizing agent for drugs, that are subject to base-catalyzed degradations, probably because of the electric repulsion between anionic charges of the sulfobutyl moiety and catalytic anionic species such as hydroxide ion.

KEY WORDS: prostaglandin E_1 analogue; sulfobutyl ether β-cyclodextrin; inclusion complex; stabilization; solubilization.

INTRODUCTION

Prostaglandins are essentially long-chain fatty acids containing a substituted cyclopentanone ring (1,2). Some commercial products of prostaglandins are formulated as inclusion complexes with α- and β-cyclodextrins to improve their poor aqueous solubility and chemically labile property (3). However, the limited solubility and chemical instability of prostaglandins are still serious obstacles for the development of new formulations and preparations (4–9). For example, cyclodextrins can improve the chemical stability of E-type prostaglandins in the solid state, but they accelerate the dehydration and isomerization rates of E-type prostaglandins in aqueous neutral and alkaline solutions (10,11). This acceleration was attributable to the catalytic effect of hydroxyl groups of cyclodextrins.

Recently, various kinds of cyclodextrin derivatives were prepared to extend physicochemical, biologic, and inclusion capacities of cyclodextrins as multifunctional drug carriers (12–15). Hydrophilic cyclodextrin derivatives have been applied for the improvement of low solubility, dissolution rate, and bioavailability of poorly water-soluble drugs, whereas hydrophobic ethylated and acylated cyclodextrin derivatives are useful as slow-releasing carriers for water-soluble drugs with short biologic half-lives. Among various hydrophilic cyclodextrin derivatives, 2-hydroxypropyl-β-cyclodextrin and sulfobutyl ether β-cyclodextrin seem to be the prospective carriers for solubilization and stabilization of highly hydrophobic drugs in parenteral preparations, because of their superior safety profile (16,17). In this study, the effects of various cyclodextrin derivatives on the stability and solubility of a E_1-type prostaglandin, methyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxy-4-(*m*-methoxymethylphenyl)-1-butenyl]-5-oxocyclopentyl]-5-thiaheptanoate (MEester, Scheme 1), were investigated, in the anticipation of both stabilization and solubilization in aqueous solution. Parent α-, β-, and γ-CyDs, and biocompatible CyD derivatives, i.e., 2-hydroxypropyl-α- and -β-CyDs, sulfobutyl ether β-CyD, and maltosyl-β-CyD were mainly used in this study, but dimethyl-β-CyD was ruled out because of its relatively high hemolytic activity and muscular irritation (18).

MATERIALS AND METHOD

Materials

α-, β-, and γ-cyclodextrins (α-, β-, and γ-CyDs), 2-hydroxypropyl-α- and β-CyDs (HP-α- and -β-CyDs, degree of substitution (D.S.) = 4.1 and 4.8, respectively), and dimethyl-β-CyD (DM-β-CyD) were supplied from Japan Maize Co. (Tokyo, Japan). Sulfobutyl ether β-CyD (SBE-β-CyD, D.S. = 6.2) was donated by CyDex (Overland Park, KS). Maltosyl-β-CyD (G_2-β-CyD) was obtained from the Bio Research Corporation of Yokohama (Yokohama, Japan). MEester, its carboxylic acid (MECOOH) and the A-type MEester (MAester) were supplied from Ono Pharmaceutical Co. (Osaka, Japan). All other chemicals and solvents were of analytic reagent grade, and deionized double-distilled water was used throughout the study.

Solubility Measurements

Solubility study was carried out according to the method of Higuchi and Connors (19). The screw-capped vials containing MEester (40 mg) in excess amounts in aqueous CyD solutions (1.0 mL, pH 7.4 phosphate buffer (I = 0.2)) at various concentrations were shaken at 25°C and 37°C for 12 h, under which conditions there was neither degradations of MEester

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nor pH changes of the solution. After the shaking, the solution was centrifuged at 2500 rpm for 5 min, and the supernatant was filtered through a membrane filter (ADVANTEC DISMIC-13CP, Toyo-Rochi, Tokyo, Japan), and analyzed for MEester by using high-performance liquid chromatography (HPLC) under the following conditions: a Hitachi 655A pump, a 635-A UV detector, and an Hitachi D-2500 Chromato-integrator (Tokyo, Japan), a G.L. Science Inertsil ODS-2 column (5 μm, 4.6 mm × 150 mm, Tokyo, Japan), a mobile phase of acetonitrile/20 mM potassium dihydrogenphosphate (4:6 v/v), a flow rate of 1.0 mL/min, and a detection of 205 nm. The 1:1 stability constant (Kc) of CyD complexes was calculated from the initial straight line portion of the phase solubility diagrams, Kc = slope/S_0 (1-slope) (18), where S_0 is the intrinsic solubility of drugs.

