# Cyclodextrin-Mediated Removal of Sterols from Monolayers: Effects of Sterol Structure and Phospholipids on Desorption Rate†

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ABSTRACT: In this study, we have examined a number of parameters which affect the rate of sterol desorption from a model membrane surface (a monolayer at the air/water interface) to cyclodextrins (CD) in the aqueous subphase. The desorption experiments were carried out at a constant lateral surface pressure with a zero-order trough, which allowed for a determination of desorption rates which were unaffected by monolayer substrate concentration. At a surface pressure of 20 mN/m (30 °C), 0.9 mM $\beta$-CD caused a desorption of about 13 pmol of cholesterol per minute and square centimeter of monolayer area. The desorption of cholesterol proceeded linearly as a time function and was sensitive to the concentration of $\beta$-CD in the subphase. The rate of cholesterol desorption increased as the monolayer surface pressure increased (3 → 35 mN/m) but decreased slightly with increasing temperature (15 → 30 °C). The rate of sterol desorption appeared to be influenced by the relative polarity of the sterols. Oxidized sterols desorbed significantly faster than cholesterol (e.g., 4-cholesten-3-one desorbed 8.4-fold faster than cholesterol), whereas less polar sterols desorbed at slower rates [e.g., 20(*R*)-isoheptyl-5-pregnen-3$\beta$-ol, a cholesterol analogue with a ten-carbon branched side chain, desorbed at $^1/_{10}$ of the rate of cholesterol]. Cholesterol desorption from a monolayer membrane containing both cholesterol and a phospholipid was much slower than from a pure cholesterol monolayer. When the effect of dipalmitoylphosphatidylcholine and *N*-palmitoylsphingomyelin on cholesterol desorption rate was compared, it was found that cholesterol desorption was much more retarded from sphingomyelin monolayers as compared to that from phosphatidylcholine monolayers. Taken together, the results of this study show that the $\beta$-CD-enhanced desorption of cholesterol (and other sterols) from monolayer membranes is influenced by the polarity of the desorbing molecules, as well as by lipid/lipid interactions in the membranes. Since $\beta$-CD has no surface activity of its own, it appears to be a useful, nonintrusive catalyzer of cholesterol desorption and is expected to become a valuable probe in membrane and cell research.

Cyclodextrins (CDs[1]) are cyclic oligosaccharides which have a polar surface and a hydrophobic cavity. Commercially available CDs have six, seven, or eight α-(1 → 4)-glucopyranose units (termed α-, $\beta$-, or $\gamma$-cyclodextrins, respectively), and may contain additional functional groups which modify the hydrophilic/hydrophobic properties of the CDs (Szejtli, 1988; Pitha et al., 1988; Irie et al., 1992). CDs have been used extensively in pharmaceutical formulations to aid in the delivery and release of pharmaceutically active hydrophobic compounds in polar environments (Uekama et al., 1986; Pitha et al., 1988; Stern, 1989).

The finding that CDs can bind hydrophobic molecules to their cavity has made them potentially useful as probes in membrane research. Different derivatives of $\beta$-CDs were observed to increase the water solubility of neutral and polar lipids (Thoma et al., 1965; Bender & Komiyama, 1978; Szejtli et al., 1984; Irie et al., 1992), and hydroxypropyl-$\beta$-CD was additionally shown to efficiently extract membrane cholesterol from red blood cells (Irie et al., 1982, 1992). In other recent studies, $\beta$-CD or its 2-hydroxypropyl or methyl derivatives were used to extract cholesterol from cultured cell membranes (Kilsdonk et al., 1995; Klein et al., 1995) or to change cell membrane cholesterol levels at will (both depletion and repletion; Klein et al., 1995; Gimpl et al., 1995). In related model membrane studies, CDs have been used to extract fatty acids and monoglycerides from monolayer membranes at the gas/buffer interface (Asgharian et al., 1988; Laurent et al., 1994; Ivanova et al., 1995). The observations that cholesterol can be extracted from both cell (Kilsdonk et al., 1995; Klein et al., 1995) and monolayer membranes (Asgharian et al., 1988) by CDs are important. However, even though the previously mentioned studies were carefully performed, no attempts were made to control the quality of the membrane interface or to correlate lipid desorption with interfacial properties. Using intact cells, one has no means to control the properties of the water/membrane interface. The advantage of monolayer technology is that membrane properties can be controlled at will, and lipid desorption studies can be performed under conditions where the membrane surface pressure is kept constant (Laurent et al., 1994).

