Interaction of Tri-O-methyl-β-cyclodextrin with Drugs

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Summary

The effect of tri-O-methyl-β-cyclodextrin(methyl-β-CD) on the partition coefficients of drugs, such as p-nitrophenol, salicylic acid, benzoic acid, and aspirin, was studied at 25 °C. The partition coefficients of these drugs were increased linearly with methyl-β-CD concentration. The increase of partition coefficients was interpreted by the 1:1 complex formation between methyl-β-CD and the drug in $CHCl_3$ phase.

The interaction between p-nitrophenol and methyl-β-CD in solution was studied by UV and PMR spectroscopies. It was concluded that p-nitrophenol is included in the cavity of methyl-β-CD in both aqueous solution and $CHCl_3$ solution.

Inclusion compounds of these drugs with methyl-β-CD in the solid state were studied by X-ray diffractometry, IR spectroscopy, and DSC measurements. 1:1 crystalline inclusion compounds were obtained from hot water. It is also suggested that amorphous inclusion compound was obtained by the grinding of drug with methyl-β-CD.

The dissolution rate and the bioavailability of ketoprofen were significantly increased in the presence of methyl-β-CD. The bioavailability of ketoprofen after oral administration with methyl-β-CD to rats was 3.7 times that of ketoprofen alone.

Introduction

Cyclodextrins are known to form inclusion compounds with many kinds of drugs and have widely been studied. In the pharmaceutical field, cyclodextrins improve the physicochemical properties of unstable drugs and solubilize the slightly soluble drugs (1).

Methyl-β-CD is a permethylated β-cyclodextrin derivative and has some unusual physical properties compared with β-cyclodextrin. Methyl-β-CD is soluble not only in water but also in organic solvents,

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especially in CCl_4, and $CHCl_3$. Since methyl-β-CD has a complexing ability (2), it is suggested that the addition of methyl-β-CD affects the partition coefficients of drugs, like polysorbate 80 (3), and influences the bioavailability of drugs.

In this study, the effect of methyl-β-CD on the partition coefficients of drugs was investigated (4). The interaction between methyl-β-CD and drugs was investigated in the solid state and in solution (5). The effect of methyl-β-CD on the bioavailability of ketoprofen following oral administration to rats was examined (6).

Experimental

Materials Methyl-β-CD was synthesized by modification of the reported procedure (7) and recrystallized twice from hot water, mp 156-158 °C, $[\alpha]_D^{19}$ +160.0 ($CHCl_3$). p-Nitrophenol was purchased from Wako Pure Industries, Ltd. Aspirin, benzoic acid, and salicylic acid were of JP X grade. Ketoprofen was a gift from Iwaki Pharmaceutical Co., Ltd. All other materials were of analytical reagent grade.

Procedure for the Determination of the Partition Coefficients Aqueous phases were prepared by dissolving the drugs in 0.2 N HCl. Organic phases were prepared by dissolving methyl-β-CD (0 - 3×10^{-2}M) in $CHCl_3$. Ten ml of each solution was taken in a 50 ml glass-stoppered flask and shaken at 130 rpm for 1 h in a thermostated water bath at 25°C. The flask was left to stand, then the aqueous phase was withdrawn and the concentration of drug was determined with a Hitachi 124 double-beam spectrophotometer. The partition of methyl-β-CD between 0.2 N HCl and $CHCl_3$ was determined by polarimetry. A Nihon Bunko DPI-SL machine was used and the lower limit of detection was of the order of 10^{-5} M.

UV Absorption Study The UV absorption spectra of p-nitrophenol were measured containing various amounts of methyl-β-CD using Hitachi model 340 spectrophotometer.

PMR Study PMR spectra in D_2O were measured with JEOL-MH-100 spectrometer. Tetramethyl silane was used as an external reference.

Powder X-ray Diffraction Rigakudenki 2027 diffractometer was used. The measurement conditions were the same as described previously (8).

Differential Scanning Calorimetry Perkin Elmer Model DSC 1B differential scanning calorimeter was used. The measurement was done using the sample pan for the liquid sample at the scanning speed of 8°C/min.

IR Absorption Spectroscopy Hitachi 295 infrared spectrophotometer was used. The measurements were done according to the Nujol method.

Preparation the Inclusion Compound Inclusion compounds were prepared from hot water according to the coprecipitation method.

Preparation of the Ground Mixture Vibrational mill of Heiko Seisakusho Model TI-200 was used. Total weight of specimen was 2.0 g.

