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Some Pharmaceutical Properties of 2,3,6-partially Methylated-β-cyclodextrin and its Solubilizing and Stabilizing Abilities

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Summary

The pharmaceutical properties of 2,3,6-partially methylated-β-cyclodextrin(PMCD) were investigated. The aqueous solubility of PMCD was much higher than that of the parent β-CyD, and it exhibited endothermic dissolution in contrast to that of the conventional heptakis-(2,6-di-O-methyl)-β-cyclodextrin(DMCD). The acid-catalyzed hydrolysis rate of PMCD was faster than those of the parent β-CyD and DMCD. The hemolytic activity(human erythrocytes) of PMCD was similar to that of DMCD. PMCD was a more effective solubilizer for poorly water-soluble drugs than the parent β-CyD; however PMCD is not as effective as DMCD. The stabilizing effect of PMCD on chemically unstable drugs was higher than that of the parent β-CyD.

For 2,3,6 partially methylated-β-CyD(PMCD), in which the hydroxyl groups of cyclodextrin are substituted by a methyl group, the methylation ratios are as follows: 58~62% at the 2-position, 48~52% at the 3-position, and

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98~100% at the 6-position[1]. This novel cyclodextrin derivative is synthesized by MERCIAN CORPORATION[2]. The aqueous solubilities of conventional heptakis-(2,6-di-O-methyl)-β-cyclodextrin(DMCD) and heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin(TMCD) usually decreased with increasing temperature; however, PMCD exhibited endothermic dissolution in a manner similar to that of the parent β-cyclodextrin (β-CyD). PMCD has received considerable attention in the pharmaceutical field; therefore, in this study some of the physicochemical properties of PMCD, such as surface activity, hemolytic activity and chemical stability in acid medium were investigated. In addition, the solubilizing and stabilizing abilities of PMCD for pooly water-soluble drugs were compared with those of β-CyD and DMCD.

Experimental procedures

Materials β-CyD and DMCD were supplied by Nihon Shokuhin Kako Co.Ltd(Tokyo, Japan). PMCD was donated by MERCIAN CORPORATION(Tokyo, Japan). All other chemicals and solvents were from commercial sources and were used without further purification. The water used in all of the experiments was Milli-Q water.

Apparatus for measurement of the physicochemical properties of PMCD MF-Fab-MS spectra: JEOL SX-102A mass spectrometer (Jeol, Tokyo, Japan) with the Magic Bullet (MB) as a matrix; the acceleration was -10kV. Optical rotation measurements: SEPA-200 digital polarimeter (HORIBA, Ltd, Kyoto, Japan) with an accuracy of ±0.002°. Surface tension measurements: Wilhelmy surface tensiometer (KYOWA, KAIMENKAGAKU Co., Ltd. Tokyo, Japan) with an accuracy of ±0.2 mN/m. Distilled Milli-Q water was used, and the glass vessel was treated with 20% sulfuric acid before each measurement.

Hemolysis studies of CyDs

This was carried out in the same manner as described before[3], except that the optical density of the supernatant was measured for hemoglobin at 543 nm. The results were expressed as % total hemolysis by comparison with a sample of complete hemolysis in water(hemolysis rate is 100%).

Acid-catalyzed hydrolysis of CyDs

One gram of CyDs was dissolved in 100 ml of 1M HCl, and the reaction solution was heated in a heating bath at 60°C. The pH of the sample solution was ascertained to be the same before and after the reaction. Samples of the reaction solution (0.5 ml protions) were taken at appropriate intervals and neutralized by adding 0.5 ml of 10M NaOH containing TMCD (1%) as an internal standard for HPLC. Four ml of ethyl acetate were added to the mixture to extract the CyDs, and the samples were then shaken vigorously. Two ml of the organic layer was taken and volatilized under reduced pressure. The residue was dissolved in 0.25 ml of the mobile phase, and 0.25 μl samples were injected into the HPLC. The HPLC conditions were as follows: pump, SSC Flow System 3100J (Senshu Kagaku Co., Ltd. Tokyo, Japan); detector, ERC-7512 differential refractometer(Erma Optical Works, Tokyo, Japan); column, ODS(4.6φ×250 cm) (Showa Denko, Tokyo, Japan); mobile phase, acetonitrile-water(13:9 v/v); flow rate, 1 ml/min.

