

# Interactions of Cyclodextrins with Dipalmitoyl, Distearoyl, and Dimyristoyl Phosphatidyl Choline Liposomes. A Study by Leakage of Carboxyfluorescein in Inner Aqueous Phase of Unilamellar Liposomes

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The interaction of cyclodextrins (CDs) with L- $\alpha$ -dipalmitoyl phosphatidyl choline (DPPC), L- $\alpha$ -distearoyl phosphatidyl choline (DSPC), and L- $\alpha$ -dimyristoyl phosphatidyl choline (DMPC) unilamellar liposomes was investigated by the leakage of carboxyfluorescein (CF) entrapped in the inner aqueous phase of liposomes, at 25°C (DPPC and DSPC liposomes) and at 5°C (DMPC liposomes). The efficiency of CDs for CF leakage was remarkable in the order of heptakis (2,6-di-*O*-methyl)- $\beta$ -CD (DOM- $\beta$ -CD) >  $\alpha$ -CD > heptakis (2,3,6-tri-*O*-methyl)- $\beta$ -CD (TOM- $\beta$ -CD) from DPPC liposomes, in the order of DOM- $\beta$ -CD > TOM- $\beta$ -CD >  $\alpha$ -CD from DSPC liposomes and in the order of  $\alpha$ -CD > DOM- $\beta$ -CD > TOM- $\beta$ -CD from DMPC liposomes. The other CDs used in the present studies,  $\beta$ -CD, 2-hydroxypropyl  $\beta$ -CD, and  $\gamma$ -CD scarcely induced the CF leakage from above the three liposomes.

From the profiles of % CF leakage, together with measurements of differential scanning calorimetry, it was found that hydrophobic DOM- $\beta$ -CD penetrates the matrix of the liposomes to interact with them as well as TOM- $\beta$ -CD, and that less hydrophobic  $\alpha$ -CD exists at the surface of the membrane to interact with the liposomes. Further, it was found that the interaction of CDs with liposomes changes depending not only on the length of fatty acid chain of phospholipid (condensation force and hydrophobicity) but also the hydrophobicity and the cavity size of CD.

**Key words** cyclodextrin; liposome; dipalmitoyl phosphatidyl choline; distearoyl phosphatidyl choline; dimyristoyl phosphatidyl choline; interaction

Cyclodextrins (CDs) are widely used in the pharmaceutical, cosmetic, and food industries because of their ability to include a large number of organic molecules in their hydrophobic cavities.<sup>1,2</sup> In pharmaceuticals, CDs have been used to increase the stabilization and the solubility of drugs.<sup>3</sup> Also, it has been reported that the drugs given by oral administration in the style of the inclusion complexes are better adsorbed in the living body than those free of such complexes, if the drugs are slightly soluble.<sup>3</sup> It has also been reported that CDs induce hemolysis by reacting with the erythrocyte membrane.<sup>4</sup> Thus, the hemolytic activity of CDs severely limits their potential use as carriers in pharmaceutical applications, mainly when parenteral administration is necessary. Therefore, the interaction of phospholipids, being the main constituent of the biomembrane, with CDs is important, and the interaction of CDs with multilamellar liposomes consisting of L- $\alpha$ -dipalmitoyl phosphatidyl choline (DPPC) has already been studied using differential scanning calorimetry (DSC).<sup>5</sup>

In this paper, three kinds of unilamellar liposomes were prepared, which are more similar to biological membrane than multilamellar membrane, and consisting of DPPC, L- $\alpha$ -distearoyl phosphatidyl choline (DSPC), L- $\alpha$ -dimyristoyl phosphatidyl choline (DMPC) which entrap 5 (6) carboxyfluorescein (CF) in the inner aqueous phase of liposomes, respectively; CDs were then added to these liposomes, resulting in the leakage of CF out of the liposomes. From the amount of leaked CF, how the interaction of CDs with the liposomes is affected by the fatty acid chain length of phospholipid was studied, as well as CD structure. As CDs,  $\alpha$ -CD,  $\beta$ -CD, 2-hydroxypropyl- $\beta$ -CD (HP- $\beta$ -CD),  $\gamma$ -CD, hep-