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[1] Abbreviations: CD, cyclodextrin; di-10-PC, 1,2-didecanoyl-*sn*-glycero-3-phosphocholine; DPPC, dipalmitoyl-*sn*-glycero-3-phosphocholine; *N*-*P*-SPM, *N*-palmitoylsphingomyelin; iso-C5, 20(*R*)-isopropyl-5-pregnen-3$\beta$-ol; iso-C8, 20(*R*)-isooctyl-5-pregnen-3$\beta$-ol; iso-C10, 20(*R*)-isoheptyl-5-pregnen-3$\beta$-ol; *n*-C3, 20(*R*)-methyl-5-pregnen-3$\beta$-ol; *n*-C5, 20(*R*)-*n*-propyl-5-pregnen-3$\beta$-ol; *n*-C7, 20(*R*)-*n*-pentyl-5-pregnen-3$\beta$-ol.

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The aim of this study is twofold. First, we wanted to define the parameters which control or affect sterol desorption from membranes to CDs in the subphase. This definition is important because cholesterol is an important membrane component and because a detailed knowledge of the parameters which affect cholesterol removal from membranes can help us to better understand and possibly predict what will happen when, e.g., cell membranes are exposed to CDs. Second, we wanted to explore the usefulness of CDs as membrane probes with which one could measure cholesterol/phospholipid interaction in model membrane systems and learn more about the factors involved in this association.

## EXPERIMENTAL PROCEDURES

*Materials*. All lipids, unless otherwise stated, were obtained from Sigma Chemicals (St. Louis, MO). The side chain analogues of cholesterol were synthesized as described previously (Morisaki et al., 1980; Slotte et al., 1994). 4-Cholesten-3$\beta$-ol was obtained from Steraloids (Wilton, NH). $\alpha$- and $\beta$-cyclodextrins were obtained from Sigma Chemicals (catalog no. C4642 and C4767, respectively). TRITC-labeled phosphatidylethanolamine was obtained from Molecular Probes (Eugene, OR). Stock solutions of the lipids were prepared in hexane/2-propanol (3/2, v/v), stored in the dark at −25 °C, and warmed to ambient temperature before use. The cyclodextrin stock solutions were prepared in pure water to a concentration of 40 mM. [1$\alpha$,2$\alpha$-$^3$H]-Cholesterol (TRK330) was purchased from Amersham International (U.K.). It was purified by reverse-phase HPLC to a purity greater than 99%. The water used as subphase was purified by reverse osmosis followed by passage through a Millipore UF Plus water purification system to yield a product with a resistivity of 18.2 M$\Omega$/cm.

*Force−Area Isotherms.* Pure monolayers of each lipid were compressed on water at ambient temperature (or at 30 °C) with a KSV surface barostat (KSV Instruments Ltd., Helsinki, Finland). The barrier speed did not exceed 3.4 Å$^2$/molecule per minute during compression. Data were collected using proprietary KSV software. From these isotherms, the mean molecular area value was obtained for a given surface pressure and was used for calculations of lipid desorption rates, as indicated below.

*Removal of Monolayer Lipids to the Subphase*. Monolayers containing pure sterols (or [$^3$H]cholesterol), or a mixture of cholesterol and a phospholipid, were prepared at the air/water interface. The trough used was a zero-order type, with a reaction chamber (28 mL volume, 28.3 cm$^2$ area) separated by a glass bridge from the lipid reservoir. The reaction chamber was termostatted to a given temperature (indicated separately), and the cyclodextrin was injected into the reaction chamber whose contents had been stirred without penetrating the monolayer (in a volume not exceeding 1 mL). The removal of monolayer lipids to the subphase was determined from the area decrease of the monolayer at constant surface pressure. Knowing the mean molecular area at a given surface pressure and temperature, we could calculate the amount of lipid removed as a time function. When the removal of [$^3$H]cholesterol was determined, aliquots of the subphase (200 $\mu$L) were removed at time intervals and analyzed for [$^3$H]cholesterol radioactivity, using a scintillation counter.

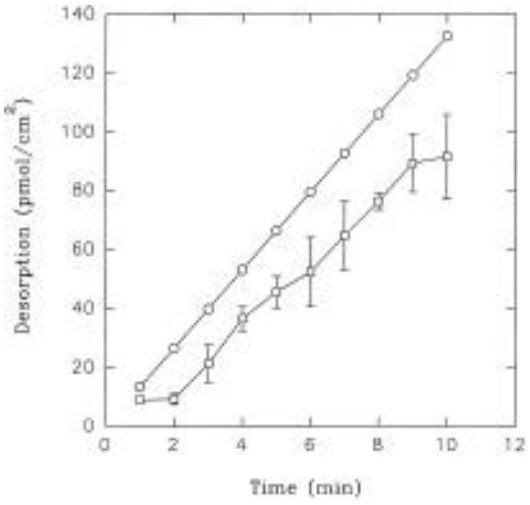


FIGURE 1: Removal of cholesterol or [$^3$H]cholesterol from pure sterol monolayers by $\beta$-CD. Pure sterol monolayers were prepared at the air/water interface and compressed to 20 mN/m (at 30 °C). While the monolayer was kept at a constant surface pressure, $\beta$-CD was injected into the stirred monolayer subphase (final concentration of 0.9 mM), and the rate of cholesterol removal (circles) was determined from the monolayer area decrease as a time function. The rate of [$^3$H]cholesterol desorption (squares) was determined by analyzing aliquots of the subphase for [$^3$H]cholesterol activity at time intervals. Values for [$^3$H]cholesterol desorption are averages from three different experiments ± SEM.