Spectroscopic Studies

Ultraviolet (UV) and circular dichroism (CD) spectra were measured at 25°C using a Hitachi U-2000 UV spectrometer (Tokyo, Japan) and a Jasco J-720 polarimeter (Tokyo, Japan), respectively. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Jeol JNM-α 500 instrument (Tokyo, Japan), operating at 500.16 MHz at 25°C. MEester and CyDs were dissolved in deuterium oxide (D_2O) or in dimethylsulfoxide-d_6 (DMSO). The chemical shifts were given as parts per million (ppm) downfield from that of tetramethylsilane. The phase-sensitive rotating frame nuclear Overhauser effect (ROESY) spectra were measured under the following conditions: sweep width of 5000 Hz, spin-lock field of 6 kHz, and mixing time of 250 ms.

Kinetic Studies

The dehydration and the ester hydrolysis of MEester were monitored by HPLC for the disappearance of MEester and the appearance of MAester and MECOOH. The reaction was initiated by the addition of a stock solution (0.04 mL) of MEester in methanol to phosphate buffer (4.0 mL, I = 0.2) at constant temperatures (generally 60°C). The initial concentrations of MEester and methanol were 5.0×10^{-5} M and 0.99% v/v, respectively. The pH of the sample after the reaction was confirmed to be identical with the initial pH. The HPLC conditions were the same as that described above. The dehydration rate constant (k_1) and the ester-hydrolysis rate constant (k_2) were determined by analyzing the time profiles of concentrations of MEester, its ester-hydrolyzed product (MECOOH) and A-type MEester (MAester), by means of a nonlinear least-squares method using the MULTI program (20) (Scheme 1).

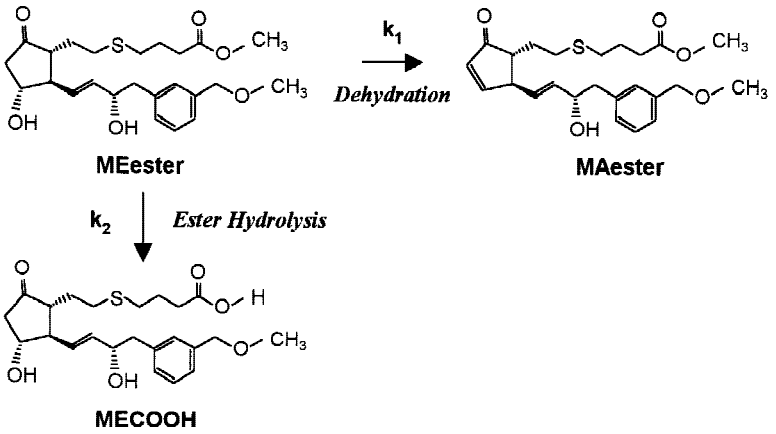
RESULTS AND DISCUSSION

Solubilizing Effects of CyDs

Figure 1 shows phase solubility diagrams of MEester with parent α-, β-, and γ-CyDs, HP-α- and -β-CyDs, G_2-β-CyD, and SBE-β-CyD in aqueous solution at 25°C. The γ-CyD system showed a typical B_S type (19) phase solubility diagram with MEester, whereas other CyDs showed A_L type (19) diagrams. The stability constants (Kc) of the complexes were calculated from the initial straight line of the diagrams and were 403 (±19) M^{-1} for α-CyD, 734 (±38) M^{-1} for β-CyD, 129 (±11) M^{-1} for γ-CyD, 235 (±15) M^{-1} for HP-α-CyD, 468 (±24) M^{-1} for SBE-β-CyD, and 308 (±21) M^{-1} for G_2-β-CyD. The Kc value for the HP-β-CyD complex could not be determined because the slope of the initial straight line of the diagram was larger than unity. The chemical analysis of the solid γ-CyD complex, which was isolated at higher CyD concentrations, indicated the 1:1 complex formation of MEester with γ-CyD. This stoichiometry coincided with that determined from the length of the plateau region in the solubility diagram. The solubilizing effect of parent β- and γ-CyDs on MEester was limited, because of the low intrinsic solubility (1.6×10^{-2} M at 25°C) (14) of β-CyD and the limited solubility of the MEester/γ-CyD complex due to the Bs-type diagram. On the other hand, parent α-CyD and the other hydrophilic CyD derivatives significantly improved the low solubility of MEester, where the solubilizing effect was HP-β-CyD > SBE-β-CyD > α-CyD > G_2-β-CyD > HP-α-CyD. For example, the solubility of MEester was enhanced about 12, 11, and 10 times by the additions of 45 mM HP-β-CyD and 60 mM SBE-β-CyD and 60 mM parent α-CyD, respectively. The solubilizing and interacting abilities of CyDs became weaker at a higher temperature (37°C, data not shown), although the type of the solubility diagrams did not change.