takis (2, 6-di-*O*-methyl)- $\beta$ -CD (DOM- $\beta$ -CD), and heptakis (2,3,6-tri-*O*-methyl)- $\beta$ -CD (TOM- $\beta$ -CD) were used.  $\alpha$ -CD,  $\beta$ -CD, and  $\gamma$ -CD contain six, seven, and eight of the glucopyranose units, respectively. The structure of the CDs is that of a truncated cone with average cavity diameters of 0.57, 0.78, and 0.95 nm for  $\alpha$ -CD,  $\beta$ -CD, and  $\gamma$ -CD.<sup>3</sup> These three CDs are less hydrophobic ones, and HP- $\beta$ -CD having the same cavity diameter as  $\beta$ -CD is even less hydrophobic. On the other hand, DOM- $\beta$ -CD and TOM- $\beta$ -CD have a deeper cavity and are more hydrophobic than  $\beta$ -CD.<sup>6</sup> Though TOM- $\beta$ -CD is more hydrophobic than DOM- $\beta$ -CD, the macrocyclic ring is remarkably distorted from a regular heptagonal structure<sup>7</sup>; also, TOM- $\beta$ -CD is bulky.<sup>7</sup> In monitoring the leakage of CF, it was hoped to clarify the interaction at time course from immediately after addition of CD to liposomes and to obtain the information on the interaction which would complement that of the DSC method.

## Experimental

**Materials**  $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD, DOM- $\beta$ -CD, TOM- $\beta$ -CD, purchased from Nacalai Tesque Co. were used after being recrystallized from water and HP- $\beta$ -CD, which was purchased from Aldrich Chemical Company, Inc. The average substitution degree of the 2-hydroxypropyl group per  $\beta$ -CD was 5.6. The CDs were dried for 12 h at 110°C in a vacuum before use. DPPC (purity >99%), DSPC (purity >99%), and DMPC (purity >99%) purchased from Sigma, were used without further purification. CF purchased from Molecular Probes was purified as follow<sup>8</sup>: the purchased CF was dissolved in Tris-HCl buffer (10 mM Tris/0.1 mM EDTA/100 mM NaCl, pH 7.4), and 250 mM, 12.5 ml was obtained. The solution was purified on a LH-20 Sephadex column (40×2.5 cm) by elution at room temperature with distilled water. CF was eluted as a well-defined dark orange-red band. The purified solution was lyophilized and stored in a refrigerator prior to use.

**Preparation of Liposomes Entrapping CF in Inner Aqueous Phase** In the case of DPPC liposomes, DPPC: dicetyl phosphate at a molar ratio of

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10:1 was dissolved in chloroform to obtain  $3.01 \times 10^{-4}$  mol/l of phospholipid. The chloroform solution 7.0 ml was placed in a 20 ml eggplant type-flask and the solvent was evaporated to dryness first under nitrogen gas by rotary evaporation to give the deposition of a thin phospholipid film on the inside wall of the flask. To this film, 2 ml of 100 mM CF dissolved in Tris-HCl buffer pH 7.4 was added. The mixture was then vigorously vortexed for approx. 20 min, during which time the mixture was warmed at 50 °C above the transition temperature of the DPPC liposomes. Unilamellar vesicles were prepared by ultrasonication of the suspension for 40 min at intervals of 1 min at 41 °C, using an ultrasonic disruptor (Model UD 201, Tomy Seiko Co., Ltd.), and by incubation at room temperature for 1 h. The net sonification time was 20 min. To remove the untrapped CF, the vesicles were gel-filtrated with PD-10 column (1.5×5.5), using Tris-HCl buffer pH 7.4 as eluants and the small unilamellar vesicles entrapped CF in their inner aqueous phase were prepared. The concentration of the phospholipid obtained was determined by Phospholipid C-Test Wako supplied by Wako Pure Chemical Industries, Ltd. to be  $1.6\text{--}1.5 \times 10^{-3}$  mol/l. In the experiment of CF leakage, the phospholipid solution diluted by one-tenth was used. In the case of DSPC liposomes, the vortex mixing and the sonification were both carried out at 60 °C, and DMPC liposomes both at 31 °C, other experimental conditions being the same as that of DPPC liposomes.

**Monitoring CF Leakage from Liposomes** Fluorescence measurements were run on a Shimadzu RF-510 fluorospectrophotometer equipped with a thermoregulated cell compartment. CF emits at 520 nm on excitation at 470 nm. A suspension of the small unilamellar vesicles containing 100 mM CF in inner aqueous phase hardly fluoresced, but fluoresced strongly upon liberation from the concentration quenching when CF was released from the inner aqueous phase to the bulk aqueous phase. Hence, the reduced barrier function of liposomal bilayers can be observed quantitatively by monitoring an increase in the fluorescence intensity of CF at 520 nm.

