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Inclusion Complexation of Prostaglandin $F_{2\alpha}$ with α- and β-Cyclodextrins in Aqueous Solution

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The interaction of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) with α- and β-cyclodextrins in aqueous solution was investigated by potentiometric titration, solubility, and carbon 13 nuclear magnetic resonance (^{13}C NMR) techniques. Changes in titration curve and solubility of $PGF_{2\alpha}$ following the binding to α- and β-cyclodextrins were quantitatively treated to obtain equilibrium constants and thermodynamic parameters. The protolytic dissociation of $PGF_{2\alpha}$ was significantly suppressed by inclusion complexation. ^{13}C NMR chemical shift changes suggested that binding of $PGF_{2\alpha}$ to α-cyclodextrin was somewhat different from that to β-cyclodextrin.

Keywords——prostaglandin $F_{2\alpha}$; cyclodextrin; inclusion complex; stability constant; thermodynamic parameter; potentiometric titration; solubility method; ^{13}C NMR spectroscopy

The prostaglandins are essentially long chain fatty acids containing a substituted cyclopentane system. The relatively hydrophobic environment of cyclodextrin cavity is particularly favorable for inclusion of the highly hydrophobic molecules of the prostaglandins in aqueous solution.[2,3] The present study deals with the details on the interaction of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)[4] with α- and β-cyclodextrins (α-CyD and β-CyD) to gain insight into the mechanism and geometry of inclusion process. The equilibrium constants and thermodynamic parameters for the complexation were determined by potentiometric titration[5] and solubility methods. Furthermore, the chemical shift changes of $PGF_{2\alpha}$ carbons following the binding to cyclodextrins were examined by carbon 13 nuclear magnetic resonance (^{13}C NMR) spectroscopy.

Experimental

Materials——$PGF_{2\alpha}$ was donated by Ono Pharmaceutical Industries Co., Ltd. α- and β-Cyclodextrins were the gift of Teijin Ltd. and were recrystallized from water. All other materials and solvents were of analytical reagent grade.

Potentiometric Titration Studies——The titration of aqueous sodium $PGF_{2\alpha}$ (20 ml of 2.0×10^{-3} M) containing various concentration of cyclodextrin (2.0×10^{-3} to 1.3×10^{-2} M) with HCl (5.0×10^{-2} M) was carried out in a thermostated beaker (10—35°) under a stream of nitrogen gas using a pH meter of Hitachi-Horiba F-7 type. Calculation of the dissociation constants was in the same manner as previously described.[5]

Solubility Studies——The procedure of the solubility measurements was same as previous paper[2] and 0.3 M sodium phosphate buffer was used as the solvent. The concentration of $PGF_{2\alpha}$ was determined gas-chromatographically following the method of Roseman and Yalkowsky.[6] The typical gas chromatographic conditions were as follow: gas chromatograph, Shimadzu GC-6A; sample volume 2 μl; column, 1% SE-52 on Gas-Chrom Q (60—80 mesh) in glass column (3 mm $\times$ 1 m); carrier gas, nitrogen (0.6 kg/cm^2); injection temp., 280°; column temp., 250°; hydrogen flame ionization detector (FID) temp., 280°, silylating reagent, 4:1 mixture of N,O-bis(trimethylsilyl)acetamide and trimethylchlorosilane containing 3 mg/ml of cholesteryl acetate as an internal standard.

1) Location: *5-1, Oe-honmachi, Kumamoto, 862, Japan.*
2) K. Uekama, F. Hirayama, K. Ikeda, and K. Inaba, *J. Pharm. Sci.*, **66**, 706 (1977).
3) K. Uekama, F. Hirayama, S. Yamasaki, M. Otagiri, and K. Ikeda, *Chem. Lett.*, **1977**, 1389.
4) (5Z, 9α, 11α, 13E, 15S)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid.
5) T. Miyaji, Y. Kurono, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 1155 (1976).
6) T.J. Roseman and S.H. Yalkowsky, *J. Pharm. Sci.*, **62**, 1680 (1973).

