Molecular Selective Associations of Cyclodextrins with Lipid or Cholesterol Multibilayers cast on a Piezoelectric Crystal

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Depending on their cavity sizes, cyclodextrins form soluble molecular selective inclusion complexes *via* their interaction with phospholipid or cholesterol multibilayers which can be directly detected by changes in frequency observed for a lipid-coated piezoelectric crystal in aqueous solution.

Recently, the interaction between cyclodextrins (CDs) and biological membrane components such as phospholipids and steroids has been studied in connection with the hemolysis of human erythrocytes. However, it is difficult to detect these interactions directly from liposome or lipid monolayer experiments, consequently some inconsistent data have been reported.[1—5]

In this paper, we report the direct detection of the selective interaction between phospholipid or cholesterol multibilayer films cast on a piezoelectric crystal plate with α-, β-, or γ-CDs in aqueous solution. Piezoelectric crystals are known to act as very sensitive mass measuring devices because of the decreases in resonance frequency which occur upon the deposition of a given mass on the electrode.[6] We have reported that a lipid bilayer-coated piezoelectric crystal can detect the selective adsorption of odorous and bitter substances in both gaseous and aqueous phases.[7—9] Lai and co-workers reported that the cyclodextrin-immobilized piezoelectric crystal can selectively adsorb benzene vapour.[10]

Dipalmitoylphosphatidylethanolamine (DPPE) or cholesterol (chol) was cast from chloroform solution on both sides of a silver coated piezoelectric crystal (9 MHz, AT cut, 8 × 8 mm). These films were 0.20 ± 0.05 μm thick (10 ± 2 μg). After aging in hot aqueous solution (60 °C, for 30 min), *X*-ray analysis and electron microscopy confirmed that DPPE and chol form multibilayer structures (spacing 3—4 nm) parallel to the plane of the film.[6—8] The cast films were stable and did not peel off the plate, even under harsh conditions in aqueous solution, this could be confirmed by frequency observations of the crystal. Calibration showed that a decrease in frequency of 1 Hz corresponded to an increase in mass of 1.27 ng on the piezoelectric crystal.

Figure 1 shows typical frequency changes (ΔF) of the DPPE or chol-coated piezoelectric crystal in aqueous solutions at 25 °C corresponding to the interaction with α-, β-, and γ-CDs. When the DPPE multibilayer-coated crystal was soaked in a high concentration of α-CD in aqueous solution (0.06 M), the frequency of the crystal gradually decreased to $\Delta F = -6000 \pm 20$ Hz within 40 min which indicates an increase in mass (the adsorption amount) of 7.6 μg. When the crystal was transferred to distilled water, the frequency increased gradually with time and then slowly reached $\Delta F = +1970 \pm 20$ Hz, where a decrease in mass of 2.5 μg is consistent with the 25% of the original DPPE cast film (10 ± 2 μg). In an aqueous solution of β- and γ-CDs (0.01—0.1 M), no frequency changes were observed within experimental error (±20 Hz). This suggests that only α-CD can selectively interact with DPPE multibilayers, and that some of the resulting inclusion molecules peel off into aqueous solution when the crystal is moved in the distilled water.

The amount of α-CD on the adsorbed DPPE multibilayers increased linearly with increasing CD concentrations in

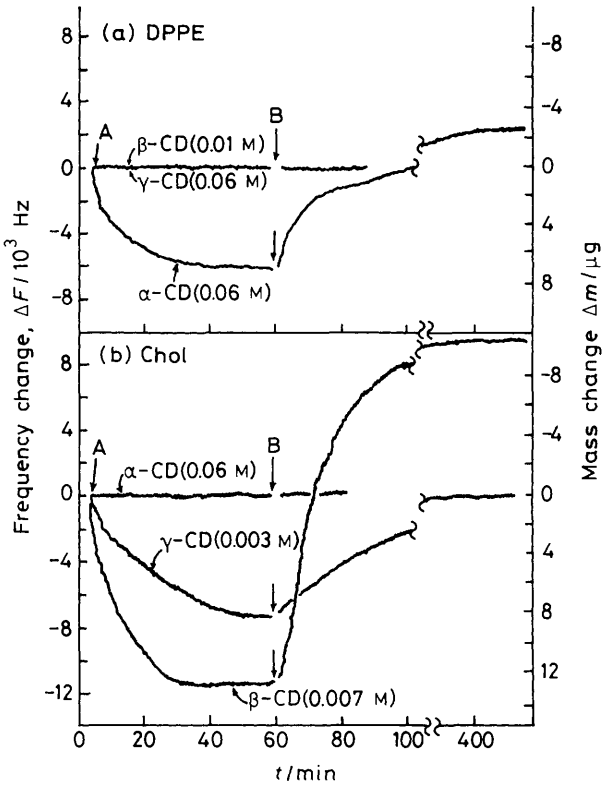

Figure 1. Frequency changes of (a) DPPE multibilayer-coated and (b) chol multibilayer-coated piezoelectric crystals responding to α-, β-, and γ-CDs in aqueous solutions at 25 °C. The crystal was immersed in aqueous solutions of CDs at the arrow A; the aqueous solution was changed to distilled water at the arrow B.