Solubility studies for poorly water soluble drugs

Excess amounts of drugs were added to aqueous solutions containing PMCD (15 mg/ml). The solutions were sonicated three times for 10 minutes at 30-minute intervals and then shaken at 25°C. After equilibrium was attained (about 24 hours), the solutions were filtered through a 0.45 μm membrane filter. A portion of each sample was diluted and analyzed by spectrophotometry at suitable wavelengths, and then their solubility was calculated. For reference, the solubilization ability of other CyDs was determined in the same way.

The effect of CyDs on the acid hydrolysis rate of Prostaglandin I_2

The acid hydrolysis rate of prostaglandin I_2 (PGI_2) in the absence and presence of CyDs was spectrophotometrically monitored by measuring the decrease in absorbance at 230 nm. The reaction was initiated by the addition of 0.1 ml of stock solution of PGI_2 sodium salt (ca. 9×10^{-3}M) into 3 ml of a phosphate buffer solution (pH 7.4, μ=0.2) containing CyDs at a constant temperature (20°C). The final concentration of PGI_2 was adjusted to ca. 3.0×10^{-4}M. The pH of the sample solution was ascertained to be the same before and after the reaction. The plot of the logarithm of the concentration against time for the acid-hydrolysis of PGI_2 was a straight

line, indicating that the reaction was first-order. The rate constant was then calculated from the slope of the line.

The effect of CyDs on the alkaline hydrolysis rate of Indomethacin

The alkaline hydrolysis rate of indomethacin in the absence and presence of CyDs was spectrophotometrically monitored by measuring the decrease in the absorbance at 319 nm. The reaction was initiated by the addition of 0.1 ml of the stock solution of indomethacin (5×10^{-4}M) into 3 ml of a Na_2HPO_4-NaOH buffer solution (pH 11.0, μ=0.2) containing CyDs at a constant temperature (20 °C). The final concentration of indomethacin was adjusted to ca. 1.6×10^{-5}M. The pH of the sample solution was ascertained to be the same before and after the reaction. The calculation of the rate constant for the alkaline hydrolysis of indomethacin was similar to that for PGI_2.

The effect of CyDs on the dehydration of Prostaglandin E_1

The dehydration of prostaglandin E_1(PGE_1) in the absence and presence of CyDs was spectrophotometrically monitored by measuring the increase in absorbance at 218 nm. The reaction was initiated by the addition of a 0.1 ml of a methanol stock solution of PGE_1(ca. 3×10^{-2}M) to 3 ml of a 0.1M HCl solution containing CyDs at a constant temperature(55°C). The pH of the sample solution was confirmed to be identical to the initial pH. The calculation of the rate constant for the dehydration of PGE_1 was similar to that for PGI_2.

The effect of CyDs on the isomerization of Prostaglandin A_1

An aqueous solution of PGE_1 was permitted to stand in the dark for at least 48 hours, so that the PGE_1 was completely converted to prostaglandin A_1(PGA_1). The solution was then adjusted to a pH of 9.0 by the addition of NaOH. Isomerization of PGA_1 in the absence and presence of CyDs was spectrophotometrically monitored by measuring the increase in absorbance at 278 nm at a constant temperature(20°C). The pH of the sample solution was confirmed to be identical to the initial pH. The calculation of the rate constant for isomerization of PGA_1 was similar to that for PGI_2.