Dissolution Studies A powder sample containing 250 mg of ketoprofen was put into 50 ml of water in a flask at 30°C. The suspension was

shaken at 110 rpm. An aliquot of the solution was pipetted through a Milipore filter (1.0 μm). One ml of the sample solution was diluted with 0.1 N HCl and analysed spectrophotometrically.

In Vivo Absorption Studies Male Wister albino rats, 270 - 310 g, were fasted for 24 h prior to drug administration. Ketoprofen powder or its physical mixture with methyl-β-CD (molar ratio ; 1:1) was freshly suspended in distilled water and administrated into the stomach of a rat through a sonde at a dose of 25 mg/kg (ketoprofen) in a constant volume of 5 ml/kg. Blood samples were obtained from the femoral artery via a cannula and immediately centrifuged at 3000 rpm to obtain plasma samples. The concentration of ketoprofen in plasma was determined according to the method described by Bannier et al (9), using Simadzu LC-3A high performance liquid chromatograph. Indomethacine was used as an internal standard.

Results and Discussion

Effect of Methyl-β-CD on the Partition Coefficients of Drugs

The partition coefficients of drugs, such as p-nitrophenol, salicylic acid, benzoic acid, and aspirin, between aqueous phase and $CHCl_3$ phase were measured at various concentrations of methyl-β-CD at 25 °C. The initial concentrations of drugs were kept $1X10^{-4}$ M to avoid the self association of drugs. The partition coefficients of these drugs were increased linearly with methyl-β-CD concentration as shown in Fig. 1. On addition of 0.03 M methyl-β-CD, the partition coefficients of p-nitrophenol, salicylic acid, benzoic acid, and aspirin were 2.53, 2.49, 1.64, 1.58 times larger than those in the absence of methyl-β-CD. Moreover, these values were independent of the pH values of aqueous phase (pH 0.5 - 6.0).

As methyl-β-CD was not detected in $CHCl_3$ phase, the increase of

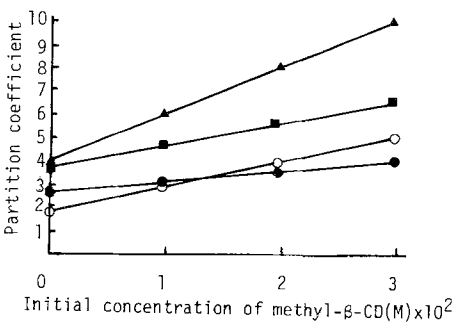

Fig. 1 Effect of Methyl-β-CD on Partition Coefficients of Drugs at 25°C
Aqueous phase: 0.2 N HCl, Organic phase: $CHCl_3$
▲: salicylic acid, ■: benzoic acid,
●: aspirin, ○: p-nitrophenol

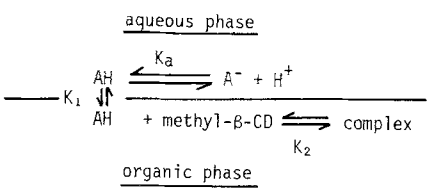

Chart 1

Table I Stability Constants of Complexes in $CHCl_3$ Phase at 25 °C

Drug	$(PC)_{MCD}/(PC)$	$K_2(M^{-1})$
p-Nitrophenol	2.53	51
Salicylic Acid	2.49	50
Benzoic Acid	1.64	21
Aspirin	1.58	19

the partition coefficients can be interpreted by the 1:1 complex formation of drug with methyl-β-CD in $CHCl_3$ phase (10). These results can be ascribed to an equilibrium of drug, methyl-β-CD, methyl-β-CD - drug complex, between aqueous phase and $CHCl_3$ phase as shown in Chart 1. AH and A^- indicate unionized drug and ionized drug, respectively. K_a, K_1, K_2 are the electrolytic dissociation constant, the partition coefficient of unionized drug, and the stability constant of the complex, respectively.
The following equations can be written

$$(PC) = \frac{[AH]_0}{[AH]_w + [A^-]_w} \qquad (1)$$

$$(PC)_{MCD} = \frac{[AH]_0' + [Complex]_0'}{[AH]_w' + [A^-]_w'} \qquad (2)$$

$$K_1 = \frac{[AH]_0}{[AH]_w} = \frac{[AH]_0'}{[AH]_w'} \qquad (3)$$

$$K_2 = \frac{[Complex]_0'}{[AH]_0'[MCD]_0'} \qquad (4)$$

$$K_a = \frac{[A^-]_w[H^+]_w}{[AH]_w} = \frac{[A^-]_w'[H^+]_w'}{[AH]_w'} \qquad (5)$$

where $(PC)_{MCD}$, and (PC) denote the partition coefficient with and without methyl-β-CD, respectively.
if $[MCD]_{ini} \gg [AH]_{ini}$, then $[MCD]_0' \fallingdotseq [MCD]_{ini}$
from equations (1) - (5), we may write

$$\frac{(PC)_{MCD}}{(PC)} = 1 + K_2[MCD]_{ini} \qquad (6)$$

Equation (6) indicates that $(PC)_{MCD}/(PC)$ ratios are dependent on the initial concentration of [MCD] and the stability constants K_2, but are independent on pH value, supporting our experimental data.

