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Enhanced Dissolution and Oral Bioavailability of α-Tocopheryl Esters by Dimethyl-β-cyclodextrin Complexation

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Abstract. Inclusion complexation of heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) with α-tocopheryl acetate and α-tocopheryl nicotinate in aqueous solution was studied by the solubility method. The aqueous solubilities of the esters were about 10^5 times increased by DM-β-CyD complexation. The phase-solubility diagram of the tocopheryl ester–DM-β-CyD systems showed a typical A_p type, and the stability constants (K) of high-order complexes were estimated by analyzing the upward curvature of the diagrams. The solid complex of α-tocopheryl nicotinate with DM-β-CyD in a molar ratio of 1 : 2 was prepared by the kneading method. The dissolution rate of the solid complex was much greater than that of the drug itself, and the rapidly dissolving form of α-tocopheryl nicotinate, as an example, showed a markedly increased bioavailability (about 70-fold) after oral administration to fasted dogs.

Key words. α-Tocopheryl acetate, α-tocopheryl nicotinate, dimethyl-β-cyclodextrin, inclusion complexation, solubility, dissolution rate, bioavailability.

1. Introduction

The bioavailability of α-tocopheryl esters (fat-soluble vitamin E derivatives) is known to be extremely low when administered orally under fasting conditions, one reason for this being their low solubility in gastrointestinal fluid [1, 2]. Chemically modified cyclodextrins have been utilized for improving various pharmaceutical properties such as solubility, chemical stability, and bioavailability of drugs [3–5]. In our preliminary study, parent α-, β- and γ-cyclodextrins were found to have little solubilizing effect on tocopheryl esters. Therefore, the present study deals with inclusion complexation of heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) with α-tocopheryl acetate and α-tocopheryl nicotinate, in anticipation of improved solubility, dissolution, and *in vivo* absorption characteristics of the drugs.

2. Experimental

2.1. MATERIALS

dl-α-Tocopheryl acetate and *dl*-α-tocopheryl nicotinate were used as supplied by Nisshin Flour Milling Co. Ltd., Japan. DM-β-CyD was supplied by Toshin Chemical Co., Japan

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and used after recrystallization from water. All other materials and solvents were of analytical reagent grade and deionized doubly-distilled water was used throughout the study.

2.2. SOLUBILITY STUDIES

Solubility measurements were carried out according to Higuchi and Connors [6]. Excess amounts of the α-tocopheryl esters were added to aqueous solutions containing various concentrations of DM-β-CyD and shaken at 25°C in the dark, under which conditions no degradation of the tocopheryl esters was observed. After equilibrium was attained (approximately 2 weeks), an aliquot was centrifuged and pipetted through a cotton plug. The filtrate was adequately diluted with the methanol and analyzed spectrophotometrically (λ_{max} = 284 and 263 nm for α-tocopheryl acetate and α-tocopheryl nicotinate, respectively).

2.3. PREPARATION OF SOLID COMPLEX

The solid complexes were prepared according to the kneading method [7]. For example, α-tocopheryl nicotinate (1 g) and DM-β-CyD (5.96 g) in a molar ratio of 1 : 2 were triturated with a small amount of water (about 10 mL) and the slurry was further kneaded thoroughly for about 40 min. The paste thus obtained was dried under a reduced pressure at room temperature for 3 days. Differential thermograms and powder X-ray diffraction patterns of the complexes were taken under conditions similar to those reported previously [8].

2.4. DISSOLUTION STUDIES

The dissolution rate was measured according to the dispersed amount method [9]. Excess amount of α-tocopheryl nicotinate (20 mg, 100 mesh) or its complex (equivalent to 20 mg of the drug) was put into 100 mL of Japanese Pharmacopoeia XI (JP XI) 1st fluid (2.0 g of NaCl and 24 mL of 10% HCl in 1000 mL of water, pH about 1.2) or into JP XI 2nd fluid (250 mL of 0.2 M KH_2PO_4 and 118 mL of 0.2 N NaOH in 1000 mL of water, pH about 6.8) at 37°C, and the dissolution medium was stirred at 91 rpm. At an appropriate interval, 3 mL of solution was sampled by a pipet with a cotton plug, and assayed spectrophotometrically using a Hitachi 556S double-wavelength spectrophotometer ($\lambda_1 = \lambda_{max}$ of each drug and λ_2 = 350 nm) because of the emulsification of the solution.