^{13}C NMR Studies——^{13}C NMR spectra were taken on a Jeol PFT-100 spectrometer operating at 25.03 MHz, interfaced with a Jeol EC-100 Fourier transform computer with 16 K memory. The NMR spectra of degassed samples in 0.1 M sodium borate buffer (pH 9.3) were obtained in 10 mm spinning tube at ambient temperature (about 38°). The chemical shifts were referenced to external tetramethylsilane with accuracy of ±0.025 ppm.

Results and Discussion

Equilibrium Constants of Inclusion Complexes

In the preceding paper,[5] the potentiometric titration method has been shown to be applicable to obtain the equilibrium constants of inclusion complexes between cyclodextrins and weakly acidic and basic guest molecules in aqueous solution. Recently, Connors and Lipari[7] also reported the effects of cyclodextrins on apparent dissociation constants of carboxylic acids and phenols by means of potentiometric titration. In the present study, this method was applied to the interaction of weak carboxylic acid $PGF_{2\alpha}$[8] with α- and β-cyclodextrins. The possible complexation process in the molar ratio of 1:1 may be represented as Chart 1, where both the ionized and unionized species of $PGF_{2\alpha}$ participate in the

$$\begin{array}{ccc} PGH + CyD & \underset{}{\overset{K_{13},\ \Delta H_{13},\ \Delta S_{13}}{\rightleftharpoons}} & PGH\text{-}CyD \\ K_{12},\ \Delta H_{12},\ \Delta S_{12} \uparrow\downarrow & & \uparrow\downarrow K_{34},\ \Delta H_{34},\ \Delta S_{34} \\ PG^- + H^+ + CyD & \underset{K_{24},\ \Delta H_{24},\ \Delta S_{24}}{\rightleftharpoons} & PG^-\text{-}CyD + H^+ \end{array}$$

Chart 1. Proposed Diagram for Interaction between Prostaglandin $F_{2\alpha}$ and Cyclodextrin

PGH: unionized $PGF_{2\alpha}$, PG^-: ionized $PGF_{2\alpha}$, CyD: cyclodextrin, PGH-CyD: unionized $PGF_{2\alpha}$-cyclodextrin complex, PG^--CyD: ionized $PGF_{2\alpha}$-cyclodextrin complex.

interaction. In this diagram, K_{12} and K_{34} are the dissociation constants for non-complexed and complexed $PGF_{2\alpha}$, respectively, and K_{13} and K_{24} are the dissociation constants for inclusion complexes of unionized and ionized $PGF_{2\alpha}$, respectively. Meanwhile, the dissociation constants and thermodynamic parameters in Chart 1 must hold the following relations.[9]

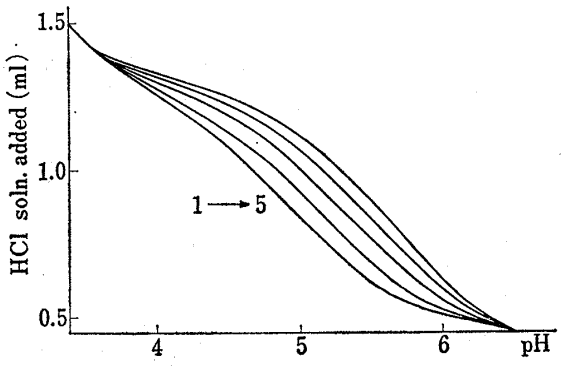


Fig. 1. Titration Curve of $PGF_{2\alpha}$ (2×10^{-3} M, 20 ml) with HCl (5×10^{-2} M) at 10°

Curve 1: in the absence of α-CyD, curves 2—5: in the presence of α-CyD (concentration of α-CyD; 2×10^{-3}, 5×10^{-3}, 8×10^{-3}, and 13×10^{-3} M for curves 2, 3, 4, and 5, respectively).

