Regular Article

Formation of the Ternary Inclusion Complex of Limaprost with α- and β-Cyclodextrins in Aqueous Solution

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The inclusion mode of Limaprost in the presence of α- and β-cyclodextrins (CDs) was investigated to gain insight into the stabilization mechanism of Limaprost-alfadex upon the addition of β-CD in the solid state. The inclusion sites of α- and β-CDs were studied by NMR spectroscopic and kinetic methods. With the addition of α- and β-CDs, displacements in ^{13}C chemical shifts of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were observed in the ω-chain and the five-membered ring, respectively, of the drug. Similar shift changes were observed with the addition of both α- and β-CDs. In two-dimensional (2D) ^{1}H-NMR spectra, intermolecular correlation peaks were observed between protons of $PGF_{2\alpha}$ and protons of both α- and β-CDs, suggesting that $PGF_{2\alpha}$ interacts with α- and β-CDs to form a ternary complex by including the ω-chain with the former CD and the five-membered ring with the latter. In kinetic studies in aqueous solution, Limaprost was degraded to 17*S*,20-dimethyl-*trans*-Δ^2-PGA_1 (11-deoxy-Δ^{10}) and 17*S*,20-dimethyl-*trans*-Δ^2-8-iso-PGE_1 (8-iso). The addition of α-CD promoted the dehydration to 11-deoxy-Δ^{10}, while β-CD promoted the isomerization to 8-iso, under these conditions. In the presence of both α- and β-CDs, dehydration and isomerization were also accelerated, supporting the formation of the ternary Limaprost/α-CD/β-CD complex.

Key words Limaprost alfadex; α-cyclodextrin; β-cyclodextrin; ternary inclusion complex; NMR spectroscopy; kinetics

α-, β- and γ-Cyclodextrins (CDs) are cyclic oligosaccharides in which six, seven and eight D-glucose units, respectively, are linked by α-1,4 glycosidic bonds.[1–6] The outside of the ring is hydrophilic, while the center cavity is hydrophobic. Because CDs form inclusion complexes by encapsulating other compounds into their hydrophobic cavity, natural and chemically modified CDs are widely used as pharmaceutical and food additives. Many studies of CD inclusion complex formations have been conducted to improve physicochemical properties of drugs, such as poor water solubility,[7–10] degradation upon heating[11–15] or by light irradiation,[16–19] and oxidation.[20] However, most of these studies have examined the effect of a single type of CD. Only a few reports focus on the effect of two types of CDs. For example, Nikouei *et al.* reported that the simultaneous addition of both α-CD and 2-hydoxypropyl-β-CD (HP-β-CD) improved the solubility of Cyclosporine A (CyA), a poorly water-soluble drug, compared to the addition of one CD.[21] A similar synergistic effect on the solubility has been reported for dexamethasone by the addition of γ-CD and HP-γ-CD.[22,23] Although these reports focused on the synergistic effect of two CDs on drug solubility, the inclusion mode between the two CDs and the drug has not been studied in detail.

Limaprost is a prostaglandin E_1 (PGE_1) derivative that is approved for "treatment of various ischemic symptoms such as ulcers, pain, and sensation of coldness of the hands and feet associated with thromboangiitis obliterans" and for "improvement in subjective symptoms and walking ability, which accompany lumbar spinal canal stenosis." Limaprost is formulated as the α-CD complex, Limaprost-alfadex, in commercially available tablets, Opalmon® tablets, to ensure its dose uniformity and to improve its aqueous solubility, because of the very low content, 5 μg, in each tablet.[24–26]

Limaprost in Opalmon® tablets is chemically stable in Press-through-Package, but once the package is opened and placed in humid conditions, Limaprost degrades to 17*S*,20-dimethyl-*trans*-Δ^2-PGA_1 (referred to as 11-deoxy-Δ^{10}) and 17*S*,20-dimethyl-*trans*-Δ^2-8-iso-PGE_1 (referred to as 8-iso), as shown in Fig. 1. We have reported previously that the addition of dextran improved the stability of Limaprost-alfadex in humid conditions,[27–29] probably due to the increased interaction of the drug with α-CD in the presence of dextran.[30,31] Furthermore, we have found that the addition of β-CD to the Limaprost/α-CD (Limaprost-alfadex) improved the stability of the drug in humid conditions.[32] The stabilization of Limaprost by employing two CDs may be due to the formation of a ternary complex with the two CDs, but the inclusion mode of the prostaglandin derivative in the presence of two CDs does not yet confirmed.

In this study, we focus on the inclusion complex formation of Limaprost with two CDs, α-CD and β-CD, in solution. The interaction between the prostaglandin derivative and the two CDs was investigated by ^{1}H- and ^{13}C-NMR spectroscopy. In addition, the effects of α-CD and β-CD on the stability of Limaprost in aqueous solution were evaluated to estimate the inclusion mode of the drug.