2.5. RELEASE STUDIES FROM CAPSULE

The drug release rates from gelatin capsules were measured by the rotating basket method for the dissolution test in JP XI at 75 rpm. For example, a test capsule (JP XI No. 00, volume = 0.95 cm^3) was filled with DM-β-CyD complex (298 mg, equivalent to 50 mg drug) or α-tocopheryl nicotinate mixed with a diluent (50 mg drug + 248 mg starch), and put into 500 mL of JP XI 1st fluid at 37°C. The concentration of α-tocophenyl nicotinate released in the medium was determined spectrophotometrically according to the method described in the dissolution study.

2.6. *IN VIVO* ABSORPTION STUDIES

Six male beagle dogs weighing 9.6–12.5 kg were fasted for about 12 h prior to drug administration. Intervals of at least two weeks were taken in a cross-over matrix to minimize the

cumulative effect of the preceding dose. A gelatin capsule containing α-tocopheryl nicotinate or its DM-β-CyD complex (equivalent to 100 mg α-tocopheryl nicotinate/body) was orally administered, and blood samples (7.0 mL) were collected at predetermined time, using sodium heparin as an anticoagulant. The α-tocopheryl nicotinate in the plasma was determined by high-performance liquid chromatography (HPLC) as previously reported [10].

3. Results and Discussion

3.1. COMPLEXATION OF α-TOCOPHERYL ESTERS WITH DM-β-CyD

Figure 1 shows the phase solubility diagrams obtained for α-tocopheryl esters with DM-β-CyD in water, where the solubility curves can be classified as type A_p [6], suggesting a high order complexation. It is worth noting that DM-β-CyD complexation remarkably improved the low solubility of esters in water. For example, the solubilization by a factor of about 10^5 was attained by the addition of 0.1M DM-β-CyD. Such large solubilization enhancements have rarely been observed in other cyclodextrin systems, so far as we know [5].

The ascending curvatures in Figure 1 were quantitatively analyzed according to the optimization technique [11] to obtain the stability constants of high-order complexes. That is, when $1:n$ (guest : host) complexes occur in a stepwise reaction (Equation 1), each stability constant ($K_{1:n}$) can be defined by Equation 2 where (G) and (C) represent molar concentrations of free guest and cyclodextrin, respectively. Since the mass balance equations are given by Equations 3 and 4 and (G) is the solubility (G_0) of G in the absence of