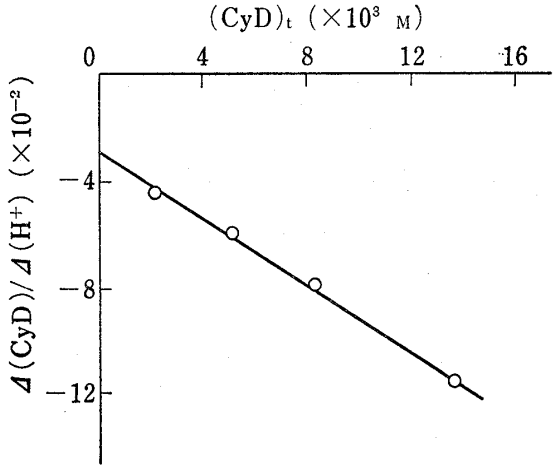


Fig. 2. Analysis of the Titration Data for Interaction between Prostaglandin $F_{2\alpha}$ and α-Cyclodextrin at 10°

See equation 8 in ref. 5.

7) K.A. Connors and J.M. Lipari, *J. Pharm. Sci.*, **65**, 379 (1976).
8) The pK_a of $PGF_{2\alpha}$ was found to be 4.83 at 25° by potentiometric titration.
9) F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967).

$$K_{12} \times K_{13} = K_{24} \times K_{34} \quad \text{(Eq. 1)}$$

$$\Delta H_{12} + \Delta H_{13} = \Delta H_{24} + \Delta H_{34} \quad \text{(Eq. 2)}$$

$$\Delta S_{12} + \Delta S_{13} = \Delta S_{24} + \Delta S_{34} \quad \text{(Eq. 3)}$$

Figure 1 shows the titration curves of $PGF_{2\alpha}$ with HCl in the presence and absence of α-CyD, as an example, where the curve shifts to alkaline side as concentration of α-CyD increases. The hydrogen ion concentrations at the half-neutralization, $(H^+)_h$, as a function of cyclodextrin concentration were quantitatively treated to obtain the dissociation constants in the same manner as previously described[5] (see Fig. 2). The linear plot obtained in Fig. 2 verifies a 1:1 stoichiometry in Chart 1. The dissociation constants and thermodynamic parameters determined by potentiometric titration are shown in Table I. The relations in

Table I. Equilibrium Constants and Thermodynamic Parameters for Complexation of Prostaglandin $F_{2\alpha}$ with α- and β-Cyclodextrins Determined by Potentiometric Titration at 25°

System	Equilibrium constant (M)	ΔH (kcal/mol)	ΔS (e.u.)
α-CyD-$PGF_{2\alpha}$	K_{12}: 1.51×10^{-5}	−0.23	−22.1
	K_{34}: 1.80×10^{-6}	0.71	−23.9
	K_{13}: 5.50×10^{-3}	6.34	10.9
	K_{24}: 4.63×10^{-2}	5.31	11.7
β-CyD-$PGF_{2\alpha}$	K_{12}: 1.51×10^{-5}	−0.23	−22.1
	K_{34}: 7.40×10^{-6}	3.00	−13.4
	K_{13}: 1.45×10^{-3}	5.01	3.82
	K_{24}: 3.10×10^{-3}	2.27	−3.86

Eqs. 1, 2, and 3 are exactly fulfilled for α-CyD system $[(K_{12}\times K_{13})/(K_{24}\times K_{34})=0.997$, $\Delta\Delta H=0.09$ kcal/mol, $\Delta\Delta S=1.0$ e.u.] and β-CyD system $[(K_{12}\times K_{13})/(K_{24}\times K_{34})=0.954$, $\Delta\Delta H=0.49$ kcal/mol, $\Delta\Delta S=1.0$ e.u.] within experimental error. As seen in Table I, the values of K_{12} are larger than those of K_{34}, indicating that the protolytic dissociation of $PGF_{2\alpha}$ is suppressed by the complex formation. Similar results were obtained for barbiturates[5] and benzoic acid[7] following the binding to cyclodextrins.