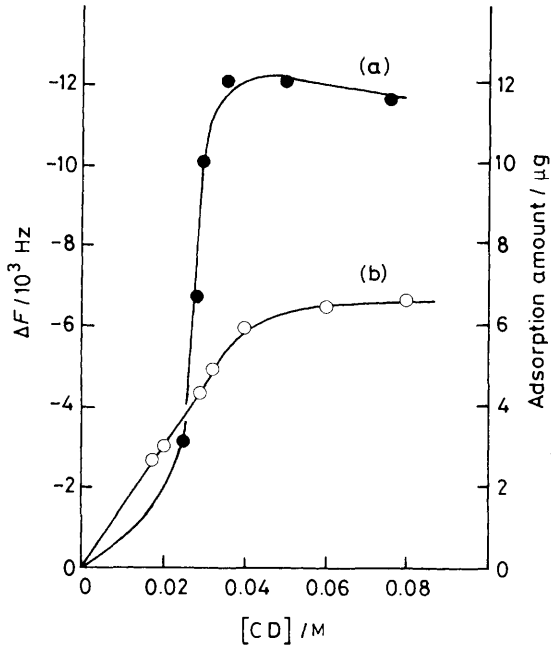

Figure 2. Dependence of the amount of CDs adsorbed on the concentration of CDs in aqueous solutions during interactions of (a) α-CD to DPPE multibilayers, and (b) β-CD to chol multibilayers at 25 °C.

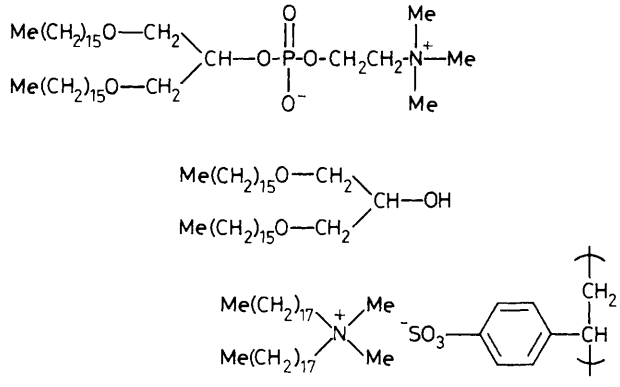

Figure 3

aqueous solution and reached a plateau at [α-CD] = 0.05 M [Figure 2 curve(a)]. The molecular ratio of α-CD/DPPE was calculated to be 0.6 ± 0.1 at the plateau region. The amount adsorbed (0.5—2.0 g) also increased linearly with increasing thickness of the cast film (0.01—0.4 μm). This indicates that α-CD adsorbs and penetrates deeply into the DPPE multibilayers.

α-CD molecules can also adsorb on multibilayer films from other dialkyl-chain amphiphiles (Figure 3), independent of structures of the hydrophilic head group.

The inner cavity size of CDs can be estimated to be 0.20 ± 0.01, 0.32 ± 0.02, and 0.50 ± 0.02 nm² for α, β-, and γ-CDs, respectively, from Corey–Pauling–Koltun models.[11] α-CD can include only one alkyl chain, whose cross-sectional area is calculated to be 0.20 nm², in the cavity. From these data, α-CD is thought to penetrate into lipid layers and include one alkyl chain from the hydrophobic tail part of DPPE molecules. The α-CD/DPPE inclusion complex is hydrophilic, can disturb bilayer structures, and peels from the crystal plate [Figure 1 (a)].

In the case of a chol multibilayer-coated crystal, β- and γ-CDs adsorbed onto the cholesterol layers, but α-CD did not [Figure 1(b)]. When the crystal was soaked in distilled water, γ-CD could reversibly desorb from the chol layers. However, β-CD was removed together with the cholesterol molecules, and most of the multibilayer material (9.5 μg) quickly peeled away from the crystal plate. A plot of the amount of β-CD adsorbed onto the chol multibilayers against the concentration of β-CD in aqueous solution also produced a saturation curve with a plateau region associated with an estimated 1 : 1 molecular complex [Figure 2, curve (b)]. The amount of β- and γ-CDs adsorbed on the chol layers increased linearly with increasing thickness of the cast film, which corresponds to the penetration of CD molecules into the cholesterol multibilayers.

From the data of the cross-sectional area of a cholesterol molecule (0.39 ± 0.01 nm²), γ-CD can be estimated to include the cholesterol skeleton in the cavity of size 0.50 ± 0.02 nm². In contrast, β-CD (cavity size 0.32 + 0.02 nm²) can probably only include the side chain of cholesterol in the inner cavity. γ-CD can be reversibly removed from the inclusion complex without disturbing multibilayer structures [Figure 1(b)]. When

the side chain of cholesterol was included with β-CD at a molar ratio of 1 : 1, the inclusion complex becomes hydrophilic and is easily removed from the plate.

These findings are consistent with results obtained by a monolayer at the air–water interface.[12] The surface pressure of a cholesterol monolayer decreases when CDs are dissolved in the subphase in the order β- > γ- > α-CD. Cyclodextrins in the aqueous phase can interact with the monolayer of dimiristoylphosphatidylcholine (DMPC) and decrease the surface pressure in the order α- > β- > γ-CD.

In conclusion, α-, β-, and β-CDs can selectively penetrate and interact with DPPE or cholesterol multibilayers and, depending on their cavity sizes, render lipid molecules from the plate soluble. This may be related to the cyclodextrin-induced hemolysis of human erythrocytes. The lipid-coated crystal becomes a useful tool to detect the molecular selective interaction between lipid membranes and various chemicals.[7–9]

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