*Monolayer Fluorescence Microscopy.* The presence of cholesterol-rich and laterally condensed domains in a cholesterol/di-10-PC mixed monolayer at the air/water interface was documented using monolayer fluorescence microscopy (Slotte & Mattjus, 1994; Slotte, 1995a). The monolayer, which was equimolar with respect to cholesterol and phosphatidylcholine, also contained 0.5 mol % TRITC-PE as a fluorescent reporter molecule. The monolayer was kept at a constant lateral surface pressure of 18 mN/m at 20 °C. To remove cholesterol from the monolayer, $\beta$-CD was injected under the monolayer without penetrating it. Micrographs of the monolayer surface texture were obtained before and at time intervals after $\beta$-CD addition, using a sensitive video camera attached to a DT3851 digitizing board in a personal computer, as described previously (Slotte, 1995a).

## RESULTS

*Characteristics of Cholesterol Removal from Monolayers by Cyclodextrins.* To examine the factors which affect cholesterol removal from monolayers to CDs in the subphase, monolayers were kept at constant surface pressure during the removal experiments. In this way, the removal of cholesterol from the monolayers could be followed directly from the decrease in monolayer area, which was continuously monitored. Knowing the mean molecular area of cholesterol at a given surface pressure, we could convert monolayer area decreases to picomoles of cholesterol desorbed per time unit and squared centimeters of monolayer area exposed to $\beta$-CD. A typical experiment is shown in Figure 1, which gives the desorption of cholesterol from a pure cholesterol monolayer (30 °C, 20 mN/m) to $\beta$-CD in the subphase (0.9 mM). The average desorption rate for cholesterol under these conditions was 13 pmol desorbed min$^{-1}$ cm$^{-2}$. The $\beta$-CD that was injected below the monolayer was not surface active by itself, since the surface tensions of pure water and $\beta$-CD-containing water (2 mM) were similar (data not shown). To obtain

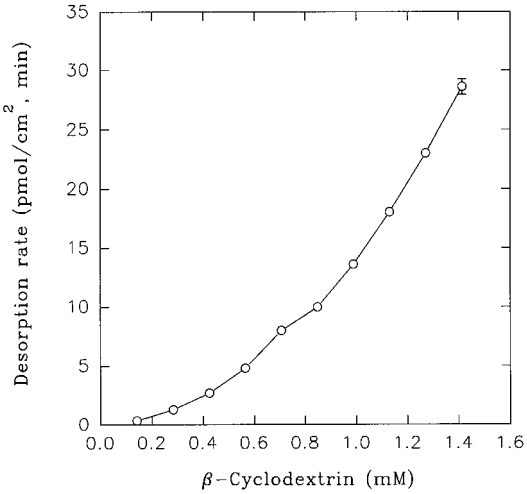


FIGURE 2: Effect of $\beta$-CD subphase concentration on cholesterol removal from monolayers. Pure cholesterol monolayers were prepared at the air/water interface and compressed to 20 mN/m (at 30 °C). While the monolayer was kept at a constant surface pressure, different amounts of $\beta$-CD were injected into the stirred subphase. The rate of cholesterol removal was determined from the monolayer area decrease as a time function.

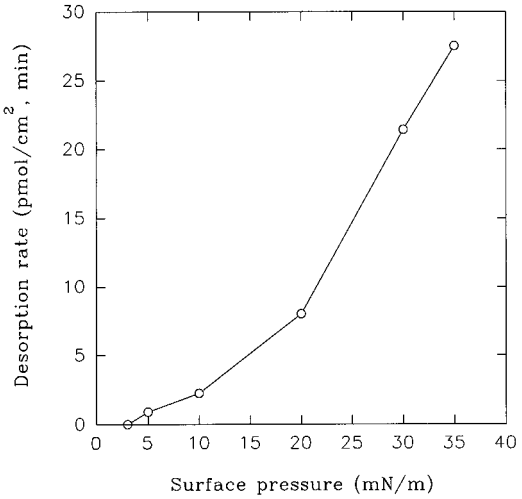


FIGURE 3: Effect of monolayer lateral surface pressure on cholesterol removal by $\beta$-CD. Pure cholesterol monolayers were prepared at the air/water interface and compressed to different surface pressures (at 30 °C). While the monolayer was kept at a constant surface pressure, $\beta$-CD was injected into the stirred subphase (final concentration of 0.7 mM), and the rate of cholesterol removal was determined from the monolayer area decrease as a time function.