Inclusion Complexation of MEester with CyDs

UV and CD spectroscopic studies were conducted to gain insight into the interaction of MEester with α-, β-, and



Scheme 1. Degradation pathway of MEester under the experimental conditions.

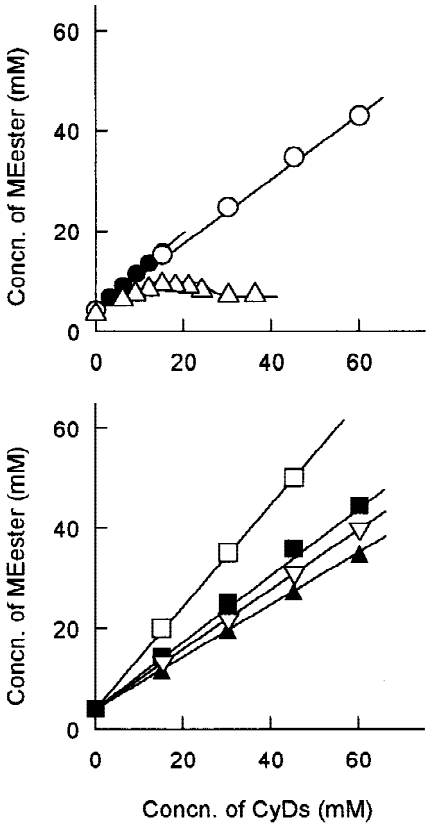


Fig. 1. Phase solubility diagrams of MEester/CyD systems in phosphate buffer (pH 7.4, $I = 0.2$) at 25°C. Key: (○) α-CyD, (●) β-CyD, (△) γ-CyD, (■) SBE-β-CyD, (▲) HP-α-CyD, (□) HP-β-CyD, and (▽) G_2-β-CyD.

γ-CyDs and SBE-β-CyD in aqueous solution (pH 7.4, 25°C, data not shown). MEester gave a UV absorption maximum (λmax) at 205 nm and a shoulder around 215 nm. The UV intensity of MEester at 205 nm was significantly decreased by the addition of β-CyD, whereas it was increased by SBE-β-CyDs. On the other hand, α- and γ-CyDs only slightly affected the UV spectrum of MEester (γ-CyD > α-CyD). There was no quantitative relationship between magnitudes of the spectral change and the stability constant of the complexes. MEester gave a negative Cotton effect at 287 nm owing to the n-π* transition (21) in the CD spectrum. This CD intensity increased by the addition of CyDs, with the change in the order of γ-CyD > α-CyD > β-CyD, whereas it was decreased by the addition of SBE-β-CyD with a concomitant shift of the λ_{max} to longer wavelength. No relationship between magnitudes of the CD change and the stability constant was observed, suggesting different inclusion modes and/or sites of the complexes.

^{1}H NMR spectroscopic studies were carried out to gain an insight into the inclusion mode of MEester/CyD complexes in aqueous solution. Figure 2A shows the ^{1}H NMR spectrum of MEester in D_2O solution. The signals were assigned as indicated, according to the reports on PGE homologues and two-dimensional ^{1}H COSY spectroscopic data (22,23). Figures 2B–E show changes in ^{1}H chemical shifts of MEester by the addition of α-, β-, and γ-CyDs and SBE-β-CyD (3.0, 6.0, and 15.0 mM), where the positive sign is for low-field shift and the negative sign for up-field shift. Unfortunately, the change of protons other than those listed in Fig. 2B–E, could not accurately be measured, because of the overlapping and broadening of signals. Upon the binding to α-CyDs, a large low-field shift was observed at the H3 proton in the α-side chain of MEester, whereas other protons were little affected. On the other hand, the signals of the H7, H8, H13, and H16 protons around the five-membered ring and the ω-side chain significantly shifted, through the binding of β-CyD, γ-CyD, and SBE-β-CyD, where the β-CyD-induced shifts were larger than the γ-CyD-induced shifts. The shift changes augmented as CyD concentrations increased (3.0, 6.0, and 15.0 mM). The stoichiometry of the complexes was estimated by the continuous variation method (24), by monitoring the chemical shifts of the H3 or H16 protons of MEester. The continuous variation plots gave a maximum at 0.5 host/guest molar ratio, indicating that MEester forms inclusion complexes with these CyDs in a molar ratio of 1:1 in aqueous solution. These results suggested that α-CyD with the smaller cavity interacts preferably with the α-side chain of MEester, whereas the β- and γ-CyD, having larger cavities interact with the five-membered ring and the ω-side chain. The SBE-β-CyD complex may have a similar inclusion structure as that of the β-CyD complex, because the similar chemical shift change was observed for both complexes, as shown in Figs. 2C and 2E.