All runs were initiated by adding 20  $\mu$ l of liposome suspension to a buffered solution of CD, a buffered solution, and 0.5% Triton X-100 buffered solution, respectively, which were preincubated for 10 min in thermoregulated cells for fluorescence measurement, each being 3.4 ml. The amount of released CF was calculated by the following equation: %CF leakage =  $(I_t - I_0)/(I_\infty - I_0)$ , where  $I_0$  is the fluorescence intensity of CF-loaded liposome suspension at 520 nm in a buffered solution of the absence of any CD, while  $I_\infty$  is the fluorescence intensity at 520 nm after completely destroying the liposomes by Triton X-100.  $I_t$  stands for the fluorescence intensity at 520 nm at t time after adding liposome suspension to a CD solution.

**DSC Measurements** For preparation of the samples used for DSC, a given amount of DPPC was dissolved in chloroform, and the solvent was evaporated to dryness under nitrogen gas. A thin phospholipid film obtained was dried in a vacuum overnight. Tris-HCl buffer pH 7.4 was added to the film and the mixture was subsequently vigorously vortexed for approx. 40 min, during which time it was warmed above the transition temperature of the DPPC liposomes at 50 °C. Then, aliquots of the obtained multilamellar (Mul) liposomes (4.0  $\mu$ l) and each CD buffer solution (11.0  $\mu$ l) were added to the aluminum sample cell, sealed and the sealed mixture was incubated for 1–5 h at the vortexed temperature. Calorimetric scans were performed at a rate of 2.0 °C/min, using the buffer as reference, with a differential scanning calorimeter, Rigaku DSC 8280, controlled by thermal analysis station TAS 100.

## Results and Discussions

**Leakage of CF from DPPC Liposomes** The leakage of CF from the interior of DPPC liposomes was monitored in the presence of CDs as a function of time at 25 °C, changing the concentrations of CDs. Six kinds of CDs,  $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD, DOM- $\beta$ -CD, TOM- $\beta$ -CD, and HP- $\beta$ -CD were used. The results on the additions of CDs are shown in Fig. 1. DOM- $\beta$ -CD induced more remarkable leakage of CF immediately after addition, at a concentration higher than  $5.0 \times 10^{-3}$  M. Also, at  $2.5 \times 10^{-3}$  M concentration of DOM- $\beta$ -CD, 1 min after addition of the CD, % CF leakage increased significantly, followed by a slow leakage which finally leveled off. In the case of  $\alpha$ -CD, % CF leakage occurred after an induction period and increased with the increase in  $\alpha$ -CD concentration added and with time after addition of  $\alpha$ -CD. As the  $\alpha$ -CD concentration added decreased, the induction period

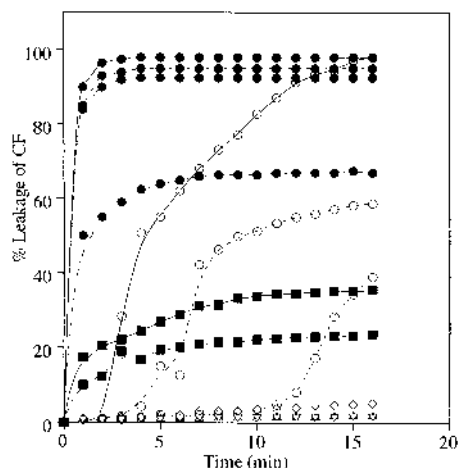


Fig. 1. Time Course of CF Leakage from DPPC Liposomes at 25 °C

—●—, DOM- $\beta$ -CD  $1.0 \times 10^{-2}$  M; ---●---, DOM- $\beta$ -CD  $7.5 \times 10^{-3}$  M; ...●..., DOM- $\beta$ -CD  $5.0 \times 10^{-3}$  M; -●-, DOM- $\beta$ -CD  $2.5 \times 10^{-3}$  M; —○—,  $\alpha$ -CD  $1.0 \times 10^{-2}$  M; --○--,  $\alpha$ -CD  $7.5 \times 10^{-3}$  M; ···○···,  $\alpha$ -CD  $5.0 \times 10^{-3}$  M; —■—, TOM- $\beta$ -CD  $1.0 \times 10^{-2}$  M; ---■---, TOM- $\beta$ -CD  $5.0 \times 10^{-3}$  M; ◇,  $\gamma$ -CD  $1.0 \times 10^{-2}$  M; △, HP- $\beta$ -CD  $1.0 \times 10^{-2}$  M.

