# Octyl-$\beta$-D-Glucopyranoside Partitioning into Lipid Bilayers: Thermodynamics of Binding and Structural Changes of the Bilayer

Markus R. Wenk, Thomas Alt, Anna Seelig, and Joachim Seelig
Department of Biophysical Chemistry, Biocenter of the University of Basel, CH-4056 Basel, Switzerland

ABSTRACT  The interaction of the nonionic detergent octyl-$\beta$-D-glucopyranoside (OG) with lipid bilayers was studied with high-sensitivity isothermal titration calorimetry (ITC) and solid-state $^2$H-NMR spectroscopy. The transfer of OG from the aqueous phase to lipid bilayers composed of 1-palmitoyl-2-oleoyl-$sn$-glycero-3-phosphocholine (POPC) can be investigated by employing detergent at concentrations below the critical micellar concentration; it can be defined by a surface partition equilibrium with a partition coefficient of $K = 120 \pm 10$ M$^{-1}$, a molar binding enthalpy of $\Delta H_D^{\circ} = 1.3 \pm 0.15$ kcal/mol, and a free energy of binding of $\Delta G_D^{\circ} = -5.2$ kcal/mol. The heat of transfer is temperature dependent, with a molar heat capacity of $\Delta C_p = -75$ cal K$^{-1}$ mol$^{-1}$. The large heat capacity and the near-zero $\Delta H$ are typical for a hydrophobic binding equilibrium. The partition constant $K$ decreased to ~100 M$^{-1}$ for POPC membranes mixed with either negatively charged lipids or cholesterol, but was independent of membrane curvature. In contrast, a much larger variation was observed in the partition enthalpy. $\Delta H_D^{\circ}$ increased by about 50% for large vesicles and by 75% for membranes containing 50 mol% cholesterol. Structural changes in the lipid bilayer were investigated with solid-state $^2$H-NMR. POPC was selectively deuterated at the headgroup segments and at different positions of the fatty acyl chains, and the measurement of the quadrupolar splittings provided information on the conformation and the order of the bilayer membrane. Addition of OG had almost no influence on the lipid headgroup region, even at concentrations close to bilayer disruption. In contrast, the fluctuations of fatty acyl chain segments located in the inner part of the bilayer increased strongly with increasing OG concentration. The $^2$H-NMR results demonstrate that the headgroup region is the most stable structural element of the lipid membrane, remaining intact until the disordering of the chains reaches a critical limit. The perturbing effect of OG is thus different from that of another nonionic detergent, octaethyleneglycol mono-$n$-dodecylether (C$_{12}$E$_8$), which produces a general disordering at all levels of the lipid bilayer. The OG-POPC interaction was also investigated with POPC monolayers, using a Langmuir trough. In the absence of lipid, the measurement of the Gibbs adsorption isotherm for pure OG solutions yielded an OG surface area of $A_S = 51 \pm 3$ Å$^2$. On the other hand, the insertion area $A_I$ of OG in a POPC monolayer was determined by a monolayer expansion technique as $A_I = 58 \pm 10$ Å$^2$. The similar area requirements with $A_S \approx A_I$ indicate an almost complete insertion of OG into the lipid monolayer. The OG partition constant for a POPC monolayer at 32 mN/m was $K_p \approx 320$ M$^{-1}$ and thus was larger than that for a POPC bilayer.

## INTRODUCTION

The solubilization of biological membranes by detergents as well as the formation of membranes from a micellar mixture of detergents and lipids have found wide application in membrane research. Examples are the reconstitution of membrane proteins in functional form (Racker, 1979), or the preparation of lipid vesicles of defined size and composition (Mimms et al., 1981; Schurtenberger et al., 1984). The nonionic detergent octyl-$\beta$-D-glucopyranoside (OG) has proved particularly useful because of its high critical micelle concentration ($c_{cm}$) of ~20–25 mM (deGrip and Bovee-Geurts, 1979; Paula et al., 1995) and its nondenaturing effect on membrane proteins (Stubbs et al., 1976). The aggregation of OG/lipid mixtures has been analyzed with a variety of methods (Jackson et al., 1982; Lichtenberg, 1985; Ollivon et al., 1988; Vinson et al., 1989; Ueno, 1989;

Almog et al., 1990; De la Maza and Parra, 1994), and the results of these studies have been summarized in a three-stage model (Lichtenberg et al., 1983). Starting at low OG concentrations, the first step is a partitioning of the detergent into the membrane bilayer (stage I). Insertion of detergent changes bilayer properties such as "fluidity" and permeability (Jackson et al., 1982; Ollivon et al., 1988; Almog et al., 1990). With increasing amounts of detergent in the bilayer, a point of saturation is reached. This marks the beginning of stage II, the conversion of detergent-saturated bilayers into mixed micelles. Mixed detergent/lipid bilayers coexist with mixed micelles. The critical molar ratio of detergent to lipid, $X_{b,crit} \approx 1.3$–1.4 mol/mol, at the onset of solubilization reflects the remarkable potential of OG to act as a membrane constituent. Increasing the detergent concentration beyond this critical limit produces a gradual disruption of the bilayer until all vesicles are transformed into micelles. In stage III, only micelles exist. The average number of lipid molecules per micelle as well as the size of the micelles decrease with increasing detergent concentration (Eidelmann et al., 1988).