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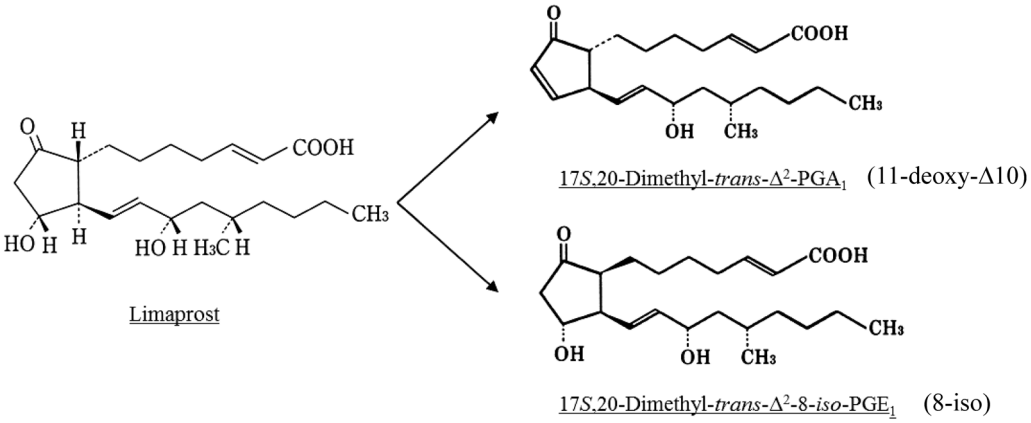

Fig. 1. Limaprost Decomposition Pathway

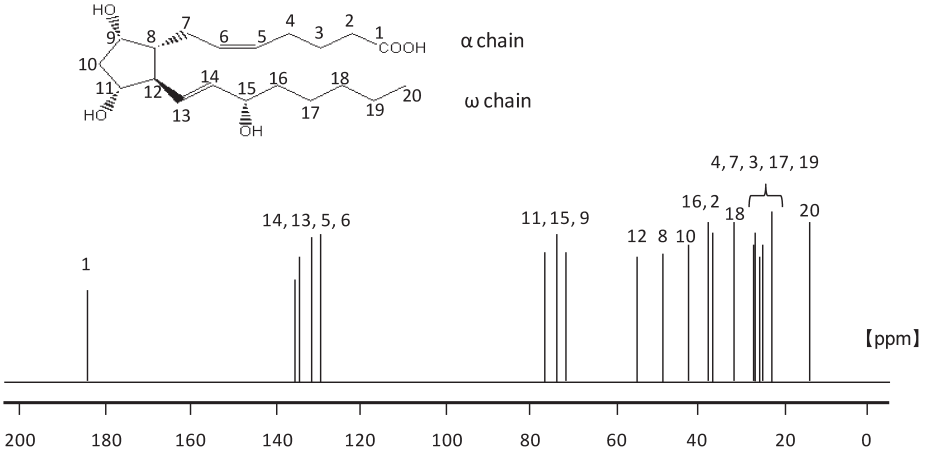

Fig. 2. ^{13}C-NMR Spectrum of $PGF_{2\alpha}$ (50 mM) in a 0.1 M Sodium Borate/D_2O Buffer (pH 9.3) at 25°C

Experimental

Materials Limaprost-alfadex is specified in the Japanese Pharmacopoeia (JP) XVI. All excipients used in this study were purchased in accordance with the specifications of JP XVI or Japanese Pharmaceutical Excipients. Deionized double-distilled water was used. All other chemicals and solvents were of analytical reagent grade.

^{13}C-NMR Studies: Interaction of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) with α- and β-CDs $PGF_{2\alpha}$ (50 mM) was dissolved in a 0.1 M sodium borate/D_2O solution (pH meter reading of 9.3). A predetermined concentration of α- or β-CD (0–50 mM) was added to the solution. ^{13}C-NMR measurements of the solution were acquired on a JEOL JNM-A500 NMR system (external magnetic field of 500 MHz) at 25°C and accumulated 10000 times. Chemical shifts were given as parts per million (ppm) down field from that of trimethylsilyl propionic acid sodium salt as an external reference with an accuracy of 0.005 ppm.

^{13}C-NMR Studies: Effect of β-CD on $PGF_{2\alpha}$/α-CD System and That of α-CD on $PGF_{2\alpha}$/β-CD System $PGF_{2\alpha}$ (10 mM) and α-CD (10 mM) were dissolved in a 0.1 M sodium borate/D_2O solution (pH 9.3). β-CD (0–20 mM) was subsequently added to the solution, and then the ^{13}C-NMR spectra were acquired. Similarly, α-CD (0–20 mM) was added to a solution of $PGF_{2\alpha}$ (10 mM) and β-CD (10 mM), and then ^{13}C-NMR measurements were performed. All measurements were taken on a JEOL JNM-A500 NMR system (external magnetic field of 500 MHz) at 25°C and accumulated 10000 times.

Two-Dimensional (2D) ^{1}H-NMR Studies $PGF_{2\alpha}$ (10 mM) was dissolved in a 0.1 M sodium borate/D_2O solution (pH meter reading of 9.3) and α- or β-CDs (10 mM) was added to the solution. 2D-NMR measurements (rotating frame nuclear Overhauser enhancement spectroscopy (ROESY)) were conducted for the sample solution on a JEOL JNM-ECA500 NMR system (external magnetic field of 500 MHz) at 30°C and accumulated 32 times.