The two-dimensional ROESY spectroscopic studies were carried out to confirm the inclusion mode of the CyD complexes. Figures 3A and 3B show partial counter plots of ROESY spectra of MEester (10 mM)/α- and β-CyDs (10 mM) systems in 50% v/v DMSO-d_6/D_2O solution. The mixed solvent of DMSO-d_6/D_2O was used to obtain concentrations of MEester high enough for measurements of high-quality spectra. The essential feature of the interaction in the mixed solvent may be similar to that in water, because the difference in dielectric constant between DMSO ($\epsilon = 46.8$) and water (78.5) becomes relatively smaller or negligible when highly hydrophobic guest molecules such as MEester are used (25,26). As shown in Fig. 3A, the H2 and H4 protons of the α-side chain of MEester gave a correlation peak with the H3′ proton, which locates inside the cavity of α-CyD, whereas the Hg and Hh protons of the ω-side chain of the guest gave correlation peaks with the H1′ proton, which locates outside the cavity. These results indicate that α-CyD with a smaller cavity includes preferably the α-side chain of MEester, and the ω-side chain is located outside the cavity. On the other hand, no apparent correlation peaks between the inner protons of β-CyD and the protons of MEester were observed, probably because the interatomic distance between the guest and the host is longer due to the large cavity of β-CyD. However, the outer H1′ proton of β-CyD gave correlation peaks to the benzene protons (Ha, Hc, and He) and the α-side chain protons (H2 and H4) of MEester, as shown in Fig. 3B. These results suggest that the α- and ω-side chains of the guest are located outside of the β-CyD cavity. Together with the NMR spectroscopic results reported previously (27,28), we concluded the inclusion mode of MEester/α- and β-CyD complexes, as shown in Fig. 4, in which the α-side chain of MEester is preferably included in the α-CyD cavity, whereas the portion around the five-membered ring is included in the β-CyD cavity.

Stabilizing Effects of CyDs

The effects of various CyDs on the degradation of MEester were investigated, in anticipation of improving its

