Solubilization of Lipid-Soluble Vitamins by Complexation with Glucosyl-β-cyclodextrin[1)]

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Inclusion complex formation of eight kinds of lipid-soluble vitamins with 6-*O*-α-D-glucopyranosyl-β-cyclodextrin (G-β-CD) in aqueous solution and in solid phase were assessed by the solubility method and thermal analysis. All lipid-soluble vitamins were highly solubilized in water by complexation with G-β-CD. From analysis of the phase solubility diagrams, the stoichiometric ratio of the main complex in water was estimated to be 1:2 for vitamin (V) A alcohol/G-β-CD, 1:2 for V D_2/G-β-CD, 1:1 for V D_3/G-β-CD, 1:3 for V E/G-β-CD, 1:4 for V E nicotinate/G-β-CD, 1:3 for V K_1/G-β-CD, 1:3 for V K_2/G-β-CD, and 1:1 for V K_3/G-β-CD. The stabilities of lipid-soluble vitamins in water containing G-β-CD were examined. A V E nicotinate –G-β-CD complex solution was stable even under irradiation with light.

Keywords glucosyl-β-cyclodextrin; lipid-soluble vitamin; inclusion complex; solubility method; solubilization; differential scanning calorimetry; stability

Branched cyclodextrins (CDs) which have one or more branches of an α-D-glucopyranosyl unit or a (1→4)-α-D-glucan at carbon 6 of glucose residues in CD have many advantages over their parent CDs: high solubility both in water and in organic solvents and decreasing hemolytic activities with the elongation of the side chain.[2–5)] The complexation abilities of branched and parent CDs appear to be almost the same, however, the enhancement of solubility of water-insoluble compounds by complexation with branched CDs was much more remarkable than that with parent CDs, particularly in the β-CD series.[2–5)]

This paper deals with the solubilization of lipid-soluble vitamins in water by complexation with 6-*O*-α-D-glucopyranosyl-β-CD (G-β-CD), and also with stabilities of these complexes in water.

Experimental

Materials G-β-CD was isolated and purified by high-performance liquid chromatography (HPLC) according to the reported method.[5)] β-CD was used after recrystallization from water. All lipid-soluble vitamins used were obtained from commercial sources. Deionized and double distilled water was used throughout this experiment. Reagent-grade organic solvents used for HPLC were freshly distilled and filtered through a 0.45-μm membrane filter.

General Method HPLC analyses of lipid-soluble vitamins were performed using a Familic-300S HPLC pump and a model VL-614 injector (all from JASCO, Tokyo, Japan). The columns used were a Finepak SIL C_{18} (250 × 4.6 mm i.d.) (JASCO) and a YMC-Pack A-802 C_4 (150 × 4.6 mm i.d.) (Yamamura Chemical, Kyoto, Japan).

Solubility Studies Estimation of complex-forming ability of G-β-CD by the solubility method[6)] was conducted according to the procedure described previously.[2)]

Preparation of Solid Complexes In vitamin (V) D_2 and V D_3–G-β-CD systems which showed B_S type phase solubility diagrams,[6)] the solid complexes were prepared by mixing appropriate amounts of G-β-CD and V D_2 or V D_3 in water. For example, 80 mg (0.2 mmol) of V D_2 and 260 mg (0.2 mmol) of G-β-CD were added to 1 ml of water, and the mixture was shaken at 30 °C for 24 h in the dark. The complexes, precipitated as microcrystalline powders, were separated from the solution by filtration and dried *in vacuo* at 25 °C for 24 h. The solid complexes were dissolved in 65% ethanol and the contents of guest and host compounds were determined by HPLC. Both V D_2/G-β-CD and V D_3/G-β-CD molar ratios in the solid complexes obtained were 1/2.

Thermal Analysis The analysis was done using a Thermo Flex DSC-8230B (Rigaku, Tokyo, Japan) at a scanning speed of 5 °C/min and scanning temperature range of 30—200 °C.

Photodegradation Study A sample solution in water was prepared by mixing equimolecular amounts of G-β-CD and lipid-soluble vitamin in water. The mixture was shaken at 30 °C for 24 h in the dark, and filtered through 0.2-μm membrane filter to remove excess lipid-soluble vitamin. The concentrations of lipid-soluble vitamins in 200 mM G-β-CD solution were 14.5 mM for V A alcohol, 2.1 mM for V E, 0.5 mM for V E nicotinate, 0.4 mM for V K_1, and 1.3 mM for V K_2. V D_2 and V D_3 concentrations in 75 mM G-β-CD solution were 5.4 and 16.2 mM. In these systems, the solution was hard to make in a higher concentration of G-β-CD, since the solid complexes tended to precipitate. Then, the sample solution was irradiated with a white lamp (Tokyo Shibaura Denki Co., 20 W, 1000 lux) at a distance of 15 cm. At regular time intervals, intact lipid-soluble vitamin in G-β-CD solution was determined by HPLC.