Degradation of Limaprost in the CD Solutions α-CD (10 mg), β-CD (10 mg) or the mixture of α- and β-CDs (10 mg each) was dissolved in 5.0 mL of phosphate buffer (pH 9.0). Then, 25 μL of an ethanoic solution of Limaprost (10.0 mg/mL) was added. The final concentrations of Limaprost and CDs were 0.13 mM, 2.0 (α-CD) and 1.8 (β-CD) mM, respectively, where CDs were more than 10-folds higher than that of Limaprost (0.13 mM) and then most of the guest was in the complex form. The solution was kept at 25°C and subjected to HPLC analysis at predetermined times. Limaprost and its degradation products, 11-deoxy-Δ^{10} and 8-iso, were analyzed using a Prominence UFLC system (Shimadzu, Kyoto, Japan), which consisted of a pump, auto-injector, UV-Vis detector, and a ODS C18 column (5 μm, 4.6 mm×15 cm; YMC, Tokyo, Japan). The mobile phase was a mixture of 0.02 M KH_2PO_4

(pH 4.2), acetonitrile and propan-2-ol at 9 : 5 : 2 ratio and a flow rate of 1.0 mL/min, and the detection was at 215 nm and 35°C.

Results and Discussion

Interaction of $PGF_{2\alpha}$ with One Type of CD in Solution Limaprost is poorly water-soluble and chemically labile in water.[27] Therefore, $PGF_{2\alpha}$ was used as a model compound in the NMR experiments, because it has a similar structure to Limaprost and is relatively stable in aqueous media. Figure 2 shows the ^{13}C-NMR spectrum of $PGF_{2\alpha}$ and Table 1 summarizes its chemical shifts. The displacements of the ^{13}C chemical shifts of $PGF_{2\alpha}$ by the addition of α- or β-CD are shown in Fig. 3. The addition of α-CD altered the chemical shifts mainly for the α- and ω-chains of $PGF_{2\alpha}$ and the displacement increased as the α-CD concentration increased (Fig. 3a). In particular, C2 and C3 in the α-chain and C18, C19, and C20 in the ω-chain exhibited significant downfield shifts, but the shift changes of the five-membered ring were minimal. These results indicate that α-CD preferentially interacts with the long alkyl chain, α- or ω-chain of $PGF_{2\alpha}$. On the other hand, the signals from the five-membered ring of $PGF_{2\alpha}$ were significantly shifted as the concentration of β-CD increased (Fig. 3b), whereas the minor changes were observed for the carbons of the ω-chain. These shift changes indicate that β-CD with a larger cavity interacts preferentially with the five-membered ring of $PGF_{2\alpha}$. The small shift changes in the α-chain may be due to intermolecular hydrogen bonding between the hydroxyl group of the β-CD and the carboxylate ion of included $PGF_{2\alpha}$, as reported previously.[33,34]

Table 1. ^{13}C-NMR Chemical Shift of $PGF_{2\alpha}$ (50 mM) in a 0.1 M Sodium Borate/D_2O Buffer (pH 9.3) at 25°C

α-Chain		Five-membered ring		ω-Chain	
Attribution (^{13}C)	$PGF2_\alpha$	Attribution (^{13}C)	$PGF2_\alpha$	Attribution (^{13}C)	$PGF2_\alpha$
1	183.54	8	48.48	13	133.72
2	36.21	9	71.09	14	134.84
3	24.91	10	42.09	15	73.11
4	26.80	11	75.96	16	37.33
5	130.66	12	54.42	17	24.69
6	128.75			18	31.11
7	26.01			19	22.19
				20	13.56

Interaction of $PGF_{2\alpha}$ with Two Types of CDs (α- and β-CD) in Solution To reveal the interaction of $PGF_{2\alpha}$ with two types of CDs, NMR measurements were conducted in the presence of α-CD and β-CD. $PGF_{2\alpha}$ (10 mM) and α-CD (10 mM) were dissolved in a 0.1 M sodium borate/D_2O solution (pH 9.3), and then β-CD was added at various concentrations (0–20 mM). Similarly, α-CD (0–20 mM) was added to a solution of $PGF_{2\alpha}$ (10 mM) and β-CD (10 mM). Figures 4a and b show the changes in the ^{13}C chemical shifts of $PGF_{2\alpha}$ upon the addition of β- and α-CDs to the $PGF_{2\alpha}$/α-CD and $PGF_{2\alpha}$/β-CD systems, respectively. In Fig. 4a, the shift changes of $PGF_{2\alpha}$ at the initial point, *i.e.*, in the absence of β-CD, were those induced by the addition of only α-CD. It was apparent from Fig. 4a that the shift displacements of the α- and ω-chains at the initial point were not significantly changed by the addition of β-CD, whereas that of C12 carbon of the five-membered ring was significantly changed and the change was more pronounced as the β-CD concentration increased. The results

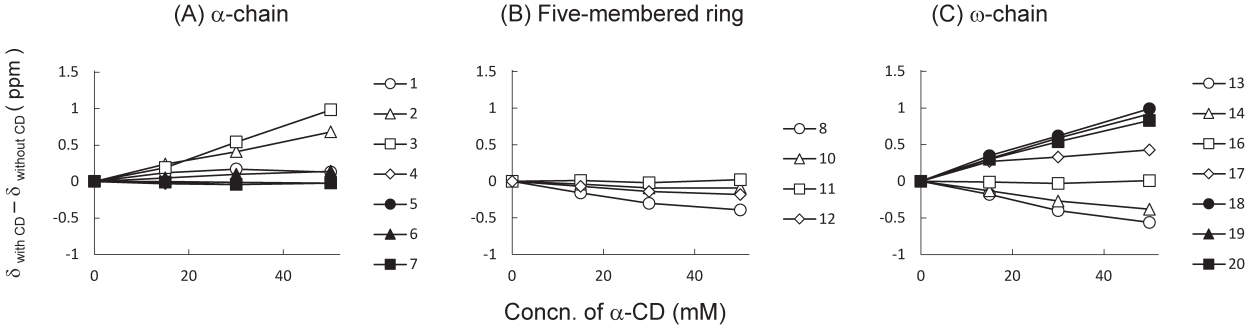

Fig. 3a. Effects of α-CD on the ^{13}C-NMR Chemical Shifts of $PGF_{2\alpha}$ (50 mM) in a 0.1 M Sodium Borate/D_2O Buffer (pH 9.3) at 25°C