was observed to become longer. With the addition of TOM- $\beta$ -CD, rapid leakage of CF was observed at the initial stage, followed by a slow leakage which finally leveled off, as in the case of DOM- $\beta$ -CD, though the extent of % CF leakage was much smaller. With the addition of  $\beta$ -CD,  $\gamma$ -CD, and HP- $\beta$ -CD at the concentration  $1.0 \times 10^{-2}$  M, leakage of CF were scarcely observed. Thus, the extent of the interaction of DPPC unilamellar liposomes with CDs was remarkable in the order of DOM- $\beta$ -CD >  $\alpha$ -CD > TOM- $\beta$ -CD. The rest of the CDs scarcely interacted with DPPC unilamellar liposomes.

We reported the interaction of DPPC liposomes (Mul) with CDs using DSC.<sup>5)</sup> Consequently, it was found that the interactions are remarkable in the order of DOM- $\beta$ -CD >  $\alpha$ -CD > TOM- $\beta$ -CD and that DOM- $\beta$ -CD forms a soluble complex and  $\alpha$ -CD forms an insoluble complex, whereas TOM- $\beta$ -CD penetrates the matrix of the liposomes, though only slightly. It was also found that  $\beta$ -CD, HP- $\beta$ -CD, and  $\gamma$ -CD scarcely interact with DPPC liposomes (Mul). Therefore, the results obtained in the present study are consistent with those using DSC. As mentioned, the profiles of % CF leakages of DOM- $\beta$ -CD and TOM- $\beta$ -CD as a function of time are similar and leakages occurred rapidly at the initial stage followed by a slow leakage which finally leveled off, though % CF leakage induced by DOM- $\beta$ -CD was much larger than that of TOM- $\beta$ -CD. Therefore, it seems apparent that there is some similarity between DOM- $\beta$ -CD and TOM- $\beta$ -CD with respect to the interaction with DPPC unilamellar liposomes. In previous a paper,<sup>5)</sup> it was reported that TOM- $\beta$ -CD penetrates the matrix of liposomes where it interacts and remains. However, it was not determined whether DOM- $\beta$ -CD penetrates the matrix of liposomes to form a soluble complex, or exists near the polar head group of liposomes to substrate DPPC molecules from the liposomes and to form a soluble complex. DSC measurements were made, with the incubation time of the mixtures of CD and DPPC liposomes (Mul) being 5 h above the phase transition temperature.<sup>5)</sup> The main peak area due to gel-to-liquid crystalline phase transition of DPPC liposomes decreased only monotonously with increasing DOM- $\beta$ -CD concentration. If the bulky DOM- $\beta$ -CD

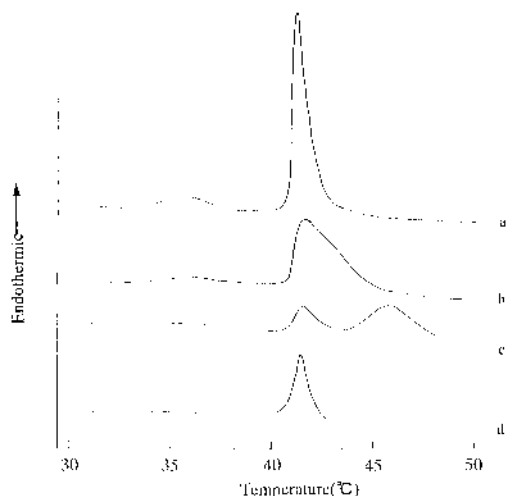


Fig. 2. Effect of Incubation Time on DSC Scans of DPPC Liposomes ( $8.0 \times 10^{-3}$  M) in the Presence of DOM- $\beta$ -CD ( $2.2 \times 10^{-3}$  M)

(a) DPPC liposomes alone (b) 1 h incubation (c) 1.5 h incubation (d) 3 h incubation.