Results and Discussion

Inclusion Complexes of Lipid-Soluble Vitamins with G-β-CD Figure 1 shows phase solubility diagrams which were obtained for lipid-soluble vitamins and G-β-CD systems in water at 30 °C. The water-solubilities of all lipid-soluble vitamins increased in the presence of G-β-CD. Parent β-CD was found to have little solubilizing effect on lipid-soluble vitamins except V K_3, because it formed insoluble complexes at lower β-CD concentrations. The V K_3–G-β-CD system indicated a typical A_L type solubility curve with linearly increasing solubility, meaning that a 1:1 complex may exist in the solution. The apparent stability constants of both V K_3–G-β-CD and V K_3–β-CD systems calculated from the initial rising portion of the solubility curves according to the method of Higuchi and Connors[6)] were 190 M^{-1}. On the other hand, solubility curves of the V A alcohol–, V E–, V E nicotinate–, V K_1–, and V K_2–G-β-CD systems were A_P type, suggesting a high order complexation. Namely, complexes were not formed at lower G-β-CD concentrations, but great solubilization enhancement due to higher-order complex formations at higher G-β-CD concentrations was observed. The stability constants of higher-order complexes of these lipid-soluble vitamins in water were estimated using the nonlinear optimization least squares technique[7)] modified by Uekama *et al.*[8)] (Table I). It is thought that these differences in molar composition sensitively reflect difference of chemical structures and physical properties of guest compounds. Both the V D_2– and V D_3–G-β-CD systems showed B_S type solubility curves in the very high concentration range of G-β-CD, a solid complex precipitating. The ratios of guest and host compounds in these microcrystalline complexes were found to be 1:2 for both V D_2/G-β-CD and V D_3/G-β-CD.

Figure 2 shows the differential scanning calorimetry thermogram of the solid complex in comparison with those

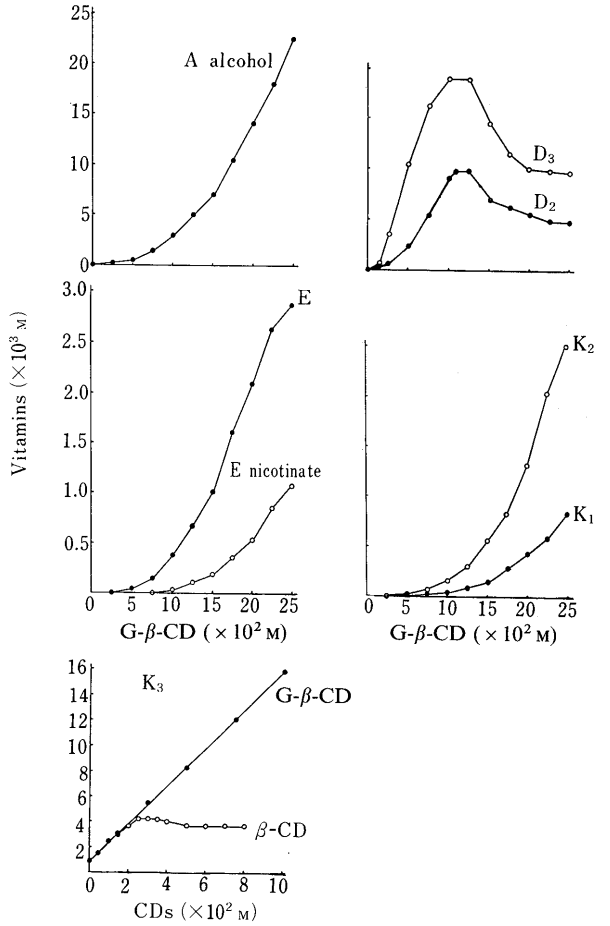

Fig. 1. Phase Solubility Diagrams of Lipid-Soluble Vitamin–G-β-CD Systems in Water at 30 °C

In cases other than that of V K_3, solubilities of lipid-soluble vitamins in water containing β-CD did not rise as vitamin–β-CD complexes precipitated.