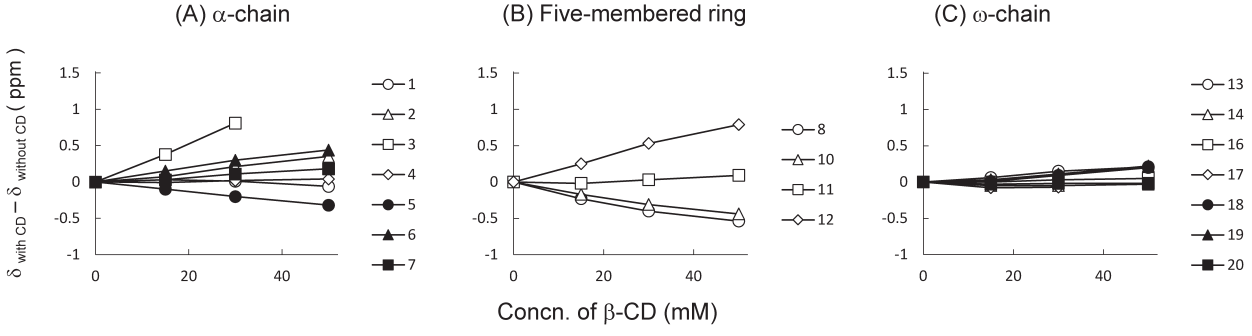

Fig. 3b. Effects of β-CD on the ^{13}C-NMR Chemical Shifts of $PGF_{2\alpha}$ (50 mM) in a 0.1 M Sodium Borate/D_2O Buffer (pH 9.3) at 25°C

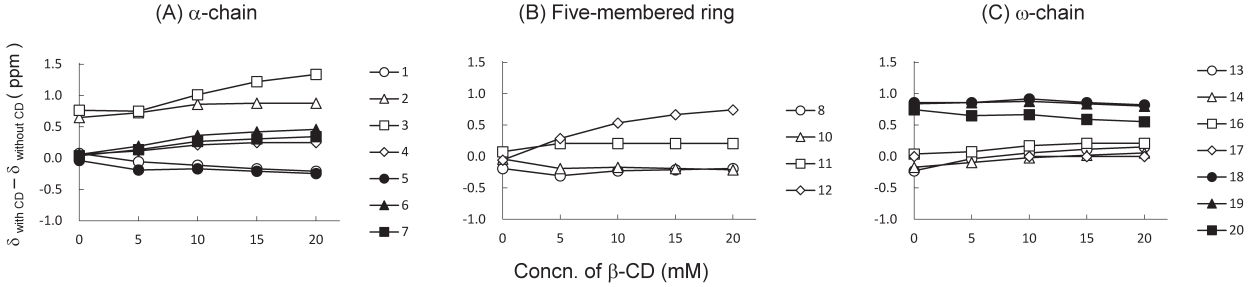

Fig. 4a. Effects of β-CD on the ^{13}C-NMR Chemical Shifts of $PGF_{2\alpha}$ (10 mM) in the Presence of α-CD (10 mM) in a 0.1 M Sodium Borate/D_2O Buffer (pH 9.3) at 25°C

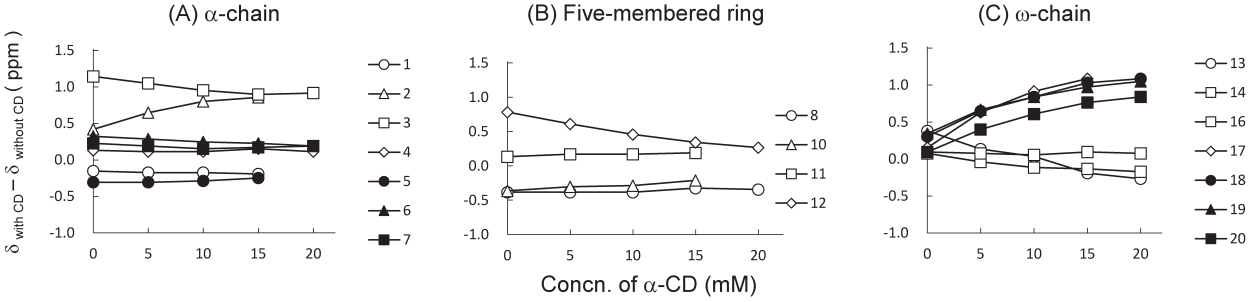

Fig. 4b. Effects of α-CD on the ^{13}C-NMR Chemical Shifts of $PGF_{2\alpha}$ (10 mM) in the Presence of β-CD (10 mM) in a 0.1 M Sodium Borate/D_2O Buffer (pH 9.3) at 25°C