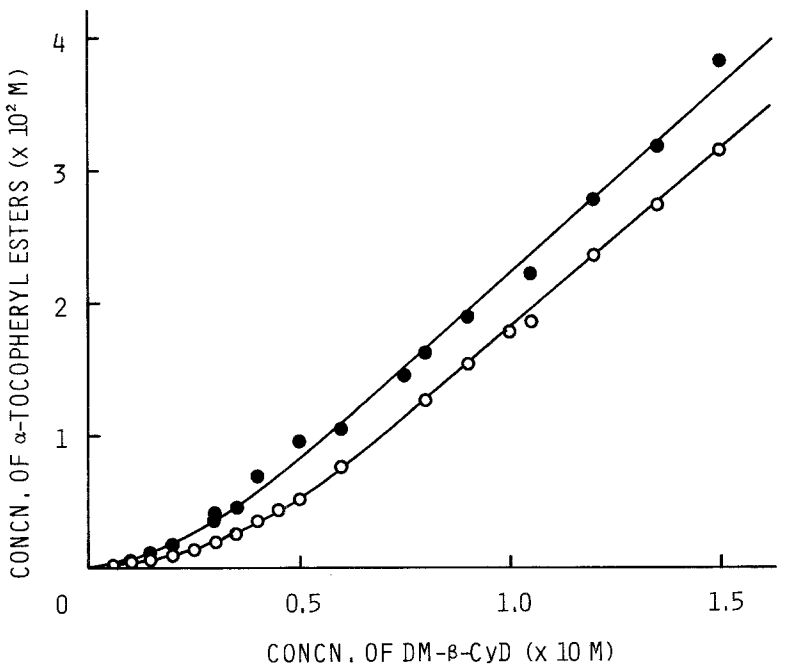

Fig. 1. Phase solubility diagrams of α-tocopheryl ester-DM-β-CyD system in water at 25°C. ○: α-tocopheryl acetate; ●: α-tocopheryl nicotinate.

C, the total concentrations of G and C (G_t and C_t, respectively) can be expressed by the polynomial equations (5 and 6) of Nth order with respect to (C). Therefore, each stability constant can be determined by analyzing Equation (5) by the least-squares method, if the free cyclodextrin concentration, (C), is known. As a first approximation, (C) was set to (C_t) and the preliminary estimate of stability constants was obtained from Equation (5) by the

$$\begin{aligned}
&(G) + (C) \overset{K_{1:1}}{\rightleftharpoons} (GC)\\
&(GC) + (C) \overset{K_{1:2}}{\rightleftharpoons} (GC_2)\\
&(GC_2) + (C) \overset{K_{1:3}}{\rightleftharpoons} (GC_3)\\
&\qquad\vdots\\
&(GC_{n-1}) + (C) \overset{K_{1:n}}{\rightleftharpoons} (GC_n)
\end{aligned} \tag{1}$$

$$\begin{aligned}
K_{1:1} &= \frac{(GC)}{(G)(C)}\\
K_{1:2} &= \frac{(GC_2)}{K_{1:1}(G)(C)^2}\\
K_{1:3} &= \frac{(GC_3)}{K_{1:1}K_{1:2}(G)(C)^3}\\
&\vdots\\
K_{1:n} &= \frac{(GC_n)}{K_{1:1}K_{1:2}\cdots K_{1:n-1}(G)\,(C)^n}
\end{aligned} \tag{2}$$

$$G_t = (G) + (GC) + (GC_2) + \cdots + (GC_n) \tag{3}$$

$$C_t = (C) + (GC) + 2(GC_2) + \cdots + n(GC_n) \tag{4}$$

$$\begin{aligned}
G_t = {} & (G_0) + K_{1:1}(G_0)(C) + K_{1:1}K_{1:2}(G_0)(C)^2 + \cdots\\
& + K_{1:1}K_{1:2}\cdots K_{1:n}(G_0)(C)^n
\end{aligned} \tag{5}$$

$$\begin{aligned}
C_t = {} & (C) + K_{1:1}(G_0)(C) + 2K_{1:1}K_{1:2}(G_0)(C)^2 + \cdots\\
& + nK_{1:1}K_{1:2}\cdots K_{1:n}(G_0)(C)^n
\end{aligned} \tag{6}$$

simplex method [12], using (C_t) and the known G_t and G_0 values. The (C) could then be calculated from Equation (6) from the initial estimate of stability constants and the known C_t and G_0. Equations (5) and (6) were used iteratively until convergent values for stability constants were obtained. In the present system, the 1 : 1, 1 : 2, and 1 : 3 complexes of α-tocopheryl esters with DM-β-CyD were assumed to form under the experimental conditions, and the results are summarized in Table I. The $K_{1:2}$ value was significantly larger than the $K_{1:1}$ and $K_{1:3}$ values in both complexes, suggesting that the 1 : 2 inclusion complex is the most predominant species under the present conditions. The 1 : 2

Table I. Stability constants of α-tocopheryl ester-DM-β-CyD complexes in water at 25°C

α-Tocopheryl ester	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-1})	$K_{1:3}$ (M^{-1})	G_0[a] (M)
α-Tocopheryl nicotinate	20	1540000	1	1.97×10^{-7}
α-Tocopheryl acetate	300	172000	70	1.07×10^{-7}

[a] Solubility of α-tocopheryl ester in water.

stoichiometry was supported also from the observations made with the space-filling Corey–Pauling–Koltun molecular models for the α-tocopheryl ester-DM-β-CyD complexes.