Results and Discussion

Table 1 presents some physicochemical and biological properties of CyDs such as aqueous solubility, specific optical rotation, surface tension and hemolytic activities. PMCD and DMCD have much higher aqueous solubilities (>50%) than the parent β-CyD. The low solubility of β-CyD may be a consequence of intermolecular hydrogen bonding between hydroxyl groups along the edges of the ring, which prevents adequate hydration by water molecules[4]. However, partial methylation of PMCD may preclude the formation of intermolecular hydrogen bonding[4]. Values for the surface tension of methylated β-CyDs were lower than those of the parent β-CyD (71 mN/m), PMCD has a surface activity analogous to that of DMCD. Fig. 1 shows the hemolytic effects of β-CyDs on human erythrocytes in an isotonic phosphate buffer(pH 7.4). The CyD-induced hemolysis was reported to be due to membrane distruption elicited by the dissolution and removal of membrane components[5]. The hemolytic activity of PMCD and DMCD were higher compared to that of the parent β-CyD. They have hydrophobic groups, so their stimulations of the erythrocyte were stronger than those of the parent β-CyD containing only hydroxyl groups[6].

CyDs are known to be fairly stable in alkaline medium; whereas, they are hydrolytically cleaved by strong acids to produce linear oligosaccharides. Fig.2 shows the logarithm of the rate constant (k) against time profiles for the degradation of PMCD and DMCD in 1M HCl at 60°C. PMCD was found to be similar to TMCD, and it also appears to be susceptible to acid hydrolysis because of the marked distortion of the macrocyclic ring conformation. The values of the acid-catalyzed hydrolysis rate of PMCD were close to those of TMCD (1.7 hr), and the acid-catalyzed hydrolysis rate of PMCD was faster than that of DMCD.

The effects of PMCD and DMCD on the solubility of drugs were studied and compared with those of the parent β-CyD. Table 2 summarizes the effects of CyDs on the solubility of slightly soluble or insoluble drugs in water at 25°C. The solubilizing ability of either PMCD or DMCD is much higher than that of the parent β-CyD because their aqueous solubilities were much higher than that of β-CyD. In spite of the higher aqueous solubility of PMCD than that of DMCD, PMCD generally exhibited a lower solubilizing ability than DMCD. The above results suggest that the steric hindrance by the methyl groups of PMCD may affect complex formation,

Table 1 Some Physicochemical Properties of β-CyD, DMCD and PMCD

CyD	Molecular Weight	Aqueous[c] solubility (g/100ml)	[c] $[\alpha]_D$	Surface[d] tension (mN/m)	Half-life of [e] degradation (hr)	50% Hemolysis (mM)
β-CyD	1135[b]	1.86	158	71.6	5.4[b]	2.0×10^{-3}
DMCD	1331[b]	>50	157.2	59.7	12.0	3.2×10^{-4}
PMCD	1340[a]	>50	164.4	56.4	2.1	4.1×10^{-4}

a)Determined by mass spectrometry(SIMS)
b)Dominique Duchêne(ed.) "Cyclodextrins and their industrial uses," Editions de santê, paris, France 1987.p 398
c)At 25°C in water
d)Concentration of CyD was 0.1w/v% in water
e)In 1M HCl at 60°C

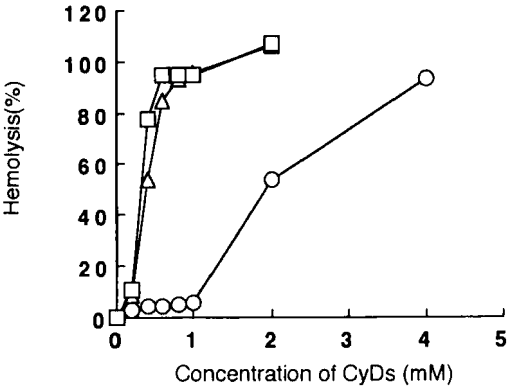

Figure1 Effects of CyDs on the Hemolysis of Human Erythrocytes in Isotonic Phosphate Buffer(pH 7.4). Key: (Δ), PMCD; (□),DMCD; (O),β-CyD.