Table 2. ^{13}C-NMR Chemical Shifts of $PGF_{2\alpha}$ (10 mM) in the Presence of α-CD (10 mM) and β-CD (10 mM) in a 0.1 M Sodium Borate/D_2O Buffer (pH 9.3) at 25°C

(A) Five-membered ring		
Attribution (^{13}C)	β-CD[a)]	β-CD with preexisting α-CD[b)]
8	−0.38	−0.23
9[e)]	—	—
10	−0.36	−0.17
11[f)]	0.13	0.21
12	0.78	0.53
(B) ω-Chain		
Attribution (^{13}C)	α-CD[c)]	α-CD with preexisting β-CD[d)]
17	0.55	0.92
18	0.86	0.84
19	0.84	0.84
20	0.74	0.61

a) ($PGF_{2\alpha}$+β-CD (10 mM))−($PGF_{2\alpha}$) (ppm): Chemical shifts of $PGF_{2\alpha}$ (10 mM) in the presence of β-CD (10 mM). *b*) ($PGF_{2\alpha}$+β-CD (10 mM)+α-CD (10 mM))−($PGF_{2\alpha}$) (ppm): Chemical shifts of $PGF_{2\alpha}$ (10 mM) in the presence of β-CD (10 mM) with preexisting α-CD (10 mM). *c*) ($PGF_{2\alpha}$+α-CD (10 mM))−($PGF_{2\alpha}$) (ppm): Chemical shifts of $PGF_{2\alpha}$ (10 mM) in the presence of α-CD (10 mM). *d*) ($PGF_{2\alpha}$+α-CD (10 mM)+β-CD (10 mM))−($PGF_{2\alpha}$) (ppm): Chemical shifts of $PGF_{2\alpha}$ (10 mM) in the presence of α-CD (10 mM) with preexisting β-CD (10 mM). *e*) Values not shown ("—") for C9 because its spectrum overlaps with that of CD. *f*) Shift did not change for C11 most likely because the chemical shift of C11 is unaffected by the addition of α- or β-CD (shift change of 0.08 with the addition of 10 mM α-CD, and 0.13 with the addition of 10 mM β-CD).

suggested that β-CD interacted with the five-membered ring of $PGF_{2\alpha}$ even in the presence of α-CD. The C3 carbon in the α-chain shifted downfield, as observed similarly in the $PGF_{2\alpha}$/β-CD binary system (Fig. 3b(A)), due to the intermolecular hydrogen bonding between the guest and host molecules, as described above. However, the shift changes of the five-membered ring in $PGF_{2\alpha}$/α-CD/β-CD system tended to be smaller than those of the $PGF_{2\alpha}$/β-CD system, as shown in Table 2(A). The ^{13}C chemical shifts of $PGF_{2\alpha}$ of the five-membered ring and the ω-chain in the presence of α-CD and β-CD are summarized in Table 2. The shift changes of C8 (−0.38 ppm), C10 (−0.36 ppm), and C12 (0.78 ppm) observed in the $PGF_{2\alpha}$/β-CD system decreased to −0.23 ppm, −0.17 ppm and 0.53 ppm, respectively, in the co-presence of both α- and β-CD, while the shift of C11 was small. These results suggest that the preexisting α-CD may slightly weaken the interaction between the five-membered ring and β-CD.

Figure 4b shows the effect of α-CD on the chemical shifts of the PGF2$_\alpha$/β-CD system. The significant downfield shifts were observed for 17–20 carbons of the ω-chain as the α-CD concentration increased. In addition, as shown in Table 2(B), the shift changes of the ω-chain (C18, 19, 20) in the binary $PGF_{2\alpha}$/α-CD system were similar to those in the ternary $PGF_{2\alpha}$/α-CD/β-CD system. This suggests that α-CD interacts preferentially with the ω-chain and the pre-existing β-CD does not significantly interfere with the interaction of α-CD with the ω-chain. The downfield shift of the C17 carbon in the ω-chain was larger in the ternary system than in the α-CD binary system. The ω-chain was included more deeply in the α-CD cavity or some conformational changes occurred at this carbon in the ternary system, compared with the binary system, because of the flexible property of the ω-chain. However, the reason for this large shift is not obvious at the present time. On the other hand, the β-CD-induced shift change of C12 of the five-membered ring slightly decreased upon the addition of α-CD (Fig. 4b). The addition of high concentrations

of α-CD may weaken the interaction between β-CD and the five-membered ring. The collective ^{13}C-NMR results showed that when adding α- and β-CDs to $PGF_{2\alpha}$ solutions, α-CD preferentially interacts with the ω-chain, while β-CD interacts with the five-membered ring, although the interaction strength of α- and β-CDs with $PGF_{2\alpha}$ in the ternary complex is different from those in the binary complex.