From equation (6), we can calculate the stability constants K_2, and the data are shown in Table I.

Interaction of Drug and Methyl-β-CD in Solution

Figure 2 shows the effect of methyl-β-CD on the UV spectra of p-nitrophenol in 0.05 N HCl (A) and in $CHCl_3$ (B) at 25 °C. With

increasing concentration of methyl-β-CD, the absorption maxima of p-nitrophenol in both solutions shifted to longer wavelength with isosbestic points. The presence of maltopentaose caused no spectral change of p-nitrophenol in aqueous solution. It is considered that methyl-β-CD forms an inclusion compound with both in aqueous solution and $CHCl_3$ solution. Since the stoichiometry of the inclusion compounds was considered to be 1:1 from the UV spectral change, stability constants were calculated according to Scott's equation (11). The stability constants of p-nitrophenol and methyl-β-CD were 120 M^{-1} in aqueous solution and 73 M^{-1} in $CHCl_3$ solution. The stability constant in $CHCl_3$ solution was about 0.6 times that in aqueous solution. This may be attributed to the difference of main driving force for complex formation (12).

Figure 3 shows the PMR spectra of p-nitrophenol in the presence and absence of methyl-β-CD in D_2O. Both the ortho and meta protons of p-nitrophenol were shifted to lower field, by 0.14 and 0.12 ppm, respectively in the presence of methyl-β-CD. It is said that factors affecting spectral shifts include the diamagnetic anisotropy of particular bonds or regions of the host, van der Waals shifts, or steric perturbation. Since appreciable shifts were not induced in the presence of maltopentaose, it can be concluded that p-nitrophenol is included in the cavity of methyl-β-CD from the results of the PMR study as well as the UV study. Bergeron et al. reported that ortho and meta protons of p-nitrophenol shifted lower field in the α-cyclodextrin system, but did not shifted in the β-cyclodextrin system on complexation (13). They inferred that in the β-cyclodextrin system, the contact between the guest and the host was not as close as in the complex of p-nitrophenolate with α-cyclodextrin.

Since lower field shifts were observed in the p-nitrophenol - methyl-β-CD system, it can be attributed to the close proximity of p-nitrophenol molecule to methyl-β-CD molecule.

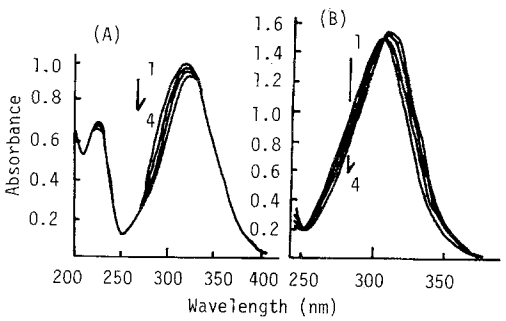

Fig. 2 Effect of Methyl-β-CD on UV Absorption Spectra of p-Nitrophenol at 25 °C

(A) in 0.05 N HCl, Concentration of methyl-β-CD curve 1; 0, 2; 1.0×10^{-3}, 3; 4.0×10^{-3}, 4; 8.0×10^{-3}M
(B) in $CHCl_3$, Concentration of methyl-β-CD curve 1; 0, 2; 5.0×10^{-3}, 3; 1.5×10^{-2}, 4; 2.5×10^{-2}M

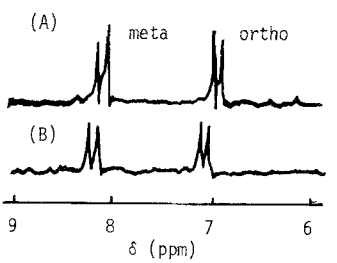

Fig. 3 Effect of Methyl-β-CD on the PMR Spectra of p-Nitrophenol in D_2O at 26.5 °C

(A): p-nitrophenol (6.4×10^{-2}M) alone
(B): p-nitrophenol (7.6×10^{-2}M) + methyl-β-CD(2.7×10^{-2}M)

Interaction of Drug with Methyl-β-CD in the Solid State

Inclusion compounds of drug with methyl-β-CD were prepared by the coprecipitation method from water. Figures 4(A), and (C) show the powder X-ray diffraction patterns of benzoic acid and methyl-β-CD system. The diffraction pattern of physical mixture was simply the superposition of each component, while the inclusion compound was apparently different from that of physical mixture and constituted a new crystalline solid.