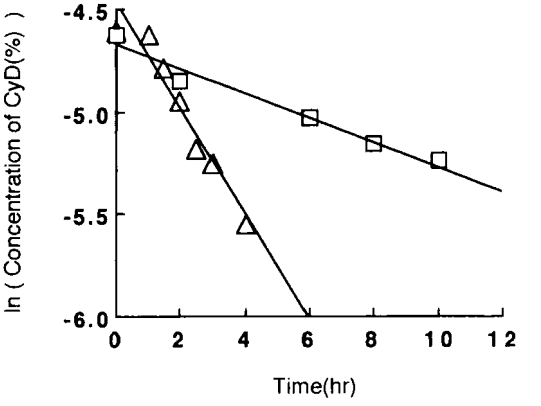

Figure 2 Time-Conversion Profiles for CyDs Acid Hydrolysis in 1M HCl at 60°C. Key: (Δ), PMCD; (□),DMCD

which is analogous with TMCD. The effect of PMCD on the solubility of indomethacin and piroxicam was greater than that of DMCD. This may be attributable to the inclusion characteristics of indomethacin and piroxicam.

Prostacyclin(PGI_2) undergoes an extremely facile hydrolysis of the vinyl ether moiety to yield 6-keto-prostaglandin $F_{1\alpha}$ in an aqueous solution, and it loses its activity within a few minutes[7]. Fig.3 summarizes

Table 2 Effects of CyDs on the solubility of slightly soluble or insoluble drugs in water at 25°C

Drug	Solubility in Water (μg/ml)	Solubility in 15 mg/ml CyDs soln. (μg/ml)		
		β-CyD	DMCD	PMCD
Teststerone Propionate	1.2	6.9 (5.7)	3460 (2845)	3038 (2598)
Nifedipine	5.4	10.8 (2.0)	27.7 (5.1)	17.2 (3.2)
Benzthiazide	5.5	44.7 (8.1)	73.4 (13)	44.6 (8.1)
Indomethacin	6.9	12.9 (1.9)	56.0 (8.0)	63.5 (11.1)
Digitoxin	9.7	533 (55)	4469 (460)	3380 (350)
Progesterone	11.7	12.5 (2.1)	2173 (186)	1441 (123)
Piroxicam	13.6	61.5 (4.5)	80.1 (5.9)	80.9 (6.0)
Polythiazide	14.6	71.1 (4.9)	187.4 (13)	95.5 (6.5)
Acetohexamide	17.4	55.9 (3.2)	130.7 (7.5)	101.8 (5.9)
Griseofulvin	21.2	26.5 (1.7)	33.5 (1.3)	27.6 (1.7)
Spironolactone	24.2	2174 (90)	4323 (180)	2512 (104)
Sulfadimethoxine	28.2	121 (4.3)	210.6 (7.5)	158.9 (5.6)
Flurbiprofen	31.3	81.4 (2.6)	1721 (55)	1939 (62)
Furosemide	32.5	67.3 (2.1)	121.2 (3.7)	108.2 (3.5)
Digoxin	33.8	5226 (154)	5193 (154)	3097 (92)
17α-Methyltestosterone	34.0	204.7 (6.0)	2340 (69)	1772 (52)
Ethiazine	53.6	75.6 (1.4)	77.6 (1.4)	72.7 (1.4)
Sulfadiazine	90.0	360.7 (4.0)	347.6 (3.9)	238.4 (2.6)
Tolbutamide	105.3	206.5 (2.0)	470.8 (4.5)	318.1 (3.0)
Tolazamide	109.3	125.4 (1.1)	216.8 (2.0)	213.9 (2.0)
Ketoprofen	133.0	1151 (8.7)	1756 (13)	1740 (13)
Trichlormethiazide	148.9	245.7 (1.7)	255.1 (1.7)	210.8 (1.4)
Carbamazepine	167.0	1108 (6.6)	1975 (12)	957.1 (5.7)
Chlorpropamide	319.0	840.4 (2.6)	920.6 (2.9)	694.1 (2.2)
Sulfathiazole	441.7	2910 (6.6)	2892 (6.5)	2593 (5.9)
Hydrochlorothiazide	617.0	1349 (2.2)	1550 (2.5)	1438 (2.3)