2D ^{1}H-NMR Spectroscopic Studies of $PGF_{2\alpha}$ in the Presence of α- and β-CDs 2D-NMR studies were conducted to investigate the inclusion sites of $PGF_{2\alpha}$ by α- and β-CDs. $PGF_{2\alpha}$, α-CD, and β-CD were dissolved in a 0.1 M sodium borate/D_2O solution (pH 9.3) at a molar ratio of 1 : 1 : 1, and the 2D-NMR (ROESY) spectra were measured. This molar ratio was selected because it is reported that $PGF_{2\alpha}$ interacts with each CD at 1 : 1 molar ratio.[35] The ROESY spectrum of $PGF_{2\alpha}$ gave the intramolecular correlation peaks between the H13 and H14 protons and the H8 and H12 protons (Fig. 5A). When α-CD was added, the additional correlation peaks ap-

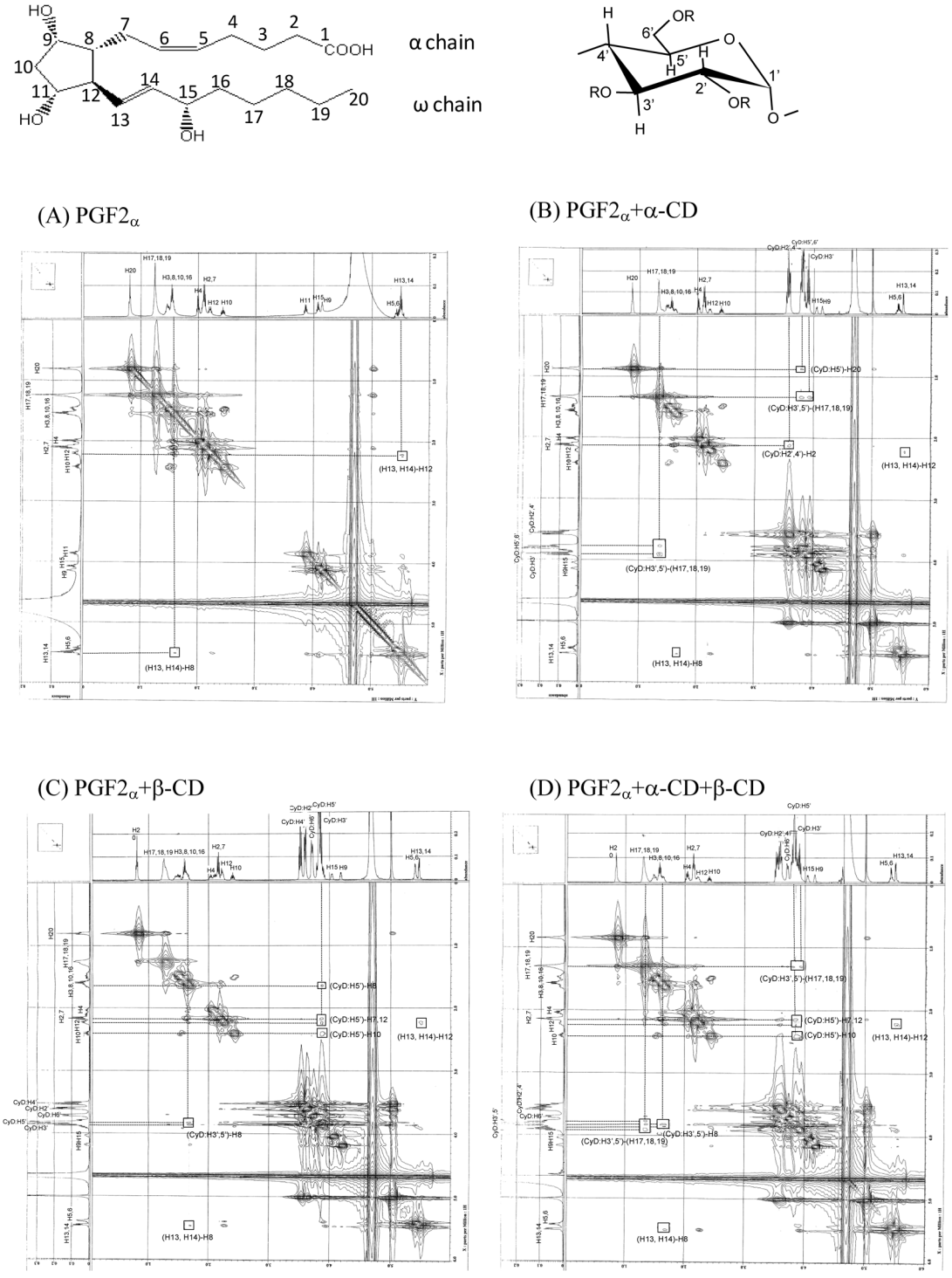

Fig. 5. 2D-ROESY Spectrum in a 0.1 M Sodium Borate/D_2O Buffer (pH Meter Reading 9.3) at 25°C

(A) $PGF_{2\alpha}$, (B) $PGF_{2\alpha}$ in the presence of α-CD, (C) $PGF_{2\alpha}$ in the presence of β-CD, (D) $PGF_{2\alpha}$ in the presence of α- and β-CDs.