TABLE I. Stability Constants of Lipid-Soluble Vitamin–G-β-CD Complexes in Water at 30 °C

Vitamin	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-1})	$K_{1:3}$ (M^{-1})	$K_{1:4}$ (M^{-1})	$K_{1:5}$ (M^{-1})
A alcohol	20	8700	0		
D_2	1100	6500	0		
D_3	11400	2200	0		
E	0	40	6300	0	
E nicotinate	0	0	0	2800	0
K_1	10	50	2200	0	
K_2	20	20	5000	0	

of the physical mixture and V D_2. V D_2 alone and the physical mixture both showed an endothermic peak at around 115 °C owing to melting; however, this peak disappeared with formation of the complex. These results imply that V D_2 interacts with G-β-CD in the solid state to form an inclusion complex.

Figure 3 shows the stabilities of lipid-soluble vitamins in G-β-CD solutions. Vitamins, D_2, D_3, E nicotinate, K_1, and K_2 in aqueous solution containing G-β-CD were very stable in the dark. A V E nicotinate–G-β-CD complex solution was stable even under irradiation with light.

Recently, Uekama *et al.*[8] investigated the inclusion complexes of V E esters with heptakis (2,6-di-*O*-methyl)-β-

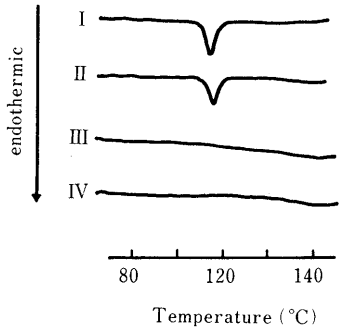

Fig. 2. DSC Thermograms of V D_2–G-β-CD Systems

I, V D_2 alone; II, physical mixture of V D_2 and G-β-CD in 1 : 2 molar ratio; III, 1 : 2 complex of V D_2 with G-β-CD; IV, G-β-CD alone.

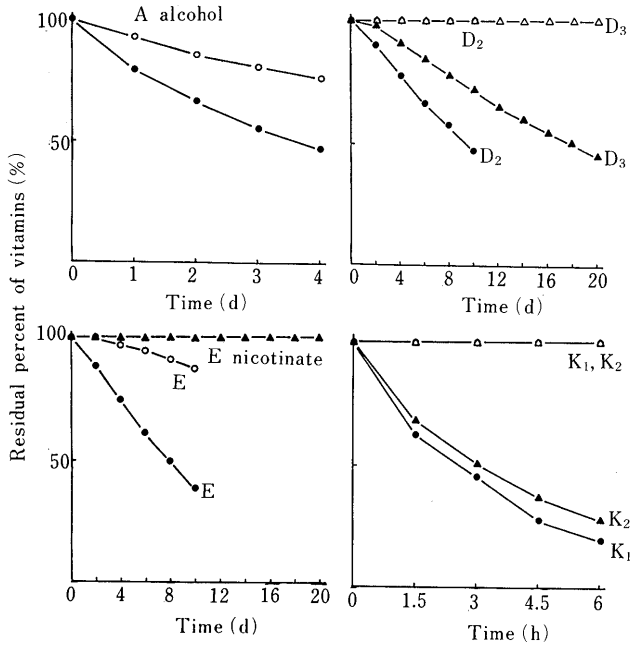

Fig. 3. Stabilities of Lipid-Soluble Vitamins in Water Containing G-β-CD

Open symbols indicate in the dark. Closed symbols indicate under light irradiation.

CD (DM-β-CD). Horiuchi *et al.*[9] also reported on V K group–DM-β-CD complexes. Both papers described that the enhanced bioavailability by complexation seemed mainly ascribable to the increase in dissolution of V E esters and V K groups due to higher-order complex formation with DM-β-CD.

It is expected that lipid-soluble vitamin–branched CD complexes give rather better results than DM-β-CD complexes, on the basis of following facts. Although solubilizing effects on lipid-soluble vitamins in water at ordinary temperature with DM-β-CD complex formation were excellent, DM-β-CD complexes would tend to precipitate at high temperature owing to the solubility of DM-β-CD in water which becomes significantly lower with rising temperature.[10,11] On the contrary, branched CDs are extremely easily dissolved in water at various temperatures,[3] and therefore it is possible to use them as solubilizing agents over a wide range of temperature. Moreover, the hemolytic activity of branched CDs is lower than that of their parent CDs[3,4,12] and DM-β-CD.[12]

These facts suggested that lipid-soluble vitamin–branched CD complexes may be practically applicable to injectable preparations. In addition, the lyophilized samples of inclusion complexes of lipid-soluble vitamins with G-β-CD may facilitate the development of solid dosage forms, and may resolve the undesirable problems encountered with the storage and handling of most lipid-soluble vitamins because of their viscous oily character.

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References and Notes

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