The thermal behaviour of methyl-β-CD and benzoic acid system was examined and their thermograms are depicted in Fig. 5. The inclusion compound showed endothermic peak at 169 °C, which was different from the melting temperatures corresponding to benzoic acid (122 °C) and methyl-β-CD (156 °C). The physical mixture showed unusual thermal behaviour, exothermic peak at 84 °C and then endothermic peak at 165 °C. To elucidate the thermogram of physical mixture, the mixture of methyl-β-CD and benzoic acid was stored in a oven at 100 °C for 2 h. X-Ray diffractogram showed that the obtained sample was changed into crystalline inclusion compound. From this result, it is reasonable to suppose that inclusion compound is formed during the heating of the physical mixture. The first endothermic peak in Fig. 5(C) is assumed to the formation of crystalline inclusion compound.

Figure 4(B) shows the powder X-ray diffraction pattern of the ground mixture of methyl-β-CD and benzoic acid system. Grinding of benzoic acid alone and with methyl-β-CD using vibrational mill showed that benzoic acid alone did not change to amorphous form, but with

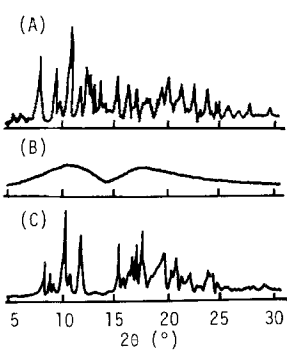

Fig. 4 X-ray Diffraction Patterns of Methyl-β-CD and Benzoic Acid System (molar ratio 1:1)
(A): physical mixture
(B): ground mixture
(C): inclusion compound

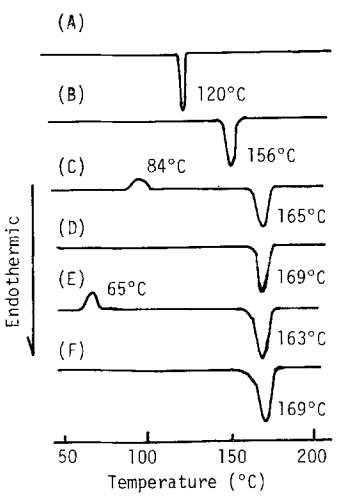

Fig. 5 DSC Curves of Methyl-β-CD and Benzoic Acid System (molar ratio 1:1)
(A): benzoic acid, (B): Methyl-β-CD, (C): physical mixture, (D): inclusion compound, (E): ground mixture, (F): after heated at 90°C of physical mixture

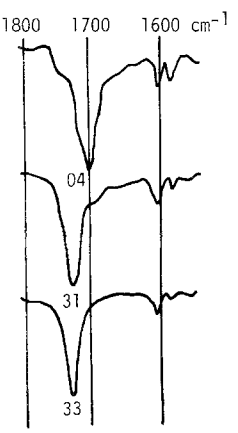

Fig. 6 IR Spectra of Methyl-β-CD and Benzoic Acid System (molar ratio 1:1)
(A): physical mixture
(B): ground mixture
(C): inclusion compound

methyl-β-CD, it was changed to amorphous form.

Figure 5(E) shows DSC curves of the ground mixture. The ground mixture represents exothermic peak at 65 °C and the endothermic peak at 163 °C. After the ground mixture was heated up to 90 °C, a second DSC measurement was carried out. The exothermic peak at 65 °C had disappeared and only endothermic peak at 169 °C was observed (Fig. 5(F)). From these observations, it is suggested that the exothermic peak at 65 °C and the endothermic peak at 163 °C correspond to the crystallization of amorphous into inclusion compound and the melting of crystalline inclusion compound, respectively.

Figure 6 shows the IR spectra of methyl-β-CD and benzoic acid system. As illustrated in Fig. 6(A), peak at 1704 cm^{-1} (14) was assigned to carbonyl stretching band of cyclic dimer structure of benzoic acid molecules. In the case of crystalline inclusion compound, the 1704 cm^{-1} band shifted to 1733 cm^{-1}, suggesting the inclusion of monomer benzoic acid molecule into the hydrophobic cavity of methyl-β-CD. In the case of ground mixture, carbonyl band was similarly higher frequency shift and observed at 1731 cm^{-1}. Similar results for powder X-ray diffraction patterns, DSC thermograms, and IR spectra were observed for other drugs examined in this study. From these results, it was suggested that drug molecule was included in the cavity of methyl-β-CD by the grinding.