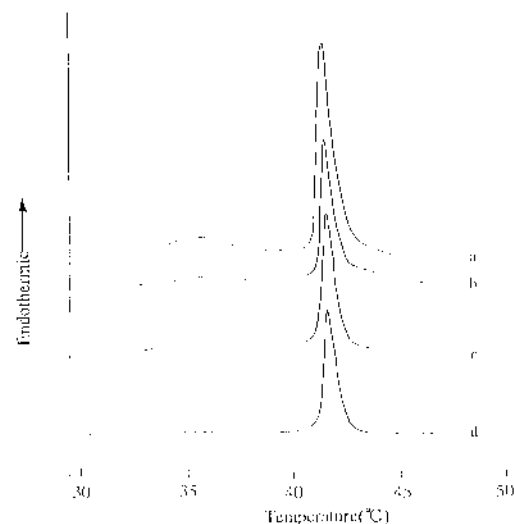


Fig. 3. Effect of Incubation Time on DSC Scans of DPPC Liposomes ( $8.0 \times 10^{-3}$  M) in the Presence of  $\alpha$ -CD ( $2.2 \times 10^{-3}$  M)

(a) DPPC liposomes alone (b) 1 h incubation (c) 3 h incubation (d) 5 h incubation.

molecule penetrates the matrix of the liposomes, the liposome structure may be remarkably disturbed, requiring some time to recover. Thus, DSC measurements of the mixtures of DOM- $\beta$ -CD and DPPC liposomes (Mul) were carried out by varying the incubation time.

**DSC Measurements** The results are shown in Fig. 2. For 1 h incubation time (Fig. 2b), a sharp endothermic peak due to gel-to-liquid crystalline phase transition changed to a broad one at only slightly higher temperature region than that of pure DPPC liposomes (Mul) shown in Fig. 2a. For 1.5 h incubation time (Fig. 2c), two endothermic peaks were observed, one being in the same temperature region as that of pure DPPC liposomes (Mul), and the other being in a higher temperature region. For 3 h incubation time (Fig. 2d), only one sharp endothermic peak coinciding with that of pure DPPC liposomes (Mul) was observed. For the incubation time from 3 up to 5 h, the form and area of the peak changed little. These results suggest that DOM- $\beta$ -CD is adsorbed on the surface of the liposomes and reams the space between the polar head groups of DPPC molecules constituting the liposomes to penetrate the matrix of the liposomes, because the cavity of the DOM- $\beta$ -CD is not large enough to pass through head group of DPPC molecule. This penetration of DOM- $\beta$ -CD to the matrix of the liposomes, which is most likely based on the hydrophobicity of DOM- $\beta$ -CD and its inclusion ability for fatty acid chain, would produce the phase separation between a relatively unperturbed bulk phospholipid domain and a DOM- $\beta$ -CD-rich domain. Further, it is assumed that with longer incubation time DOM- $\beta$ -CDs include the more fatty acid chain moiety of DPPC, resulting in the appearance of a high-temperature component in DSC scans, though the complex formed in the matrix of the liposomes effuses progressively into the bulk aqueous solution because of a soluble complex (Fig. 2c). Also, it is assumed that for a 3 h incubation period, almost all DOM- $\beta$ -CDs penetrating the matrix of the liposomes effuse to the bulk aqueous solution by forming a soluble complex with DPPC, and the perturbed membrane structure is restored almost completely during this period (Fig. 2d). In the method monitoring the

leakage of CF, the interaction of DOM- $\beta$ -CD with liposomes occurred in units of a minute and but, in hourly units in DSC measurements. These are believed to be because in the method of CF leakage, the molar ratio of DOM- $\beta$ -CD to DPPC is about 50 times that in DSC measurements, when the concentration of DOM- $\beta$ -CD is  $2.2 \times 10^{-3}$  M.

Therefore, rapid leakage of CF on the addition of DOM- $\beta$ -CD and TOM- $\beta$ -CD at the initial stage shown in Fig. 1 is probably produced by perturbation of the bilayer structure upon permeation of the bulky DOM- $\beta$ -CD and TOM- $\beta$ -CD molecules from the membrane surface to the membrane matrix. Subsequent slow leakage would be induced by a decreased barrier function of the membranes by increased perturbation of the membrane structure when CDs penetrate and diffuse into bilayer membranes, and when they form a soluble complex.