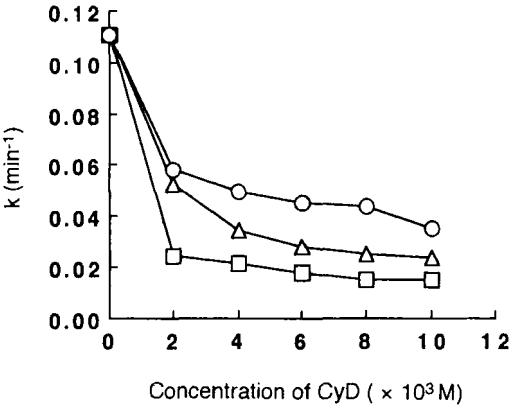

Figure 3 Rate Constants for the Hydrolysis of Prostacyclin(PGI_2) as a Function of CyDs Concentration in Phosphate Buffer(pH 7.4, μ=0.2) at 20°C. Key: (Δ), PMCD; (□), DMCD; (O), β-CyD.

the effects of CyDs on the hydrolysis rate of PGI_2. All the CyDs examined in this study slowed the hydrolysis rate. Among those CyDs, DMCD exhibited the largest stabilization effect. From this it can be assumed that the inclusion ability between DMCD and PGI_2 is greater than those of other CyDs.

Indomethacin is stable under acidic conditions, but it is immediately hydrolyzed under neutral or alkaline conditions[8]. Fig.4 summarizes the effects of CyDs on the hydrolysis rate of indomethacin. Among those CyDs, PMCD exhibited the largest stabilization effect. This order was in accordance with that of the solubilizing ability.

As shown in Fig.5, It is well known that Prostaglandin E_1 undergo dehydration and isomerization in an aqueous solution[9]. It was comfirmed that the reaction of $PGE_1 \rightarrow PGA_1$ obeyed the first-order rate law under these experimental conditions. Fig.6 shows the effects of CyDs on the dehydration of PGE_1 in 0.1M HCl at 55°C. β-CyD exhibited the largest stabilization effect.

Above a pH of 4, the isomerization of $PGA_1 \rightarrow PGB_1$ became significant. Fig.7 shows the effects of CyDs on the isomerization of PGA_1 at a pH of 9 at 20°C. DMCD exhibited the largest stabilization effect, and β-CyD showed a minimal stabilization effect. PMCD did not show any stabilization effect on the isomerization of PGA_1. It was theorized that the included position

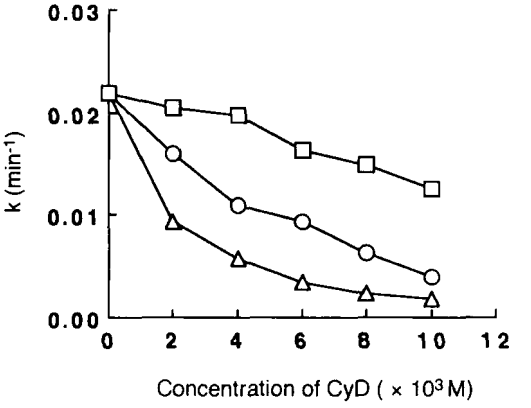

Figure 4 Rate Constants for the Hydrolysis of Indomethacin as a Function of CyDs Concentration in Phosphate Buffer(pH11.0, μ=0.2). Key: (Δ), PMCD; (□), DMCD; (○), β-CyD.