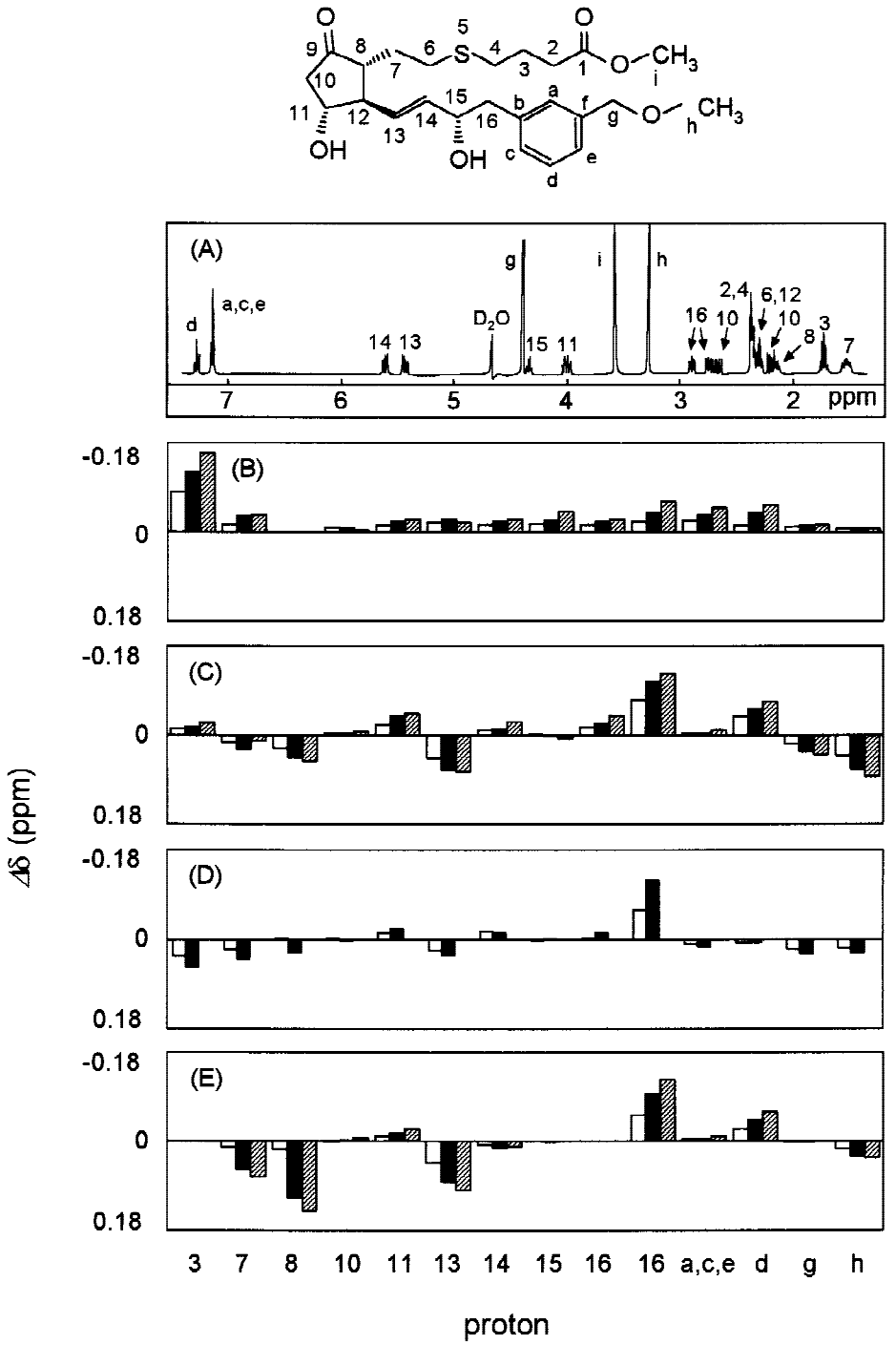


Fig. 2. Displacements of ^{1}H NMR chemical shifts of MEester (3.0×10^{-3} M) by the addition of CyDs in D_2O at 25°C: (A) ^{1}H-NMR spectrum of MEester alone, (B–E) Shift displacements ($\Delta\delta = \delta_{\text{with CyD}} - \delta_{\text{without CyDs}}$) induced by the addition of α-, β-, and γ-CyDs and SBE-β-CyD, respectively. Concentrations of CyDs were □ 3.0×10^{-3} M, ■ 6.0×10^{-3} M, ▨ 15.0×10^{-3} M.

chemical stability through the inclusion complex formation in aqueous solution. The β-hydroxyketo moiety of E-type prostaglandins is susceptible to dehydration in acidic and alkaline conditions to form A-type prostaglandins, which are isomerized consecutively to form B-type prostaglandins with a loss of pharmacologic activities (1). Because MEester has an additional reactive site, methyl ester, in a molecule, the degradation will proceed simultaneously via the dehydration and isomerization of the five-membered ring and the hydrolysis of methyl ester in the α-side chain. HPLC monitoring revealed that MEester degrades mainly to the A-type MEester (MAester) and the hydrolysis product (MECOOH), whereas negligibly to the B-type prostaglandin (< about 1%) and the hydrolysis product of MAester (< about 5%). These results indicated that MEester degrades according to Scheme 1 under the experimental conditions. The effects of CyDs on the degradation of MEester in weak alkaline and acidic solutions were investigated.

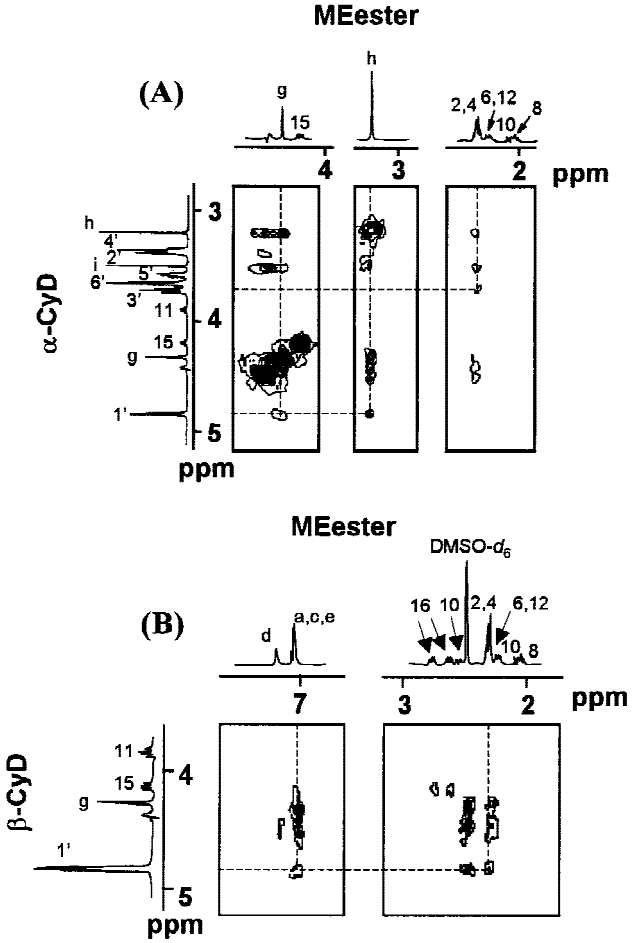


Fig. 3. Partial counter plots of ROESY spectra of MEester/α-CyD (A) and /β-CyD (B) systems in 50% v/v DMSO-d_6/D_2O solution at 25°C. The concentrations of MEester and CyDs were 1.0×10^{-2} M.