Therefore, the solid complexes of α-tocopheryl esters with DM-β-CyD in a molar ratio of 1 : 2 were prepared by the kneading method [7], and their complexation was ascertained by differential thermal analysis (DTA) and powder X-ray diffractometry. Figure 2 shows the DTA thermograms of the α-tocopheryl nicotinate-DM-β-CyD system, as an example, where the endothermic peak around 45°C corresponding to the melting temperature of the ester completely disappeared upon complexation. Furthermore, the X-ray diffraction pattern of the complex was apparently different from that of a physical mixture of the guest and host molecules, as shown in Figure 3. These data clearly indicate that the tocopheryl ester-DM-β-CyD complexes exist in the solid state [5].

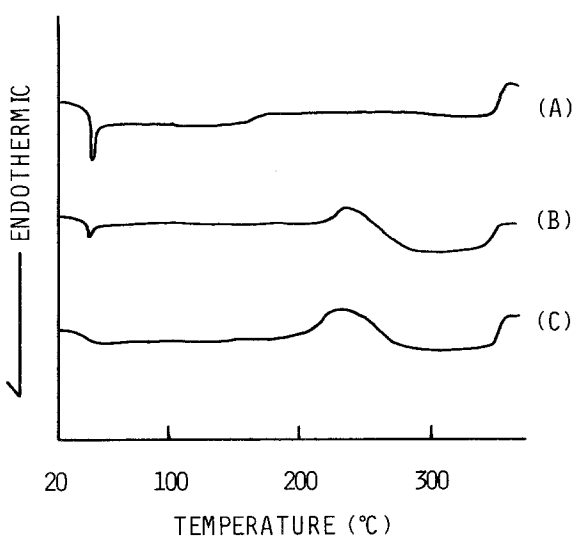

Fig. 2. DTA thermogram of the α-tocopheryl nicotinate-DM-β-CyD system. (A): α-tocopheryl nicotinate; (B): physical mixture of α-tocopheryl nicotinate and DM-β-CyD; (C): complex of α-tocopheryl nicotinate with DM-β-CyD.

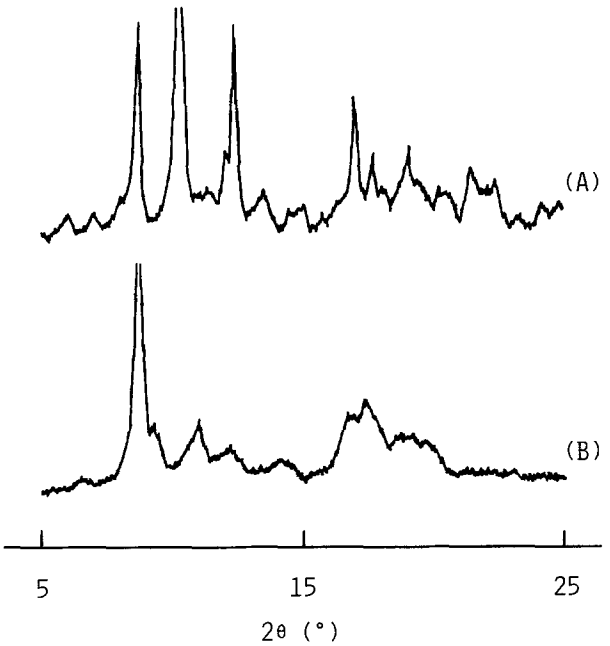

Fig. 3. Powder X-ray diffraction patterns of α-tocopheryl nicotinate-DM-β-CyD system. (A): physical mixture of α-tocopheryl nicotinate and DM-β-CyD; (B): complex of α-tocopheryl nicotinate with DM-β-CyD.