additional proof that $\beta$-CD really pulled out cholesterol from the monolayers into the subphase, experiments were performed using [$^3$H]cholesterol as the monolayer sterol, since its desorption into the subphase could be directly monitored in subphase aliquots. As shown in Figure 1, the removal of monolayer [$^3$H]cholesterol by $\beta$-CD was almost similar to the situation with unlabeled cholesterol. The small deviation in the two lines probably results from the fact that a minute fraction of the [$^3$H]cholesterol/$\beta$-CD complex adsorbed to the Teflon trough and was therefore not recovered in the bulk subphase. We also tested the capacity of α-CD to effect cholesterol desorption from monolayers, but no appreciable desorption rate could be detected under comparable conditions which gave good desorption of cholesterol to $\beta$-CD (data not shown).

The removal of monolayer cholesterol by $\beta$-CD was sensitive to the subphase concentration of $\beta$-CD. This dependence in shown in Figure 2. The desorption rate was not a simple linear function of the bulk concentration of $\beta$-CD and may suggest that the effective concentration of $\beta$-CD at the interface was a nonlinear function of the bulk $\beta$-CD concentration, in the subphase. Higher concentrations than about 1.4 mM of $\beta$-CD could not be tested, because the solubility of $\beta$-CD in water and the volume of the stock solution that could be injected into the subphase became limiting. The rate of cholesterol removal by $\beta$-CD from pure sterol monolayers was observed to also be influenced by the lateral surface pressure of the monolayer membranes (Figure 3). Higher surface pressures in the monolayers resulted in higher desorption rates. This probably reflected a change in the interaction energies between molecules in the monolayer as well as at the water/lipid interface, as the monolayers were compressed (squeezing-out effect). The effect of temperature on the rate of cholesterol desorption is shown in Figure 4. Surprisingly, the rate of cholesterol desorption increased slightly as the temperature decreased (30% increase with 15 °C decrease of temperature).

*Effect of Sterol Structure on Removal Rate from Monolayers.* To gain additional information about factors which affect sterol desorption from monolayers to $\beta$-CD, one logical

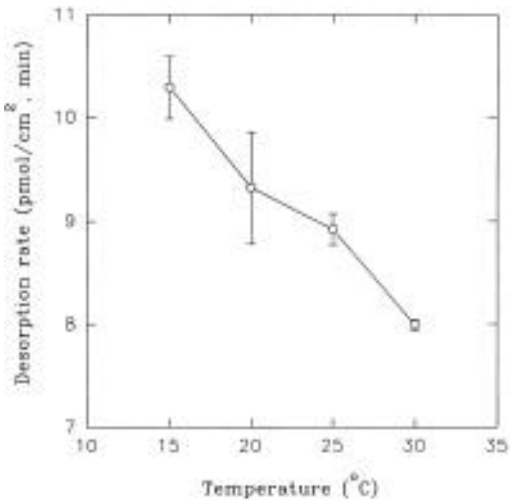


FIGURE 4: Effect of temperature on cholesterol desorption rate to $\beta$-CD. Pure cholesterol monolayers were prepared at the air/water interface and compressed to 20 mN/m. The desorption of cholesterol to $\beta$-CD (0.7 mM) was determined at different temperatures, as a time function. Values are averages ± SEM from three different experiments at each temperature.

route would be to vary the polarity of the sterols and measure how such changes would affect desorption rates to $\beta$-CD in the subphase. This was done with a number of different sterols, all of which could be spread to stable monolayers at fairly high lateral surface pressures (20 mN/m was used in these experiments). If the polarity of the 3$\beta$-OH group was decreased by replacement of it with a 3$\beta$-methoxy function, the desorption rate was slightly decreased (Table 1). However, if a 3-keto group was introduced instead of the 3$\beta$-OH group, the desorption rate increased several-fold, especially if the conformation of the A-ring was simultaneously altered (as in 4-cholesten-3-one). The introduction of an additional keto group at carbon 7 of cholesterol also increased the desorption rate (Table 1). These results suggest that the relative polarity of the sterol molecule was a significant determinant of the desorption rate when $\beta$-CD was the acceptor in the subphase.

Table 1: Effect of Sterol Structure on Desorption Rate from Monolayers to $\beta$-Cyclodextrin in the Subphase[a]

| sterol species | desorption rate (pmol cm$^{-2}$ min$^{-1}$) | rate relative to cholesterol desorption |
|---|---|---|
| variation in polar function | | |
| 3$\beta$-methoxy-5-cholestene | 6.2 ± 0.4 | 0.8 |
| 4-cholesten-3$\beta$-ol | 6.3 ± 0.5 | 0.8 |
| 5α-cholestan-3$\beta$-ol | 7.6 ± 0.3 | 0.9 |
| 5-cholesten-3$\beta$-ol | 8.0 ± 0.1 | 1.0 |
| 5-cholestene-3-one | 26.8 ± 0.1 | 3.4 |
| 7-oxo-5-cholesten-3$\beta$-ol | 30.7 ± 0.2 | 3.9 |
| 4-cholesten-3-one | 66.7 ± 2.7 | 8.4 |
| variation in side chain | | |
| iso-C10-sterol | 0.7 + 0.1 | 0.1 |
| iso-C8-sterol (cholesterol) | 8.0 + 0.1 | 1 |
| iso-C5-sterol | 24.3 + 1.5 | 3 |
| *n*-C7-sterol | 18.5 + 1.7 | 2.3 |
| *n*-C5-sterol | 70.5 + 3.2 | 8.8 |
| *n*-C3-sterol | 345.5 + 10.3 | 43 |