Dissolution Studies

Although the crystalline inclusion compound of ketoprofen with methyl-β-CD were obtained in the molar ratio of 1:1 from ether, it suddenly changed to a pasty state in water. Therefore, the physical mixture was used throughout these experiments.

The solubility of ketoprofen increased with increasing concentration of methyl-β-CD, showing A_L type phase solubility curve (15). The stability constant of the inclusion compound in water was estimated as 260 M^{-1} from the intercept and the slope. At 0.1 M

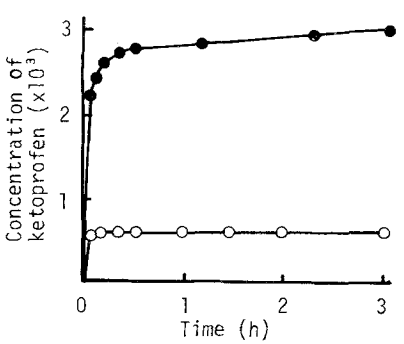

Fig. 7 Dissolution Profiles of Ketoprofen Powder and Its Physical Mixture with Methyl-β-CD in Water at 30 °C

●: physical mixture (molar ratio; 1:1)
○: ketoprofen alone

methyl-β-CD, the apparent solubility of ketoprofen was about 20 times that ketoprofen alone.

Figure 7 shows the dissolution profiles of ketoprofen from ketoprofen powder and physical mixture with methyl-β-CD (molar ratio; 1:1). A High dissolution rate of ketoprofen was observed in the presence of methyl-β-CD. The enhanced dissolution rate may be due to the increase in solubility in the presence of methyl-β-CD as expected from the phase solubility curve. Furthermore, it was found that the solubility limit was approximately attained within a few minutes in each sample.

In Vivo Absorption Studies

Figure 8 shows the plasma levels of ketoprofen after oral administration of the physical mixture and ketoprofen alone to rats. When the physical mixture was administrated to rats, the maximum plasma level of 25.1±3.2 μg/ml was attained at 10 min after oral administration, and was about 2.7 times higher than that after administration of ketoprofen alone. The initial absorption rate of ketoprofen after administration of the physical mixture was about 2.8 times larger than that of ketoprofen alone, while the elimination rate constants were similar in both cases (about 0.08 h^{-1}). Consequently, higher plasma levels were maintained for a long time in ketoprofen - methyl-β-CD system. The AUC of the physical mixture up to 19 h post administration was 220.5 gh/ml and was 3.7 times that of ketoprofen alone. The quantity of methyl-β-CD added in the in vivo studies corresponded to about 0.02 M methyl-β-CD. As the ketoprofen - methyl-β-CD system showed A_L type phase solubility diagram, a large amount of methyl-β-CD should further improve the bioavailability. The enhanced bioavailability is considered to be mainly due to high dissolution rate, i.e. high drug solubility in the case of ketoprofen - methyl-β-CD system. As methyl-β-CD has surface active properties, it might be expected to facilitate drug absorption

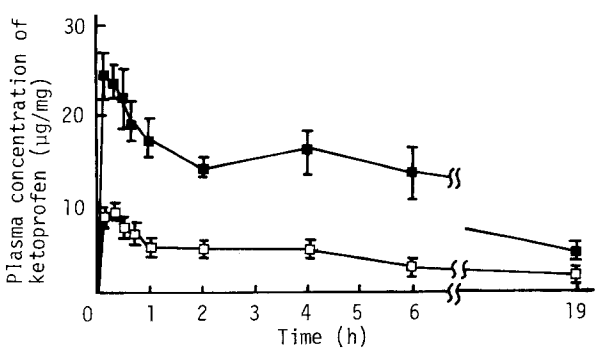

Fig. 8 Plasma Levels of Ketoprofen Following Oral Administration to Rats

Each point represents the mean±S.E. of six rats.
■: ketoprofen-methyl-β-CD physical mixture (molar ratio; 1:1), □: ketoprofen alone

through a membrane. Furthermore, it was reported that methyl-β-CD was absorbed from the intestine in rats, though slowly(16). However, we did not establish whether the inclusion complexation of ketoprofen with methyl-β-CD affected the membrane transfer.

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