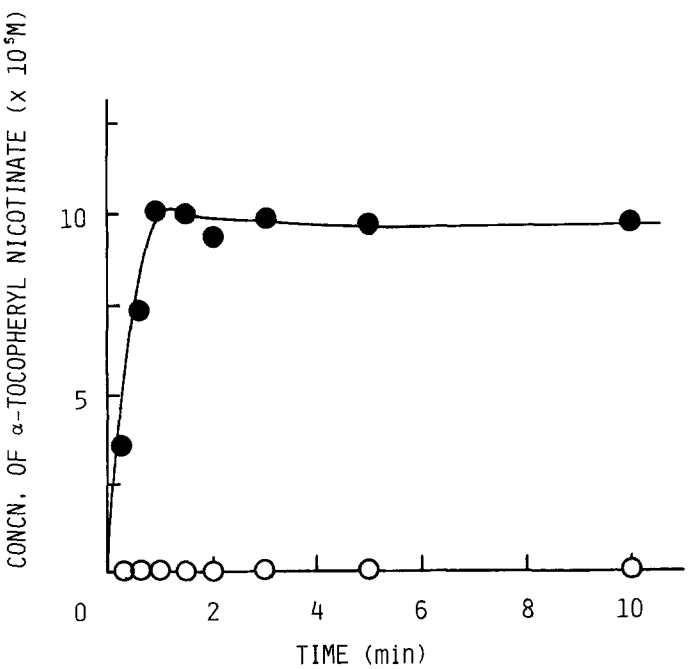

Fig. 4. Dissolution profiles of α-tocopheryl nicotinate and its DM-β-CyD complex in the medium of JP XI first fluid at 37°C, measured by the dispersed amount method. ○: α-tocopheryl nicotinate; ●: complex.

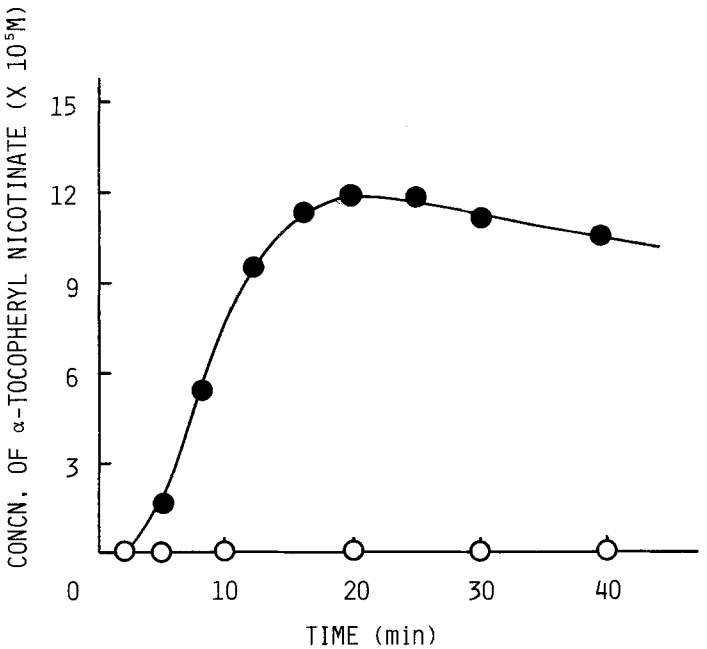

Fig. 5. Release profiles of α-tocopheryl nicotinate from capsules containing α-tocopheryl nicotinate or its DM-β-CyD complex in JP XI first fluid at 37°C, measured by the rotating basket method. ○: α-tocopheryl nicotinate; ●: complex.