In Alkaline Solution

Figure 5 shows time courses for the disappearance of MEester and the appearances of MAester and MECOOH in the absence and presence of α-CyD and SBE-β-CyD, as examples, in weak alkaline solutions (pH 8.0) at 60°C. These profiles were analyzed according to Scheme 1, to obtain the dehydration rate constant (k_1) and the ester-hydrolysis rate constant (k_2). The results on the total disappearance rate constants ($k_{obs} = k_1$ and k_2) and k_1 and k_2 values are listed in Table I. The k_1 values were larger by about 4–14 times than the k_2 values, indicating the preponderance of the dehydration over the ester hydrolysis in the degradation of MEester in neutral and alkaline solutions. It is of interest that SBE-β-CyD and DM-β-CyD significantly decelerated the dehydration (k_1), in contrast to the acceleration by parent α-, β-, and γ-CyD, HP-α- and -β-CyDs, and G_2-β-CyD. The dehydration of the β-hydroxyketo moiety of E-type prostaglandins is known to proceed through an enol intermediate, and this reaction is subject to a general-base catalysis (7,9). We previously reported that the hydroxyl groups of parent CyDs work as a general catalyst in the dehydration of E-type prostaglandins to accelerate the reaction (10,11). HP-α- and -β-CyDs and G_2-β-CyD accelerated the dehydration of MEester, probably in the same mechanism, because the

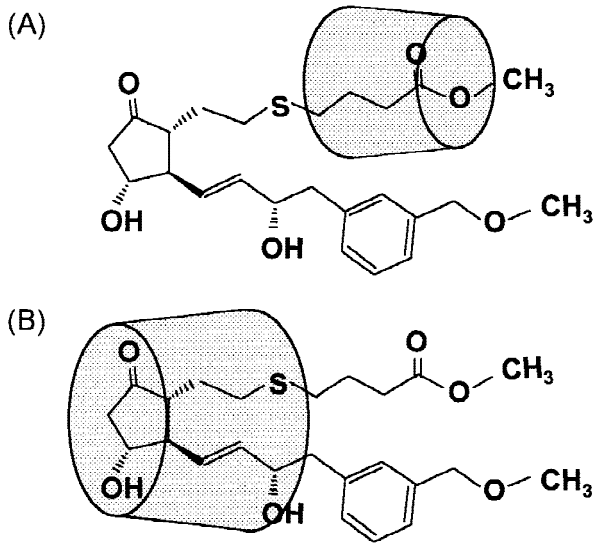


Fig. 4. Proposed inclusion mode for MEester/α-CyD (A) and β-CyD (B) complexes in aqueous solution.

former have many catalytic hydroxyl groups in the glucose moieties and in the 2-hydroxypropyl groups and the latter has 20 hydroxyl groups in the β-CyD unit and 7 hydroxyl groups in the maltose unit. On the other hand, the catalytic function of DM-β-CyD and SBE-β-CyD may be lost by the substitu-

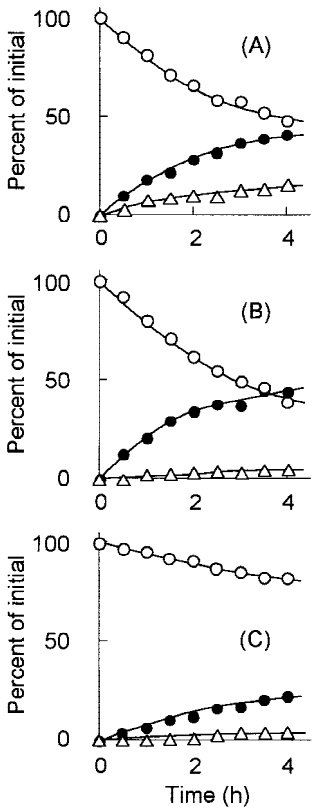


Fig. 5. Time courses for disappearance of MEester (5.0×10^{-5} M) and appearance of its degradation products, MAester and MECOOH, in the absence (A) and presence of α-CyD (B, 5.0×10^{-2} M) and SBE-β-CyD (C, 5.0×10^{-2} M) in phosphate buffer (pH 8.0, $I = 0.2$) at 60°C. Key: (○) MEester, (●) MAester, (△) MECOOH.