[a] Pure sterol monolayers were prepared at the air/water interface. The monolayer was compressed and held at a constant surface pressure of 20 mN/m at 30 °C. The rate of sterol desorption to $\beta$-CD (0.7 mM) in the subphase was determined from the rate of monolayer area decrease. The value for desorption rate is the mean ± SEM of at least three different measurements.

To further show this relationship between hydrophobicity and desorption rate, cholesterol analogues with variable side chain lengths and conformations were tested. If the side chain was ten carbons in length instead of the eight-carbon side chain of cholesterol, the desorption rate decreased to $^1/_{10}$ of that measured for cholesterol (Table 1). Shortening the side chain increased the desorption rate (Table 1). The desorption rate was further found to be faster with unbranched side chain analogues (i.e., *n*-C5) than with sterols having branched side chains (i.e., iso-C5; Table 1).

*Visualization of Cholesterol Removal from Mixed Monolayers.* Having defined some of the parameters which affect sterol desorption from pure sterol monolayers to $\beta$-CD in the subphase, we next went on with characterizations of the effect of monolayer phospholipids on the desorption of cholesterol. First, we determined the effect of cholesterol removal from a binary cholesterol/phospholipid monolayer on the disappearance of lateral cholesterol-rich domains, using monolayer fluorescence microscopy (Slotte, 1995a). By preparing a cholesterol/di-10-PC monolayer (equimolar in both components), we could visually observe the presence of cholesterol-rich condensed domains at a fairly high surface pressure (18 mN/m; Slotte, 1995b). Since a pure di-10-PC monolayer is completely expanded (Slotte, 1995b), and a 1/1 cholesterol/di-10-PC monolayer is partially condensed, one can visually follow the removal of monolayer cholesterol by determining the appearance of expanded domains in the mixed monolayer. This experiment was done at 20 °C with 1.6 mM $\beta$-CD, and the resulting micrographs are shown in Figure 5. The monolayer surface texture, before addition of $\beta$-CD to the subphase, indicates the presence of condensed domains (cholesterol-rich) and expanded inclusions (cholesterol-poor and probe-rich domains; Figure 5A). Within about 13 min, the presence of $\beta$-CD in the subphase had markedly changed the monolayer surface texture (Figure 5C). The expanded domains became more clearly defined, and the condensed domain area diminished considerably. With longer exposure times, the monolayers contained more expanded domains and less condensed domains, and after about 25 min, very little cholesterol-rich domains were left in the monolayer area exposed to $\beta$-CD (Figure 5F). These micrographs also show that cholesterol was removed from the monolayer by $\beta$-CD, since the cholesterol-rich domains disappeared. It was observed that $\beta$-CD increased slightly the monolayer desorption of that di-10-PC (Table 2), although its removal rate was so slow that it did not markedly affect the results obtained with monolayer fluorescence microscopy.

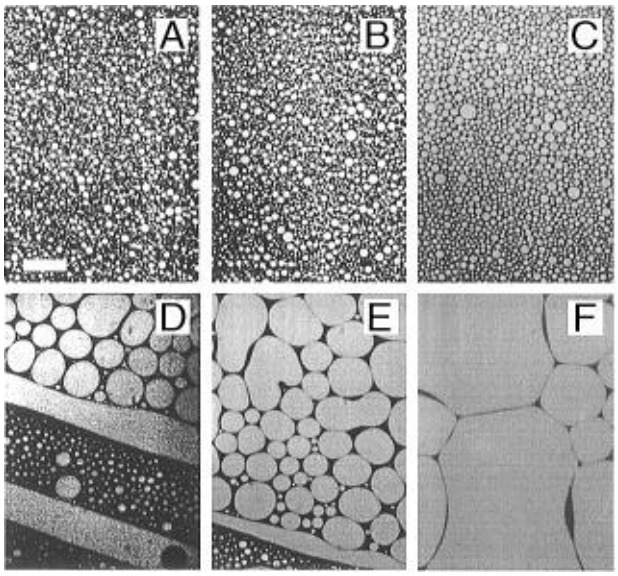


FIGURE 5: Visualization of cholesterol removal from mixed cholesterol/phospholipid monolayers by $\beta$-CD. A monolayer containing equimolar amounts of cholesterol and di-10-PC, and 0.5 mol % TRITC-PE as a fluorophore, was prepared at the air/water interface and compressed to 18 mN/m (at 20 °C). The monolayer was kept at a constant surface pressure. Panel A depicts the monolayer surface texture before the addition of $\beta$-CD. Panel B shows the monolayer 0.5 min after addition of $\beta$-CD to the subphase (1.6 mM). C is after 13 min. D is after 20 min, and E and F are after 25 min of $\beta$-CD addition. The scale bar represents 100 $\mu$m.