Figure 3 shows the solubility changes of $PGF_{2\alpha}$ as a function of cyclodextrin concentration. The stability constants of unionized $PGF_{2\alpha}$ with cyclodextrins, K_f, were calculated on the basis of 1:1 from the straight line portion of the solubility diagram at pH 3.0. Table II summarizes the stability constants obtained by two methods, where K_f and K_i by potentiometric titration method are equal to $1/K_{13}$ and $1/K_{24}$, respectively. In all cases, the stability constants for β-CyD complexes are larger than those for α-CyD complexes, indicating that the smaller cavity of α-CyD allows little penetration of bulky $PGF_{2\alpha}$ molecule. It is also noted that K_f values are larger than K_i values, suggesting that the ionized $PGF_{2\alpha}$ is unfavorable for inclusion complex formation compared to the unionized one because of the hydration. This trend is particularly significant in α-CyD complex.

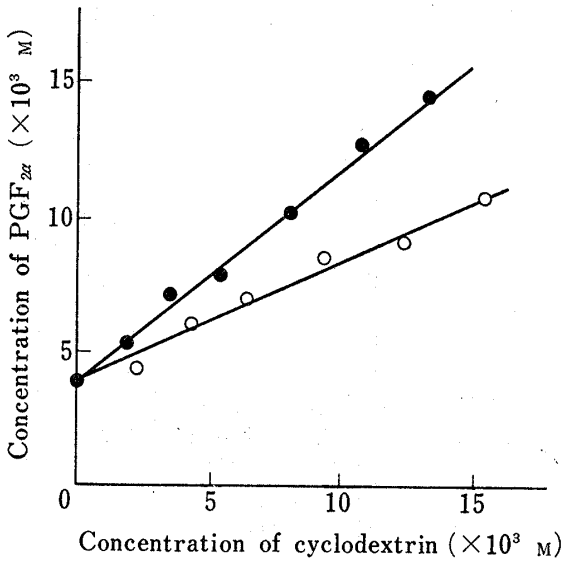


Fig. 3. Solubilities of Prostaglandin $F_{2\alpha}$ as a Function of Cyclodextrin Concentration in 0.3 M Phosphate Buffer (pH 3.0) at 25°

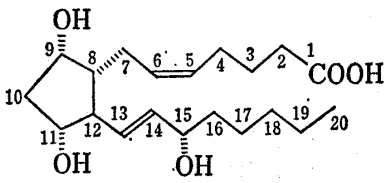
○: α-cyclodextrin, ●: β-cyclodextrin.

Table II. Stability Constants (M^{-1}) of Prostaglandin $F_{2\alpha}$ with α- and β-Cyclodextrins at 25°

	Potentiometric titration method		Solubility method	
	α-CyD	β-CyD	α-CyD	β-CyD
K_f[a]	182	691	254	1240
K_i[b]	21.6	322		

a) Stability constant of unionized $PGF_{2\alpha}$ with cyclodextrin.
b) Stability constant of ionized $PGF_{2\alpha}$ with cyclodextrin.

Table III. Effects of Cyclodextrins on the ^{13}C NMR Chemical Shifts[a] of Prostaglandin $F_{2\alpha}$ in 0.1 M Borate Buffer[b]