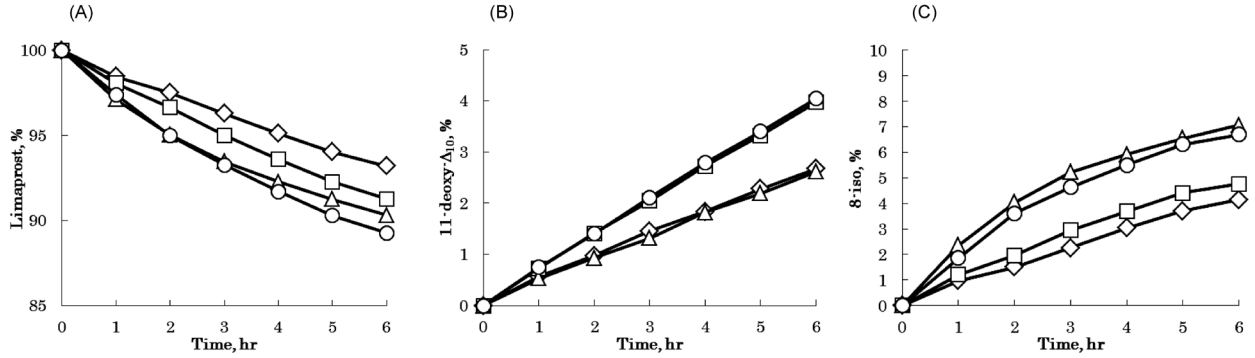

Fig. 6. Changes in (A) Limaprost, (B) 11-Deoxy-Δ_{10}, and (C) 8-Iso in the Presence of CDs in a Phosphate Buffer (pH 9.0)
◇: Limaprost, □: Limaprost+α-CD, △: Limaprost+β-CD, ○: Limaprost+α-CD+β-CD.

peared between the ω-chain protons (H17, 18, 19, 20) of $PGF_{2\alpha}$ and the H3′ and H5′ protons of α-CD (Fig. 5B). Since the H3′ and H5′ protons of α-CD are located inside of the cavity, these correlation peaks indicated that α-CD included the ω-chain of $PGF_{2\alpha}$. Additionally, the correlation peaks were observed between the H2 proton of $PGF_{2\alpha}$ and the H2′ and H4′ protons of α-CD which are located outside of the cavity. This is probably due to the interaction of the α-chain of $PGF_{2\alpha}$ with the outside of α-CD cavity, because the α-chain is not significantly involved in the inclusion complexation. Figure 5C shows the ROESY measurements after adding β-CD to $PGF_{2\alpha}$. The protons on the five-membered ring of $PGF_{2\alpha}$ (H7, H8, H10, H12) showed the correlation peaks with the internal protons of the β-CD cavity (H3′, H5′), indicating that β-CD included the five-membered ring of $PGF_{2\alpha}$. When both α- and β-CDs are added, the correlation peaks appeared between the H3′ and H5′ protons of the CDs and the protons of the ω-chain, as well as the five-membered ring of $PGF_{2\alpha}$, as shown in Fig. 5D. These results clearly demonstrate that both the ω-chain and five-membered ring of $PGF_{2\alpha}$ participate in the ternary complexation with α- and β-CDs.

Effect of CDs on the Stability of Limaprost in Solution It is reported that prostaglandins are chemically labile in aqueous media and their rates of degradation are affected by CDs.[36,37] For example, sulfobutylether–β-CD (SBE–β-CD) suppresses the degradation of PGE_1 derivatives in acid or basic conditions, while other CDs accelerate the dehydration of the five-membered ring of the derivatives.[38] To gain insight into the inclusion mode for the Limaprost/α-CD/β-CD ternary complex, the effects of α- and β-CDs on the stability of Limaprost in alkaline solutions (pH 9.0 phosphate buffer) were investigated. As shown in Fig. 6, Limaprost degraded in the alkaline solution, producing the two main products, 11-deoxy-Δ^{10} and 8-iso, where the rate to 8-iso was slightly faster than that of 11-deoxy. The degradation of Limaprost was accelerated by the addition of CDs in the condition. The degradation rates (k) determined from Fig. 6A were $1.19\times10^{-2}\,\mu g\cdot mL^{-1}\cdot h^{-1}$ without CD, $1.58\times10^{-2}\,\mu g\cdot mL^{-1}\cdot h^{-1}$ with 2.0 mg/mL α-CD, $1.88\times10^{-2}\,\mu g\cdot mL^{-1}\cdot h^{-1}$ with 2.0 mg/mL β-CD, and $2.04\times10^{-2}\,\mu g\cdot mL^{-1}\cdot h^{-1}$ with both 2.0 mg/mL α-CD and 2.0 mg/mL β-CD. The degradation rate of the ternary system was faster than those of the binary system. It was noteworthy that α-CD promoted the degradation to 11-deoxy-Δ^{10}, but negligibly affected the degradation to 8-iso. On the other hand, β-CD showed the opposite effect on the degradation, *i.e.*, it accelerated the degradation to 8-iso, whereas negligibly to 11-deoxy, as shown in Figs. 6B and C. When both α- and β-CDs were added, the degradations to 11-deoxy-Δ^{10} and 8-iso were both accelerated and the profiles of the degradation rates were almost the same as those for adding a single CD.