Table 2: Desorption Rates of Cholesterol, di-10-PC, DPPC, and *P*-SPM from Pure Monolayers to $\beta$-Cyclodextrin in the Subphase[a]

| lipid species | desorption rate (pmol cm$^{-2}$ min$^{-1}$) | rate relative to cholesterol desorption |
|---|---|---|
| cholesterol[b] | 10.0 ± 0.5 | 1 |
| di-10-PC[b] | 3.5 ± 0.05 | 0.35 |
| cholesterol[c] | 64.5 ± 0.6 | 1 |
| P-SPM | 3.5 ± 0.2 | 0.05 |
| DPPC | ~0 | 0 |

[a] Pure lipid monolayers were prepared at the air/water interface. The monolayer was compressed and held at a constant surface pressure, as indicated below. The rate of desorption to $\beta$-CD in the subphase was determined from the rate of monolayer area decrease. Values are means + SEM from at least three different monolayer experiments. [b] Determined at a lateral surface pressure of 18 mN/m (at 20 °C) with 0.8 mM $\beta$-CD. [c] Determined at a lateral surface pressure of 30 mN/m (at 30 °C) with 1.4 mM $\beta$-CD.

*Effect of Different Phospholipids on Cholesterol Removal from Monolayers.* In order to examine how cholesterol desorption rates from monolayers to $\beta$-CD in the subphase were affected by the presence of longer-chain phospholipids in the monolayer, the following experiments were performed. Mixed monolayers containing cholesterol and either DPPC or *N-P*-SPM were prepared at different ratios of cholesterol to phospholipid (C/PL from 0.5 to 9), and the rate of cholesterol desorption to $\beta$-CD (1.4 mM) at 30 °C was

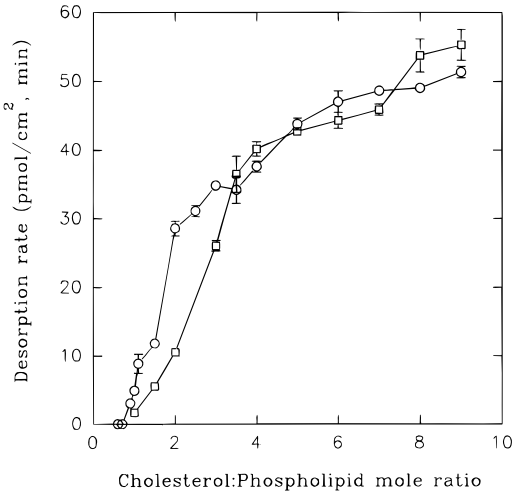


FIGURE 6: Removal of cholesterol from mixed cholesterol/phospholipid monolayers. Mixed monolayers containing cholesterol and either DPPC or *N-P*-SPM (at varying cholesterol/phospholipid molar ratios) were prepared at the air/water interface. The monolayers were compressed to 30 mN/m and maintained at a constant surface pressure (at 30 °C). Cholesterol desorption from a DPPC (circles) or an *N-P*-SPM monolayer (squares) to $\beta$-CD (1.4 mM) in the subphase was determined as a time function. Each value is normalized with regard to the surface concentration of cholesterol and is given as the mean ± SEM from three different monolayer experiments.

determined. The rate of phospholipid desorption to $\beta$-CD in the subphase was insignificant as compared to the rate of cholesterol desorption (Table 2). As shown in Figure 6, the rate of cholesterol desorption increased as the monolayer C/PL ratio increased, until a plateau level was reached. This plateau was approached at a 2/1 C/PL for the DPPC mixed monolayer, whereas a 4/1 C/PL was required with the *N-P*-SPM mixed monolayers. After the plateau was reached, desorption rates for cholesterol increased much less as the C/PL ratio increased further. The desorption rate of cholesterol was significantly faster from DPPC monolayers as compared to that from *N-P*-SPM monolayers, when comparisons were made at similar C/PL ratios (below about 3/1).