Carbon[c]	$PGF_{2\alpha}$ alone[d]	In the presence of CyD	
		α-CyD[e]	β-CyD[f]
1	184.35	184.65	184.53
2	37.52	37.77	37.61
3	26.28	26.46	26.71
4	28.03	27.95	27.99
5	131.64	131.77	131.60
6	129.77	129.94	130.08
7	27.16	27.16	27.26
8	49.79	49.47	49.37
9	72.20	72.39	72.72
10	43.28	43.19	43.03
11	77.19	77.15	77.19
12	55.62	55.45	55.98
13	134.96	134.54	134.98
14	135.99	135.73	135.88
15	74.28	73.92	74.13
16	38.48	38.50	38.53
17	26.03	26.15	25.79
18	32.38	32.86	32.30
19	23.44	23.87	23.41
20	14.84	15.21	14.71

a) Accurate to ±0.025 ppm.
b) D_2O as solvent (pH meter reading of 9.3).
c) Signal was assigned according to ref. 12.
d) 1×10^{-1} M.
e) 1×10^{-3} M.
f) 5×10^{-3} M.

^{13}C NMR Chemical Shifts Changes

^{13}C NMR spectroscopy is known to be a powerful technique for the investigation of intermolecular interactions.[10,11] This study was initiated to ascertain that $PGF_{2\alpha}$ indeed forms inclusion complexes with α- and β-cyclodextrins and to examine the effects of cyclo-

10) R.A. Dwek, "Nuclear Magnetic Resonance in Biochemistry; Applications to Enzyme Systems," Clarendon Press, Oxford, 1973.
11) M. Otagiri, T. Miyaji, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 1146 (1976); K. Uekama, F. Hirayama, and H. Koinuma, *Chem. Lett.*, **1977**, 1393.

dextrins on the ionization of $PGF_{2\alpha}$ by measuring ^{13}C chemical shifts changes in aqueous solution. The ^{13}C chemical shifts for $PGF_{2\alpha}$[12] in the presence and absence of cyclodextrins are summarized in Table III, where the magnitude and direction of the effects of α-CyD on chemical shifts of $PGF_{2\alpha}$ are different from those of β-CyD, particularly for ω-side chain (C_{13-20}) and cyclopentane ring (C_{8-12}). For example, with α-CyD the signals C_{17-20} showed a shift to downfield, whereas with β-CyD those showed a shift to opposite direction, suggesting that the binding of $PGF_{2\alpha}$ to α-CyD is somewhat different from that to β-CyD. As shown in Fig. 4, α-CyD appears to interact predominantly with ω-side chain of $PGF_{2\alpha}$ because of the smaller cavity. On the other hand, β-CyD cavity seems to be capable of inclusion of the cyclopentanone ring of $PGF_{2\alpha}$, which may result in the rigid complex with larger stability

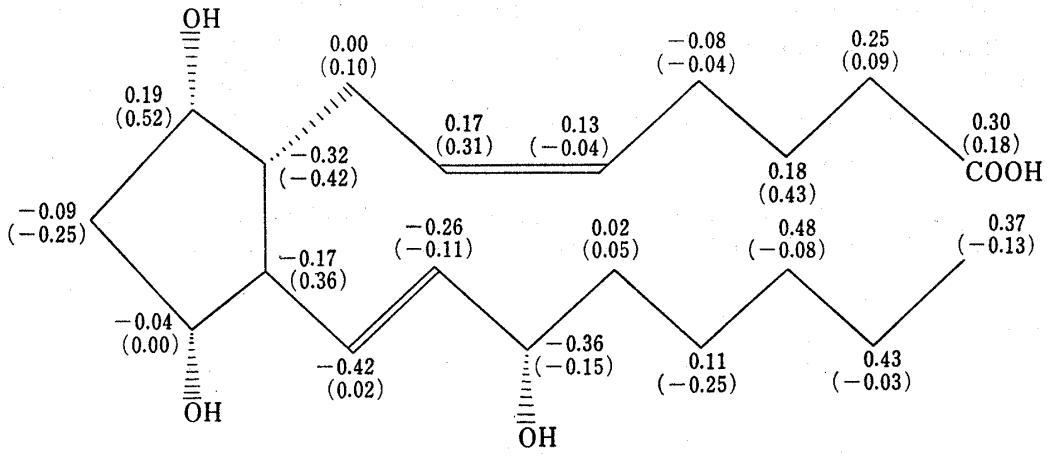
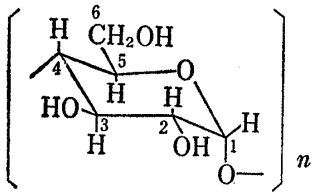