Table I. Degradation (k_{obs}), Dehydration (k_1) and Hydrolysis (k_2) Rate Constants[a] of MEester (5.0×10^{-5} M) in the Absence and Presence of CyDs (5.0×10^{-2} M) in Phosphate Buffer (pH 8.0, $I = 0.2$) at 60°C

System	k_{obs} (h^{-1})	k_1 (h^{-1})	k_2 (h^{-1})
Alone	0.170 ± 0.007 (1.0)[b]	0.138 ± 0.001 (1.0)	0.0320 ± 0.007 (1.0)
α-CyD	0.183 ± 0.002 (1.1)	0.169 ± 0.002 (1.2)	0.0133 ± 0.002 (0.4)
β-CyD[c]	0.207 ± 0.006 (1.2)	0.176 ± 0.004 (1.3)	0.0309 ± 0.002 (1.0)
γ-CyD	0.180 ± 0.001 (1.1)	0.156 ± 0.001 (1.1)	0.0241 ± 0.001 (0.8)
HP-α-CyD	0.181 ± 0.003 (1.1)	0.160 ± 0.002 (1.2)	0.0207 ± 0.002 (0.7)
HP-β-CyD	0.185 ± 0.002 (1.1)	0.163 ± 0.001 (1.2)	0.0222 ± 0.001 (0.7)
SBE7-β-CyD	0.061 ± 0.002 (0.4)	0.055 ± 0.001 (0.4)	0.0058 ± 0.001 (0.2)
G_2-β-CyD	0.214 ± 0.004 (1.2)	0.196 ± 0.002 (1.4)	0.0182 ± 0.002 (0.6)
DM-β-CyD[c]	0.047 ± 0.001 (0.3)	0.041 ± 0.001 (0.3)	0.0063 ± 0.002 (0.2)

[a] Mean ± SE of three to four experiments.
[b] Values in parentheses show rate change ratios induced by CyDs ($k_{with\ CyD}/k_{without\ CyDs}$).
[c] 1.0×10^{-2} M.

tions of the hydroxyl groups by methyl and sulfobutyl moieties, respectively. For example, most hydroxyl groups (14 of 21) were methylated in DM-β-CyD molecule, decelerating the dehydration through a steric protection of the reactive site. SBE-β-CyD decelerated the reaction, despite the low degree of the substitution 6.2. The electric repulsion against catalytic hydroxide ions and other anionic species, together with the steric protection, may be additionally operative in the deceleration, because SBE-β-CyD has anionic charges at the terminal sulfobutyl moieties. The ester hydrolysis (k_2, Table I) was decelerated by the addition of all CyDs used, where SBE-β-CyD, DM-β-CyD and parent α-CyD had larger decelerating effects. The hydrophobic and anionic environments of DM-β-CyD and SBE-β-CyD, respectively, may make an access of catalytic anionic ions to the reactive site difficult. The deceleration by α-CyD is attributable to the inclusion of the methyl ester of MEester.

In Acidic Solution

Table II shows the dehydration (k_1) and ester-hydrolysis (k_2) rate constants of MEester in an acidic condition (pH 2.4). Both degradation pathways equally contributed to the total degradation of MEester ($k_1 = 0.0205\ h^{-1}$ and $k_2 = 0.0153\ h^{-1}$), in contrast to the case of pH 8.0. Parent α-CyD significantly inhibited the ester hydrolysis, which can be ascribed to the inclusion of the reactive site, methyl ester moiety. In sharp contrast to the case of the alkaline degradation, the decelerating effect of SBE-β-CyD was smaller than those of α- and β-CyDs. The deceleration may be partially canceled out by the acceleration through enrichment of catalytic hydrogen ions around the guest molecule, due to an electric attraction with anion charges of the host molecule.

Effects of CyD Concentration and pH on Degradation of MEester

Figure 6A shows the changes in the degradation rate constant ($k_{obs} = k_1 + k_2$) of MEester as a function of α-CyD and SBE-β-CyD at pH 7.4 where the dehydration (k_1) was predominant over the ester hydrolysis (k_2). The degradation rate increased and decreased with augmenting concentrations of the former and latter CyDs, respectively, and saturated at higher CyD concentrations. The CyD concentration dependency of k_{obs} values was treated by Colter's equation [Eq. (1)] (29) derived on the basis of 1:1 complex formation, to obtain the stability constant (Kc) and the rate constant (kc) in the complex:

$$[CyD]_t/(k_o - k_{obs}) = (1/(k_o - kc))[CyD]_t + 1/(Kc(k_o - kc)) \quad (1)$$

where $[CyD]_t$ is total concentration of CyDs and k_o and k_{obs} are the rate constants in the absence and presence of CyDs, respectively. The plots of the lefthand side vs. CyD concentrations according to Eq. (1) gave straight lines ($r > 0.998$), from which the following parameters were obtained: $Kc = 65\ M^{-1}$ and $kc = 0.111\ h^{-1}$ for the α-CyD complex and $Kc = 103\ M^{-1}$ and $kc = 0.0438\ h^{-1}$ for the SBE-β-CyD complex. Therefore, the degradation rate of MEester was decreased to one half of the rate without CyDs ($k_o = 0.0842\ h^{-1}$) by the com-

Table II. Degradation (k_{obs}), Dehydration (k_1) and Hydrolysis (k_2) Rate Constants[a] of MEester (5.0×10^{-5} M) in the Absence and Presence of CyDs (5.0×10^{-2} M) in Phosphate Buffer (pH 2.4, $I = 0.2$) at 60°C