The degradation to 11-deoxy-Δ^{10} is thought to proceed *via* the E2 elimination mechanism (Chart 1A); the deprotonation occurs at the C10 position on Limaprost, and the hydroxyl group at the C11 position is subsequently eliminated. We confirmed that α-CD preferentially includes the ω-chain of Limaprost. The secondary hydroxyl group of α-CD located near the five membered ring may act as a general base catalyst in aqueous solutions to promote the dehydration to 11-deoxy-Δ^{10}. On the other hand, the degradation to 8-iso likely proceeds *via* the enol intermediate (8-ene-9-ol) or its conjugate base (Chart 1B). The epimerizing C8 carbon in the intermediate has a planar conformation, *i.e.*, sp^2 hybrid orbital (Chart 1B). Therefore, when the C8 carbon is forced to adapt this conformation, the epimerization is accelerated. Monkhouse *et al.* reported[39] that the isomerization of PGA_2 was faster than that of PGA_1. This is due to that two double bonds in a PGA_2 molecule force the α- and ω-chains to align in a parallel conformation through a nonbonding interaction, while PGA_1 with one double bond has no interaction. In this conformation, the reactive C12 carbon is apt to take the planar conformation, accelerating the isomerization. Furthermore, NMR spectroscopic and potentiometric studies suggested that the terminal carboxylic acid of $PGF_{2\alpha}$ interacts with the hydroxyl groups of β-CD by hydrogen bonding,[33,34] as described above. Therefore, when β-CD preferentially encapsulates the five-membered ring of Limaprost, it may fix the α-side chain of Limaprost in a conformation favorable for enol formation, which promoting the isomerization. As shown in Fig. 6B, β-CD had the negligible effect on the dehydration into 11-deoxy-Δ^{10}, which is probably due to its larger cavity diameter and to free access of catalytic water molecules to Limaprost. These results suggest that the respective inclusion sites of α- and β-CDs on Limaprost do not change in the co-presence of the two CDs, *i.e.*, α- and β-CDs still encapsulate the ω-chain and the five-membered ring of Limaprost, respectively. Figure 7 schematically depicts the inclusion mode estimated from the above results. α- and β-CDs preferentially encapsulates the ω-chain and the five-membered ring of Limaprost, respectively, to form the ternary complex, although the fraction of two binding sites changes depending

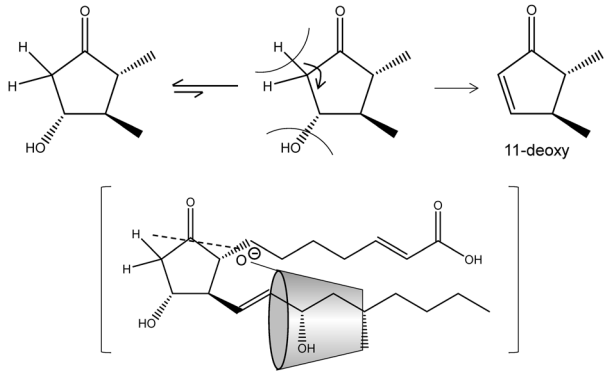

Chart 1A. Reaction Mechanism for Dehydration of Limaprost

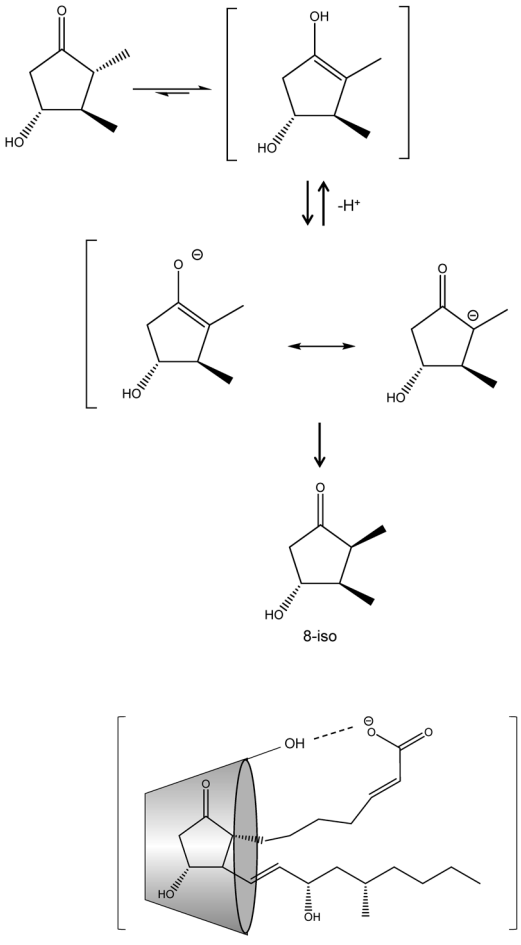

Chart 1B. Reaction Mechanism for Isomerization of Limaprost

on concentrations of CDs.

Conclusion

On the basis of NMR spectroscopic and kinetic studies, we found that α- and β-CDs, respectively, included the ω-chain and the five-membered ring of Limaprost to form the ternary complex. In a previous study, we reported that the chemical stability of Limaprost in the solid state was significantly improved by the co-addition of both α- and β-CDs.[32] The ternary complex formation of Limaprost with α- and β-CDs probably contributes to the stabilization of Limaprost in the solid state, although the degradation mechanism of the drug in the solid state is different from that in aqueous solution.

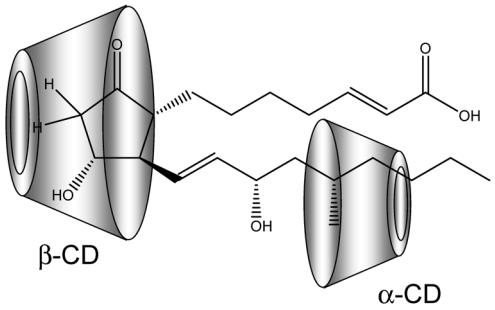

Fig. 7. Proposed Inclusion Modes of the Limaprost/α-CD/β-CD Ternary Complex in Water

Conflict of Interest Yasuo Inoue, Noboru Sekiya and Masanobu Yamamoto are employees of Ono Pharmaceutical Co., Ltd.

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