3.2. DISSOLUTION AND RELEASE BEHAVIORS

Figures 4 and 5 show the dissolution and release profiles of α-tocopheryl nicotinate and its DM-β-CyD complex, respectively, in JP XI 1st fluid (pH about 1.2). In the case of α-tocopheryl nicotinate alone, concentrations of the drug dissolved in the medium were much too low to be determined under these experimental conditions. On the other hand, both dissolution and release rates of the complex increased markedly, due to its higher aqueous solubility as expected from Figure 1. The decrease in concentration of the drug following the peak may have resulted from the dissociation of the complex to the free host and guest molecules after supersaturation [13]. The rapid dissolution of α-tocopheryl ester-DM-β-CyD complexes was also observed when JP XI 2nd fluid was used as a dissolution medium.

3.3. *IN VIVO* ABSORPTION BEHAVIOR

α-Tocopheryl nicotinate and its DM-β-CyD complex were orally administered to fasted dogs to evaluate their absorption characteristics. Figure 6 shows the mean plasma levels of α-tocopheryl nicotinate following the oral administration of capsules containing the drug or its DM-β-CyD complex (equivalent to 100 mg drug/body). It is apparent that the extent of absorption of α-tocopheryl nicotinate was extremely low when orally administered under fasted condition as reported previously [1, 2]. On the other hand, the plasma levels of α-tocopheryl nicotinate increased remarkably when the drug was administered as the complexed form of DM-β-CyD. The maximum plasma level (C_{max}) of 0.020 ± 0.008 μg/mL was observed at 3.67 ± 0.80 h in the case of the drug alone, while the complex provided

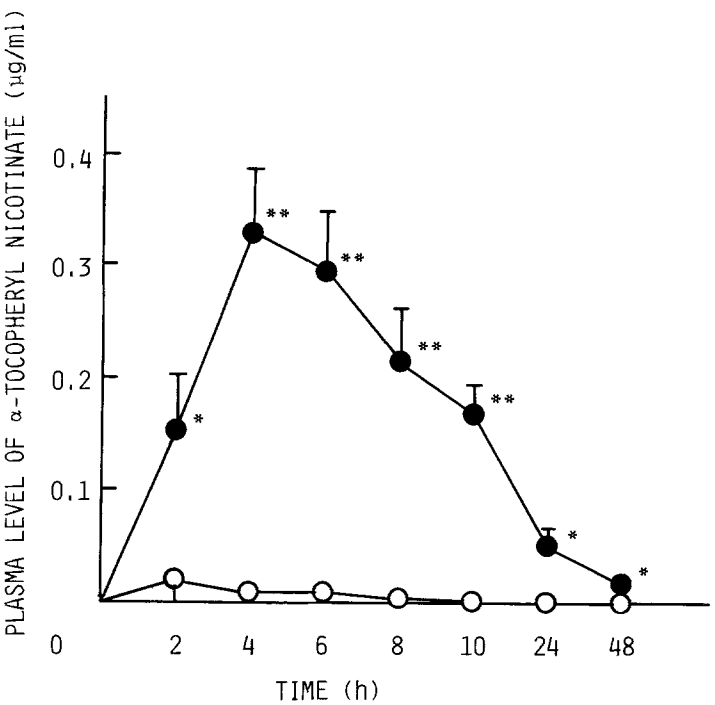

Fig. 6. Plasma levels of α-tocopheryl nicotinate following oral administration of capsules containing α-tocopheryl nicotinate or its DM-β-CyD complex (equivalent to 100 mg drug) to dogs. ○: α-tocopheryl nicotinate; ●: complex. $*p < 0.05$ in ● versus ○; $**p < 0.01$ ● versus ○. Values represent the mean ± S.E. of 6 dogs.

a C_{max} value of 0.344 ± 0.039 μg/mL at 4.00 ± 0.44 h. The area under the plasma concentration-time curve of the complex up to 48 h post-administration was about 70 times as much as that of α-tocopheryl nicotinate alone.

All the results clearly indicate that DM-β-CyD has great utility for improving the solubility, dissolution and bioavailability of α-tocopheryl esters, and may be applicable to other fat-soluble vitamins.

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