Similarly, DSC measurements of the mixture of  $\alpha$ -CD and DPPC liposomes (Mul) were carried out by being incubated for 1 h. The results shown in Fig. 3b indicate that only one main peak due to gel-to-liquid crystalline phase transition of the liposomes appears at the same temperature region as pure DPPC liposomes (Mul) and the peak area decreases with increasing incubation time up to 5 h (Figs. 3c and 3d). Also, it has been reported that the change in surface pressure of a monolayer of DMPC spread on  $\alpha$ -CD solution is no more than a monotonous decrease with time.<sup>6)</sup>

From these experimental results, the CF leakage profiles from DPPC liposomes on addition of  $\alpha$ -CD were assumed to be as follows:  $\alpha$ -CD does not permeate the matrix of DPPC liposomes but is adsorbed on their surface to subtract the DPPC molecules from the liposomes. From the fact that the induction period for CF leakage is especially observed with addition of low concentration of  $\alpha$ -CD, the adsorption of given  $\alpha$ -CD molecules on the surface of the membrane which serves to decrease the condensation force of the DPPC molecules may be required to subtract DPPC molecules from DPPC liposomes. It can also be explained that in this mechanism of  $\alpha$ -CD and DPPC liposomes, the main endothermic peak decreases monotonously with time of the incubation,

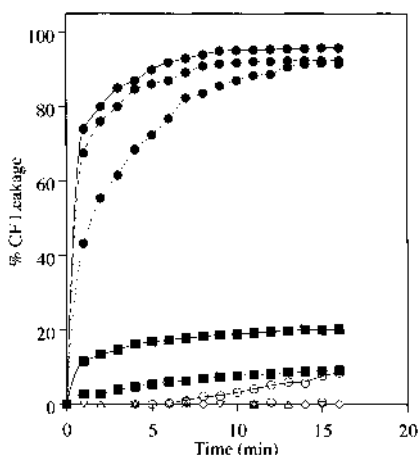


Fig. 4. Time Course of CF Leakage from DSPC Liposomes at 25 °C

—●—, DOM-β-CD  $1.0 \times 10^{-2}$  M; ---●---, DOM-β-CD  $7.5 \times 10^{-3}$  M; ···●···, DOM-β-CD  $5.0 \times 10^{-3}$  M; —○—, α-CD  $1.0 \times 10^{-2}$  M; ○, α-CD  $7.5 \times 10^{-3}$  M; ···○···, α-CD  $5.0 \times 10^{-3}$  M; —■—, TOM-β-CD  $1.0 \times 10^{-2}$  M; ---■---, TOM-β-CD  $5.0 \times 10^{-3}$  M; ◇, γ-CD  $1.0 \times 10^{-2}$  M; △, HP-β-CD  $1.0 \times 10^{-2}$  M; ▽, β-CD  $1.0 \times 10^{-2}$  M.

because as the α-CD subtracts DPPC molecule at the surface of the liposomes, the disturbance of the membrane structure is slight, resulting in the rapid recovery of the liposome structure, in comparison with the DOM-β-CD permeating the matrix of the liposomes.

**CF Leakage from DSPC Liposomes** The time courses of % CF leakage from DSPC liposomes at 25 °C are shown in Fig. 4. With the addition of DOM-β-CD, rapid leakage of CF was observed in the initial stage, followed by a slow leakage which finally leveled off. However, with the decrease in added concentration of DOM-β-CD, % CF leakage tended to decrease. The addition of TOM-β-CD also caused rapid leakage of CF was observed at the initial stage, followed by a slow leakage, though the extent of % CF leakage was much smaller than when DOM-β-CD was added. Particularly interesting was the fact that upon addition of α-CD  $1.0 \times 10^{-2}$  M, the leakage of CF began after induction period of several minutes and occurred slowly and only slightly. This leakage of CF was significantly different from that observed using DPPC liposomes. When β-CD, γ-CD, or HP-β-CD was added, the CF leakage scarcely occurred as in the case of DPPC liposomes.

Thus, the profiles of the time course of CF leakage with respect to DSPC liposomes were similar to those of DPPC liposomes, but the extent of interaction of CDs with DSPC liposomes generally became weaker than that of DPPC liposomes in under experimental conditions used. This can easily be attributed to the stronger condensation force of DSPC liposomes due to longer fatty acid chain than that of DPPC at the same temperature of 25 °C. Particularly, the CF leakage on the addition of α-CD occurred only slightly and slowly with time course after a long induction period. Thus, it was found that when the condensation force of phospholipid constituting liposomes becomes stronger, the force of α-CD subtracting phospholipid from liposomes becomes much weaker. Similarly, it is apparent that the permeations of DOM-β-CD and TOM-β-CD into the matrix of the liposomes are depressed by the stronger condensation force of DSPC molecules.

