Communications to the Editor

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COMPLEX FORMATION BETWEEN DI- AND MONOPHOSPHATIDYLCHOLINES AND CYCLODEXTRINS IN WATER

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α-, β- and γ- Cyclodextrins(CDs) interact with phospholipid membranes causing an appreciable decrease in the surface pressure of monolayer films of dimyristoylphosphatidylcholine and a leakage of the marker, calcein, in the inner aqueous phase of a liposome which is composed of diacylphosphatidylcholine(DAPC) and dicetylphosphate. The α- and γ-CDs form white amorphous solids with DAPC in the unilamellar state in aqueous solutions. These solids consist of CD and DAPC. The molar ratio of CD to DAPC depends on the kinds of CD and the acyl chain length of DAPC. From the composition of the CD-DAPC complex, several molecules of CD include two acyl chains of DAPC to make complexes such as alkanoic acid-CD complexes.

KEYWORDS ———— cyclodextrin; diacylphosphatidylcholine(lecithin); monoacylphosphatidylcholine(lysolecithin); inclusion compound; liposome; monolayer; surface pressure; leakage

Diacylphosphatidylcholine(DAPC), the typical phospholipid of cell membranes, forms several kinds of vesicles called liposomes in aqueous solutions. The physicochemical properties of liposomes have been extensively studied in connection with model membranes[1] and the drug carriers.[2]

On the other hand, cyclodextrins(CD) have also been studied as a model system of enzyme reaction[3] and new dosage formulation.[4]

Recently, it has been reported that CDs haemolyze human erythrocytes[5] and enhance drug absorption.[6] In this context, we investigated the interaction of CDs with lecithins of various acyl chain lengths in monolayer and lamellar states.

As seen in Fig. 1, the surface pressure of dimyristoylphosphatidylcholine(DMPC) monolayer film spread on the aqueous α-CD solutions decreases with time. The decreasing rates of surface pressure for various CDs are proportional to the surface concentration of DMPC and the rate constants lie in the order α- > β- > γ-CD. In contrast to the interaction of liposome with α- and γ-CDs in aqueous solutions, the precipitation of DMPC-CD complex did not occur in the aqueous CD phase. This may be because the concentration of the DMPC-CD complex is lower than the saturated concentration of the complex, so the complex formed at the interface dissolves in the aqueous CD phase.

As shown in Fig. 2, the calcein leaked and then a white precipitate formed when small unilamellar vesicles with calcein in the inner aqueous phase were mixed with various concentrations of aqueous CD solutions. The formation of the white precipitate and the leakage of calcein depend on the concentrations of DAPC and CD. Slight turbidity

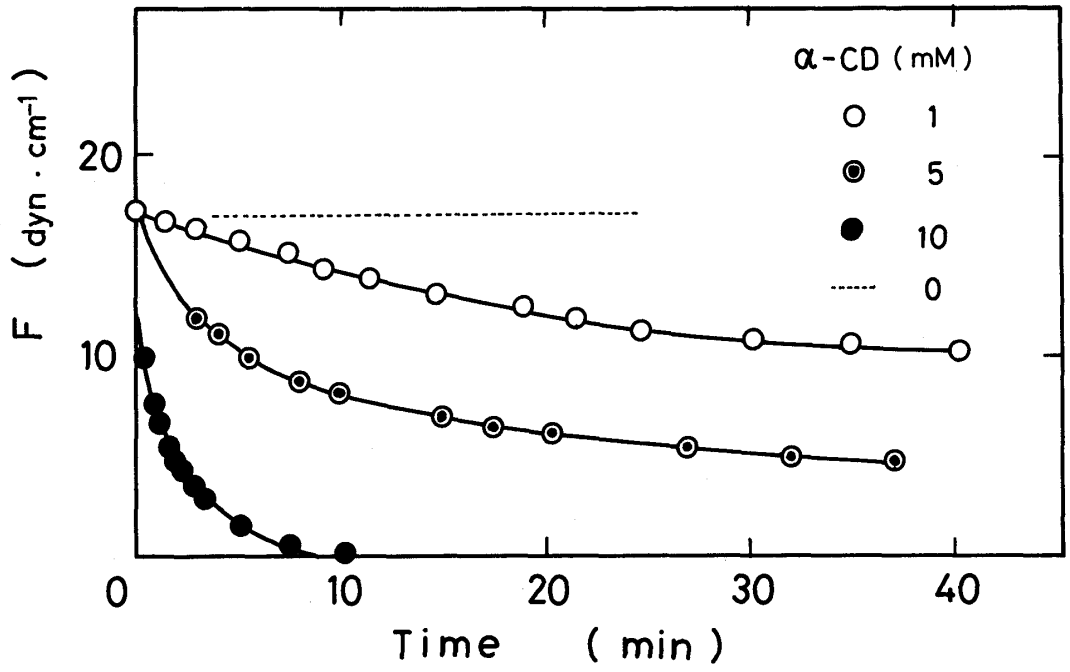

Fig. 1. Decrease in Surface Pressure of Monolayers of DMPC Spread on Various Concentrations of Aqueous α-CD Solution as a Function of Time at 25°C