## DISCUSSION

The most important advantage of this study, compared to other studies in which solubilization of sterols by cyclodextrins have been performed, is the fact that constant surface pressure was maintained in the donor membranes during sterol desorption. In a related study, cholesterol removal from monolayers to cyclodextrins in the subphase was examined at a constant monolayer area (Ohtani et al., 1989). With the constant area technique, cholesterol removal results in a lowered surface pressure and a decreased lateral packing density. As we have shown in this study, cholesterol desorption to $\beta$-CD in the subphase is highly sensitive to the lateral surface pressure of the donor membrane. Consequently, no meaningful kinetic data can be obtained if the membrane surface pressure changes as a result of a cyclodextrin-catalyzed cholesterol desorption.

Several studies, using cyclodextrins to effect lipid removal from membranes, have indicated that unsubstituted cyclodextrins are not surface active (Asgharian et al., 1988; Ohtani et al., 1989; Laurent et al., 1994). In this study, $\beta$-CD failed to affect the surface tension of pure water, indicating that at the concentrations tested it did not adsorb at the air/water interface. In addition, it was reported that $^{14}$C-labeled $\beta$-CD did not associate with red blood cells, indicating that unspecific adsorption of $\beta$-CD to erythrocyte membranes was insignificant (Ohtani et al., 1989). Therefore, one can assume that cyclodextrins do not promote lipid desorption from membranes by actively destabilizing lipid/lipid interactions but rather promote lipid desorption by shifting the monomer ↔ aggregate equilibrium to the left. However, it is not clear whether the lipid/CD complexes are monomeric with respect to either the lipid or the cyclodextrin (Asgharian et al., 1988; Kilsdonk et al., 1995).

Differently sized cyclodextrins have different capacities to solubilize lipids. In this study, α-CD (glucopyranose units) did not induce cholesterol removal from monolayers whereas $\beta$-CD (glucopyranose units) was very effective. This difference can in part be ascribed to the smaller size of the hydrophobic cavity of α-CD as compared with that of $\beta$-CD. However, $\gamma$-CD which has an even larger cavity (because the ring contains glucopyranose units) is not as efficient in removing cholesterol from monolayers as compared with $\beta$-CD (Ohtani et al., 1989). It has been suggested that the larger cavity in $\gamma$-CD is effectively less hydrophobic than the cavity of $\beta$-CD (Asgharian et al., 1988).

The evidence presented in this study suggests that sterol polarity is a major determinant of desorption rates. If the desorbing sterol was less polar than cholesterol, slower desorption rates from monolayers were seen, whereas more polar sterols desorbed faster than cholesterol to $\beta$-CD in the subphase. However, with sterols having a similar polarity (e.g., 5-cholesten-3-one and 4-cholesten-3-one), one can still see a 3-fold difference in desorption rates. This observation may be explained by the fact that 4-cholesten-3-one monolayers are more expanded than monolayers containing 5-cholesten-3-one (Slotte & Östman, 1993), suggesting that the adhesion between 5-cholesten-3-one molecules is greater than that between 4-cholesten-3-one molecules.

Our observations that sterol desorption rates did (generally) correlate with relative sterol polarity is in good agreement with related experiments in which sterol desorption rates from bilayer membranes were examined (Kan et al., 1992). It is important to note, however, that the exchange rates determined by Bittman and co-workers (Kan et al., 1992) were obtained with phospholipid-containing membranes, and consequently, bilayer sterol/phospholipid interactions also influenced the measurable rates. In a related monolayer study, it was observed that the rate of cyclodextrin-induced removal of fatty acids from monolayers (at a constant surface area!) correlated with the hydrocarbon chain length (Asgharian et al., 1988). However, in the study of Asgharian and co-workers, different temperatures were used for the different fatty acids (myristic, palmitic, and stearic) in order to have the amphiphiles in the same physical state (liquid-expanded monolayers).

Desorption rates were also markedly affected by the lateral surface pressure of the monolayers, with the rates being higher at increased surface pressures. The dependence of the cholesterol desorption rate with surface pressure was curvilinear. This finding is similar to at least one previous report, in which the desorption of lauric acid was examined as a function of monolayer lateral surface pressure (Minassian-Saraga, 1956). In this early study, the desorption of lauric acid increased curvilinearly with increasing lateral surface pressure. According to Minassian-Saraga, the de-

sorption of fatty acids from monolayers can be discussed as a two-step process, the first being the dissolution of monolayer lipids to the first molecular layers of the subphase and the second being the diffusion away from the interface (Minassian-Saraga, 1955, 1956). Cholesterol desorption can probably be described with a similar analogy. However, since $\beta$-CD it not surface active by itself, it most likely enhances monolayer desorption of sterols by removing dissolved molecules from the first molecular layers of the interfacial region.

The surface area concentration of cholesterol is a function of the lateral surface pressure, since the lateral packing density is higher at increased surface pressures. Even though a higher surface area concentration of cholesterol could possibly influence its rate of desorption (due to a more favorable cholesterol/$\beta$-CD ratio), this effect of surface concentration on desorption rates would be expected to be small, since the force−area isotherm of cholesterol is very steep. Consequently, the surface concentration of cholesterol at 20 and 25 mN/m is only 2−3% different (the mean molecular area decreasing from 39 Å$^2$ at 20 mN/m to 38 Å$^2$ at 35 mN/m).