**CF Leakage from DMPC Liposomes** The time courses

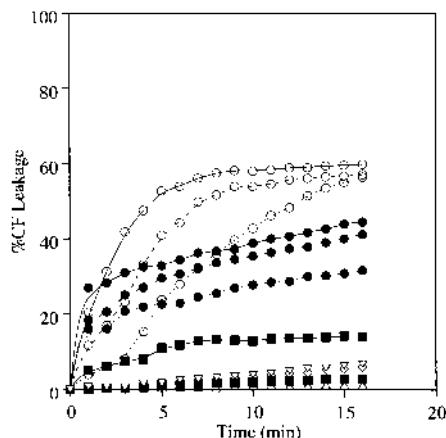


Fig. 5. Time Course of CF Leakage from DMPC Liposomes at 5 °C

—●—, DOM-β-CD  $1.0 \times 10^{-2}$  M; ---●---, DOM-β-CD  $7.5 \times 10^{-3}$  M; ···●···, DOM-β-CD  $5.0 \times 10^{-3}$  M; —○—, α-CD  $1.0 \times 10^{-2}$  M; ---○---, α-CD  $7.5 \times 10^{-3}$  M; ···○···, α-CD  $5.0 \times 10^{-3}$  M; —■—, TOM-β-CD  $1.0 \times 10^{-2}$  M; ---■---, TOM-β-CD  $5.0 \times 10^{-3}$  M; ◇, γ-CD  $1.0 \times 10^{-2}$  M; △, HP-β-CD  $1.0 \times 10^{-2}$  M; ▽, β-CD  $1.0 \times 10^{-2}$  M.

of CF leakage from DMPC liposomes were monitored at 5 °C because the condensation force between phospholipid molecules in these liposomes is weak and the CF leakage occurs in buffered solution with out CD. The results (Fig. 5) show upon the addition of α-CD, a very significant CF leakage was observed without a marked induction period, though leakage was slightly noticed with the addition of a low concentration of α-CD. Rapid leakage of CF was observed at the initial stage when DOM-β-CD was added, followed by a slow leakage, and this tendency was not dependent on the concentrations of DOM-β-CD used in the present study. Upon the addition of TOM-β-CD, a slightly faster leakage of CF occurred at the initial stage which then slowed even further. No CF leakage was observed with the additions of β-CD, γ-CD, or HP-β-CD.

Generally, the % CF leakage from DMPC liposomes with the addition of CDs was not remarkable. In the cases of DOM-β-CD and TOM-β-CD, the decreases in % CF leakage might occur because the permeations of CDs into the matrix of the liposomes are restricted due to the depressed molecular motion of CDs at low temperature and due to the reduction of hydrophobicity of DMPC liposomes. However, in the case of α-CD, because α-CD is adsorbed on the surface of the membrane and subtracts DMPC molecules from the liposomes, and the condensation force of phospholipid constituting DMPC liposomes is weakest among three phospholipids, it is assumed that α-CD is able to subtract relatively easily the DMPC molecules from the liposomes, in spite of the low temperature.

**Conclusion**

From the profiles of % CF leakage, together with the results of DSC measurements, hydrophobic DOM-β-CD penetrates the matrix of the liposomes to interact with those as does TOM-β-CD. Also, α-CD whose cavity size fits that of a fatty chain of phospholipid exists at the surface of the membrane to interact with the liposome, as the CD is less hydrophobic. β-CD, HP-β-CD, and γ-CD, in contrast, scarcely interacted with liposomes, because they are less hydrophobic and have larger cavity sizes than that of the fatty acid chain. Further, it was found that the interaction of CDs with lipo-

somes changes depending on the length of the fatty acid chain of phospholipid (condensation force and hydrophobicity).

It has been reported that the hemolytic effects of CDs on human erythrocytes are large in the order of DOM- $\beta$ -CD > TOM- $\beta$ -CD >  $\beta$ -CD > HP- $\beta$ -CD >  $\alpha$ -CD >  $\gamma$ -CD.<sup>9)</sup> The results obtained in the present study suggest that DOM- $\beta$ -CD,  $\alpha$ -CD, and TOM- $\beta$ -CD induce hemolysis by reacting with the phospholipids, whereas  $\beta$ -CD, HP- $\beta$ -CD, and  $\gamma$ -CD do so by reacting with cholesterol and /or proteins in the erythrocyte membrane.

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