Fig. 4. ^{13}C NMR Chemical Shift Changes of Prostaglandin $F_{2\alpha}$ Following the Binding to α-Cyclodextrin and β-Cyclodextrin at pH 9.3

A chemical shift change in the downfield direction is expressed as a positive value and an opposite change is expressed as a negative value. Value in parenthesis is β-cyclodextrin-induced chemical shift change.

TABLE IV. Effects of Prostaglandin $F_{2\alpha}$ on the ^{13}C NMR Chemical Shifts[a] of α- and β-Cyclodextrins[b]



Carbon[c]	α-CyD (n=6)			β-CyD (n=7)		
	Without $PGF_{2\alpha}$ (δ_0)	With $PGF_{2\alpha}$ (δ)	$\delta-\delta_0$	Without $PGF_{2\alpha}$ (δ_0)	With $PGF_{2\alpha}$ (δ)	$\delta-\delta_0$
1	102.56	103.06	0.50	102.95	103.39	0.44
2	74.47	74.92	0.45	74.24	74.56	0.32
3	73.20	73.15	−0.05	73.40	73.33	−0.07
4	82.42	82.60	0.18	82.36	82.61	0.25
5	72.91	72.91	0.00	73.20	73.18	−0.02
6	61.60	61.27	−0.33	61.59	61.24	−0.35

a) Accurate to ±0.025 ppm.
b) Experimental conditions were the same as Table III.
c) Signal was assigned according to ref. 14.

12) W.W. Conover and J. Fried, *Proc. Natl. Acad. Sci.*, U.S.A., **71**, 2157 (1974).

constant compared to α-CyD complex. It is noteworthy that C_1 and C_2 signals of $PGF_{2\alpha}$ following the binding to cyclodextrins showed a shift to downfield, in which the effects of α-CyD was larger than those of β-CyD. This may be rationalized in the following way. Since carboxylate group is expected to be less electronegative than the carboxylic acid,[13] the α (and other) carbons in $PGF_{2\alpha}$ are expected to show a shift to downfield by the suppression of protolytic dissociation of carboxylic acid. This is exactly the case of what is observed in Table III, and the magnitude of the chemical shift changes is well correlated to K_f/K_i values of α- and β-cyclodextrins, respectively, in Table II.

Table IV summarizes the effects of $PGF_{2\alpha}$ on the ^{13}C chemical shifts of α- and β-cyclodextrins.[14] In ring carbons (C_{1-5}), C_1 and C_4 signals showed a shift to downfield, while C_3 and C_5 signals showed a shift to opposite direction. Since C_3–H and C_5–H groups are oriented at the center of the cavity,[15] these carbons may be particularly susceptible to the shielding due to hydrophobic interactions.[12] The C_6 carbon located at the exterior of the torus also showed a significant shift to upfield. These results apparently indicate that $PGF_{2\alpha}$ may interact with cyclodextrins not only at interior of the cavity but also at outside of the cavity to form a rigid complex.

Acknowledgement The authors are grateful to Dr. Hideomi Koinuma, Faculty of Engineering, the Tokyo University, for valuable advice and measurements of ^{13}C NMR spectra.

13) It is proposed that the variations of the shielding of carbons α to most substituent groups are mainly dominated by the electronegativity of the substituents (R. Hagen and J.D. Roberts, *J. Am. Chem. Soc.*, **91**, 4504 (1969)).
14) K. Takeo, K. Hirose, and T. Kuge, *Chem. Lett.*, **1973**, 1233.
15) K. Harata, *Bull. Chem. Soc. Jpn.*, **50**, 1259 (1977).