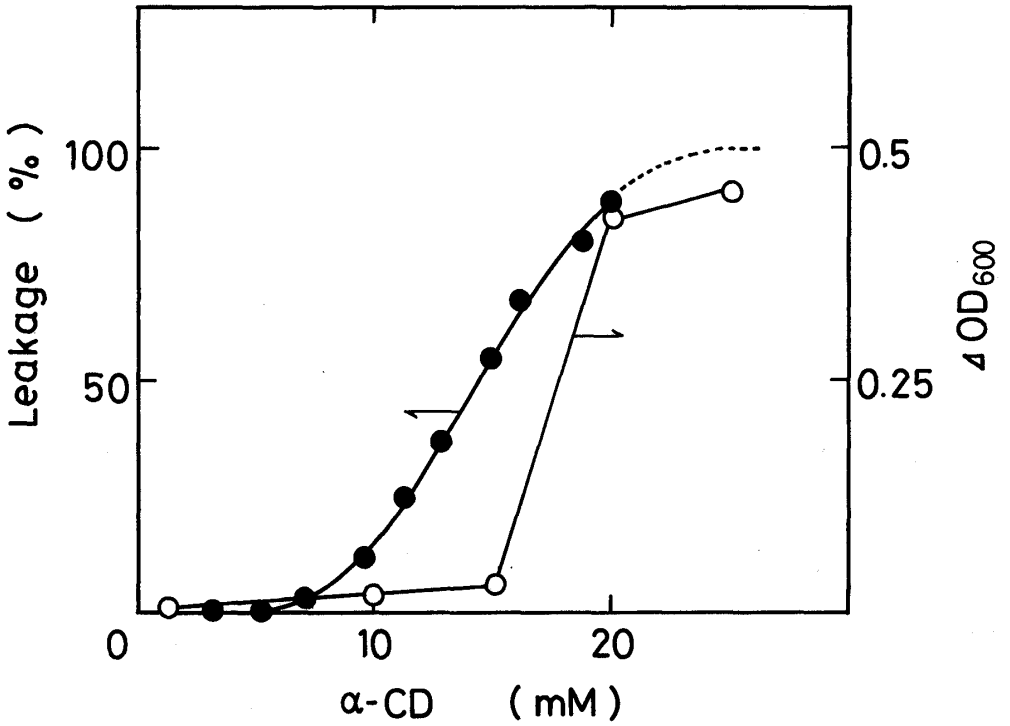

Fig. 2. Leakage of Calcein and the Change of Optical Density after Mixing an Aqueous Suspension of Liposome with Various Concentrations of α-CD Solutions at Room Temperature
●: percent leakage 10 min after mixing, o: change of optical density (600 nm) 30 min after mixing.

was observed when DAPC-liposome was mixed with an aqueous solution of β-CD, but there was little precipitate. This may be due mainly to the low solubility of β-CD. After separation of the white precipitate prepared by mixing DAPC-liposome and aqueous solutions of α- or γ-CD, the precipitate was rinsed twice with water and dried for several days in vacuo at room temperature.

The precipitate thus obtained was examined by infrared spectroscopy. The KBr disk of white amorphous solid shows absorption at 1725 cm^{-1} due to the stretching vibration of the carbonyl group, which is absent in the KBr disk of CD. This suggests the formation of a DAPC-CD complex. So we carried out an elementary analysis and an analysis of the phosphorus content by the phosphomolybdenic acid method[7] to determine the composition of CD and DAPC in the complex.

Table I. Composition of CD-Lecithin Complexes

Phospholipids	Mole of CD / Mole of phospholipid	
	α-CD	γ-CD
Dilauloyl-L-α-phosphatidylcholine	4.2 ± 0.3	1.5 ± 0.1
Dimyristoyl-L-α-phosphatidylcholine	4.2 ± 0.4	1.7 ± 0.4
Dipalmytoyl-L-α-phosphatidylcholine	5.1 ± 0.4	1.8 ± 0.2
Distearoyl-L-α-phosphatidylcholine	5.5 ± 0.7	n.d.
Palmitoyl-L-α-lysophosphatidylcholine	2.9 ± 0.4	1.1 ± 0.1
Myristoyl-L-α-lysophosphatidylcholine	2.8 ± 0.4	n.d.

n.d. : not determined.

As shown in Table I, the α-CD content is much larger than the γ-CD content in the complexes. The molar ratio of CD to DAPC increases non-stoichiometrically with acyl chain length, indicating the inclusion complexes of a torus type alkanoic acid-CD complex.[8] Lysolecithins also form complexes with α- and γ-CDs in aqueous solutions.

Assuming that the α-CD molecule interacts with the alkyl groups of the acyl chains, a molecule of α-CD covers 6.3 units of methylene chain, which correspond to ca. 7.4 Å in trans zig-zag form. The distance from head to tail of the CD molecule is about 7.8 Å,[9] indicating that α-CD molecules cover almost completely the methylene chains of the two acyl groups. However the mole ratios of γ-CD and DAPC in the complexes are less than half of those in the α-CD-DAPC complexes, suggesting a weak interaction between the alkyl groups of DAPC and γ-CD. The pore size of γ-CD is much larger than that of α-CD.

A white precipitate is also formed at interfaces on mixing aqueous solutions of β- or γ-CD and an ethereal solution of cholesterol. The white precipitate thus obtained contains cholesterol. The detailed composition of the precipitate is now being examined.

In conclusion CDs form inclusion complexes with DAPC, causing the destruction of lipid membranes. These facts seem to be closely related to the hemolysis and to the absorption of drug through biomembranes.

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