The effect of temperature on observable desorption rates may arise from at least two different effects. One is the direct effect of temperature on interlipid association in membranes, since an increased temperature also leads to an increased kinetic energy of membrane components, and consequently results in increased desorption rates. This relationship has been demonstrated by determining cholesterol desorption kinetics from phospholipid membranes as a function of temperature (Bhuvaneswaran & Mitropoulos, 1986; Lund-Katz et al., 1988). The other effect of temperature involves the stability of the acceptor, specifically the lipid/cyclodextrin complex. It is known that hydrogen bonds become more stable as the temperature decreases (Boggs, 1987). Further, it is possible that hydrogen bonds stabilize the lipid/cyclodextrin complex, since both the amphiphilic lipids and the cyclodextrins contain functional groups which can participate in hydrogen bond formation (Boggs, 1987). Our observation that cholesterol desorption rates from pure cholesterol monolayers to $\beta$-CD increased with decreasing experimental temperature can be understood, if the cholesterol/cyclodextrin complex was stabilized by hydrogen bonds. Otherwise, one would expect faster desorption at higher temperatures. If the assumption of hydrogen bond stabilization is correct, then it can also be argued that stabilization of the cholesterol/cyclodextrin complex at lower temperatures had a larger impact on observable cholesterol desorption rates than the simultaneously occurring stabilization of cholesterol/cholesterol interactions in the monolayer membrane. However, membrane stability is still a major factor in determining cholesterol desorption, since the desorption rates were so largely influenced by the monolayer lateral surface pressure.

It has been suggested that cyclodextrins cannot remove monolayer cholesterol away from the monolayer into the bulk phase and that cholesterol instead would pull cyclodextrins into the interfacial region (Asgharian et al., 1988). Our data clearly show that $\beta$-CD in the subphase extracts cholesterol from the monolayer. First, we could localize cholesterol in the bulk aqueous phase using [$^3$H]cholesterol. Second, we could visually observe the removal of cholesterol from mixed monolayers containing both the sterol and di-10-PC. Since the laterally condensed domains present in mixed cholesterol/di-10-PC monolayers were completely different from the expanded phase present in a pure di-10-PC monolayer (Slotte, 1995b), the gradual and ultimate removal of cholesterol was easily observable using monolayer fluorescence microscopy.

The desorption of cholesterol from a mixed monolayer containing phospholipids was much slower as compared to the desorption from a pure cholesterol monolayer, even when corrections were made for the different surface area concentrations of cholesterol at different cholesterol/phospholipid molar ratios. This finding clearly shows that the retarding effect of membrane phospholipids on the cholesterol desorption rate was evident even when cholesterol desorption was facilitated by $\beta$-CD. It was further noticed that cholesterol desorption to $\beta$-CD was faster from phosphatidylcholine mixed monolayers than from *N*-P-SPM-containing monolayers (under comparable conditions). Analogous effects of the phospholipid class on cholesterol desorption rates from bilayer membranes have been presented (Fugler et al., 1985; Bhuvaneswaran & Mitropoulos, 1985; Yeagle & Young, 1986; Lund-Katz et al., 1988; Thomas & Poznansky, 1988; Kan et al., 1991). The long-range interactions in the cholesterol/DPPC monolayers were clearly different compared to the interactions in cholesterol/*N*-*P*-SPM monolayers, since cholesterol desorption was differently sensitive to the C/PL mole ratio. An analogous situation has been reported previously by us, when the oxidation susceptibilities of cholesterol in mixed DPPC or *N*-*P*-SPM monolayers were examined as a function of the C/PL mole ratio (Slotte, 1992). However, when cholesterol oxidase was used to probe the stoichiometry at which the oxidation susceptibility changed, the C/PL was 1 for cholesterol/DPPC and 2 for cholesterol/*N*-*P*-SPM. The difference in stoichiometry between these two model systems must in part result from the use of different probes. Cholesterol oxidase is an intrusive probe in the sense that its reaction product (4-cholesten-3-one) remains in the monolayer and may influence the behavior of unreacted lipids, whereas $\beta$-CD is not intrusive.

In conclusion, the results presented in this work clearly show that $\beta$-CD can selectively facilitate cholesterol desorption from membranes containing long-chain phospholipids (the desorption of DPPC or *N*-*P*-SPM was insignificant as compared to the rate seen with cholesterol). The unique aspect of this study is that cholesterol desorption from monolayers to $\beta$-CD in the subphase can be examined at a constant surface pressure, and consequently, desorption rates are kinetically meaningful, since the interfacial quality is maintained more or less unchanged during the process. Therefore, with this technique, $\beta$-CD appears to be a very useful probe by which one can study cholesterol/phospholipid interactions.

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