System	k_{obs} (h^{-1})	k_1 (h^{-1})	k_2 (h^{-1})
Alone	0.0358 ± 0.002 (1.0)[b]	0.0205 ± 0.001 (1.0)	0.0153 ± 0.001 (1.0)
α-CyD	0.0179 ± 0.002 (0.5)	0.0135 ± 0.001 (0.7)	0.0044 ± 0.001 (0.3)
β-CyD[c]	0.0251 ± 0.001 (0.7)	0.0166 ± 0.001 (0.8)	0.0085 ± 0.001 (0.6)
SBE7-β-CyD	0.0349 ± 0.003 (1.0)	0.0223 ± 0.002 (1.1)	0.0126 ± 0.002 (0.8)

[a] Mean ± SE of three to four experiments.
[b] Values in parentheses show rate change ratios induced by CyDs ($k_{with\ CyD}/k_{without\ CyDs}$).
[c] 1.0×10^{-2} M.

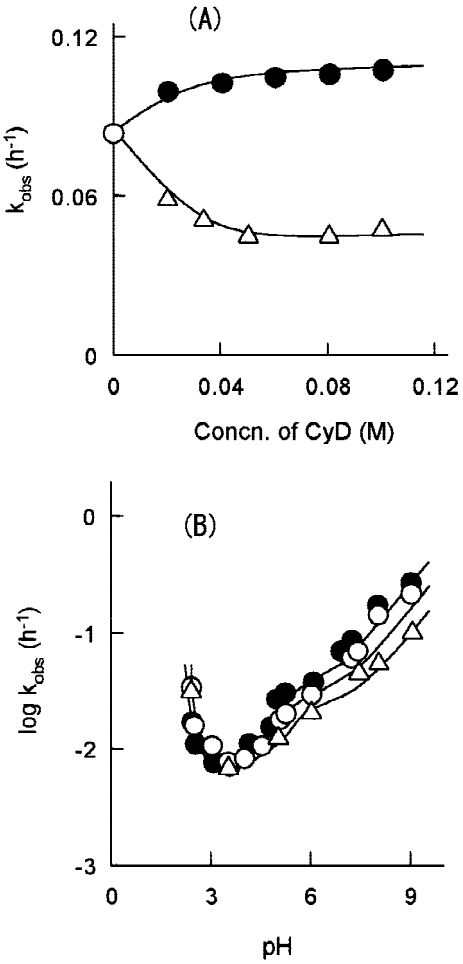


Fig. 6. (A) Degradation rate constants (k_{obs}, pH 7.4, I = 0.2, 60°C) of MEester (5.0×10^{-5} M) as a function of CyD concentrations and (B) pH profiles for k_{obs} of MEester (5.0×10^{-5} M) in the absence and presence of CyDs (5.0×10^{-2} M) in phosphate buffer (I = 0.2) at 60°C. Key: (○) without CyDs, (●) with α-CyD, (△) with SBE-β-CyD.

plexation with SBE-β-CyD, whereas it was accelerated by about 1.3 times by the complexation with α-CyD. Figure 6B shows the pH profiles for the degradation rates (k_{obs}) of MEester in the absence and presence of α-CyD and SBE-β-CyD. MEester was most stable at pH 3.5 (60°C) (8). The degradation of MEester above pH 4 was accelerated by the addition of parent α-CyD, whereas it was decelerated by the addition of SBE-β-CyD. On the other hand, the degradation below pH 3 was decelerated by the addition of α-CyD, whereas it was only slightly decelerated by SBE-β-CyD.

CONCLUSIONS

The limited solubility and chemical instability of prostaglandins are still serious obstacles for the development of new formulations and preparations, resulting in a substantial challenge to pharmaceutical scientists. In this study, we found that the limited solubility of MEester in water is markedly improved by the complexation with α-CyD, HP-α- and -β-CyDs, G_2-β-CyD, and SBE-β-CyD. However, only DM-β-CyD and SBE-β-CyD can improve the chemical instability of MEester in physiologic pH conditions, whereas other CyDs having catalytic hydroxyl groups in a molecule accelerated the degradation. Biopharmaceutical properties of SBE-β-CyD were well documented, and its safety is demonstrated, e.g., low hemolytic activity and fast elimination from blood to urine after intravenous administration (17,30). Therefore, SBE-β-CyD may be a promising candidate as a drug carrier for both solubilization and stabilization of E-type prostaglandins including MEester. Furthermore, SBE-β-CyD can be useful as a stabilizing agent for drugs that are subject to base-catalyzed degradations, probably because of the electric repulsion between anionic charges of the sulfobutyl moiety and catalytic anionic species such as hydroxide ion.

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