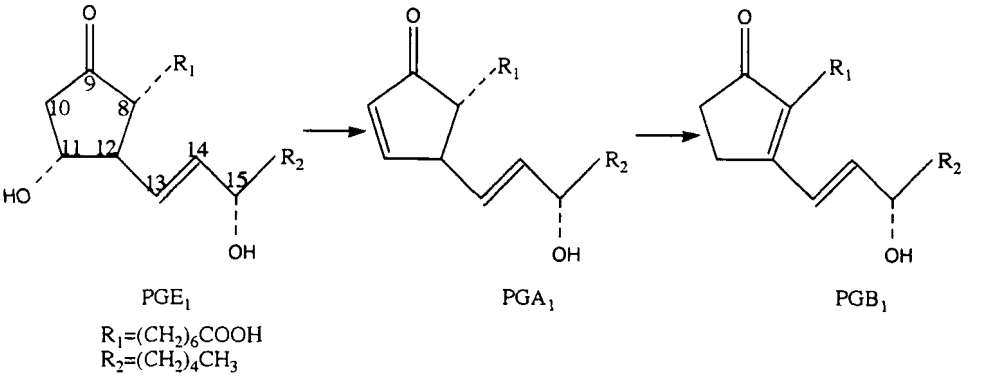

Figure 5 Dehydration and Isomerization of Prostaglandin E_1

which affected the dehydration and isomerization of PGE_1 was different from that of CyDs. So the effect of CyDs on dehydration and isomerization of PGE_1 yielded different results.

In conclusion, PMCD exhibited the inclusion behavior toward most chemical compounds similar to β-CyD and DMCD. Its inclusion ability for poorly water-soluble drugs was superior to that of β-CyD, but the effect of PMCD was slightly less than that of DMCD. PMCD prevented the hydrolysis of prostacyclin and indomethacin and the dehydration of PGE_1 in a manner similar to β-CyD and DMCD. The property of the exothermic dissolution of

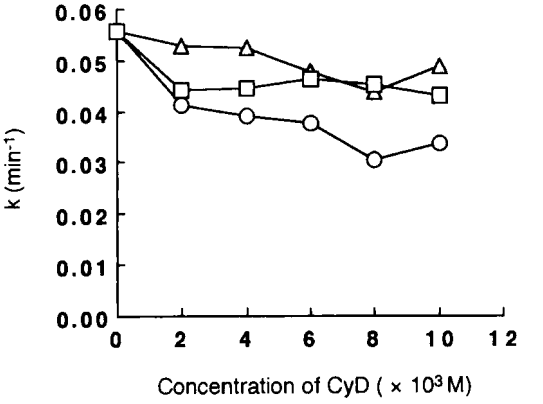

Figure 6 Rate Constants for the Dehydration of Prostaglandin E_1 as a Function of CyDs Concentration in Hydrochloric Acid(pH 1) at 55°C. Key: (Δ), PMCD; (□), DMCD; (O), β-CyD.

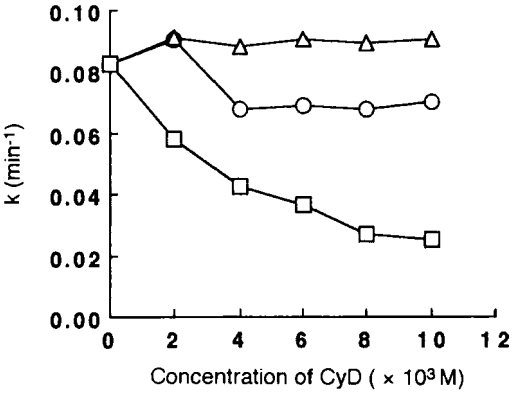

Figure 7 Rate Constants for the Isomerization of Prostaglandin A_1 as a Function of CyDs Concentration in Sodium Hydroxide(pH 9) at 20°C. Key: (Δ), PMCD; (□), DMCD; (O), β-CyD.

DMCD was modified by PMCD; therefore, it can be expected that PMCD will have various uses in medicine in place of DMCD.

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