In this study we have investigated the interaction of octyl-$\beta$-D-glucopyranoside with lipid vesicles composed of 1-palmitoyl-2-oleoyl-$sn$-glycero-3-phosphocholine    (POPC),

either in pure form or in mixture with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), negatively charged 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), or cholesterol. Using high-sensitivity titration calorimetry, we have measured the heat of binding, $\Delta H_D^o$, as well as the binding isotherm at OG concentrations well below the critical micellar concentration. The binding constant, $K$, and the free energy of binding, $\Delta G_D^o$, were derived from the analysis of the binding isotherm. Titration calorimetry hence yields a complete thermodynamic description of OG partitioning into lipid bilayers. In addition, the influence of temperature and membrane curvature was investigated. Structural changes of the lipid bilayer induced by OG binding were detected with solid-state $^2$H-NMR spectroscopy. The quadrupolar splittings of selectively deuterated lipids in mixtures with OG served to elucidate the membrane-disordering potential of this detergent. POPC selectively deuterated at the choline headgroup and at different segments of the fatty acyl chain was used in these studies, providing a detailed picture of the perturbation of the membrane with a segment-to-segment resolution. The response of the lipid headgroup region to detergent incorporation is distinctly different from that of the fatty acyl chains. Finally, we have employed a Langmuir trough with a Wilhelmy balance to derive quantitative estimates for the area requirements of OG at the air-water interface and in a tightly packed lipid monolayer and to measure the OG partition constant for a POPC monolayer.

## MATERIALS AND METHODS

### Materials

Octyl-$\beta$-D-glucopyranoside (OG) was purchased from Fluka (purity > 99% thin-layer chromatography). 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), and egg lecithin (egg PC), dissolved in chloroform, were from Avanti Polar Lipids (Birmingham, AL). The cholesterol was from Sigma. All chemicals were used without further purification. Buffers were prepared from 18 M$\Omega$ water obtained from a Nanopure A filtration system.

Most deuterium NMR experiments were carried out with selectively deuterated POPC, synthesized as described before (Tamm and Seelig, 1983; Seelig and Seelig, 1977; Seelig and Waespe-Sarcevic, 1978). For convenience, the deuterated methylene segments of the choline moiety are denoted $\alpha$ and $\beta$:

$$O^\ominus$$
$$|$$
$$-O-POCH_2CH_2\overset{\oplus}{N}(CH_3)_3$$
$$\parallel \quad \vdots \quad \vdots$$
$$O \quad \alpha \quad \beta$$
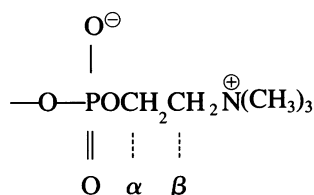
To investigate the influence of OG on double bonds, a further series of experiments was performed with 1,2-dielaidoyl-sn-glycero-3-phosphocholine (DEPC) deuterated at the 9'-10' position of the *trans* double bond (Seelig and Waespe-Sarcevic, 1978).

### Liposome preparation

Composite lipid films were prepared by appropriate mixing of the lipids in organic solvent. Small unilamellar vesicles (SUVs) were prepared as follows. A defined amount of lipid ($\sim$50 mg) was first dried under a stream of nitrogen, followed by high vacuum for 1 h at room temperature in the dark. The lipid was redissolved in dichloromethane (0.5 ml) and again dried under nitrogen. High vacuum was applied overnight. A defined amount of buffer (2 ml) was added to the dried lipid film, and the suspension was vortexed extensively. Next the lipid dispersions were sonicated for 20–50 min (10°C) until the solution became transparent. The opalescent solution was centrifuged in an Eppendorf tabletop centrifuge (8 min at 14,000 rpm) to remove metal debris.

Large unilamellar vesicles (LUVs) of diameter $d \approx 400$ or 200 nm were obtained by extrusion of multilamellar lipid suspensions through polycarbonate filters (Nuclepore, Pleasanton, CA) (Hope et al., 1985; Mayer et al., 1986). The dried lipid was suspended in buffer, vortexed, and freeze-thawed for five cycles. Unilamellar vesicles of the desired diameter were extruded under nitrogen pressure through filters by decreasing the pore size stepwise ($d = 400$ nm, $d = 200$ nm, five times for each filter).

Lipid concentrations were determined gravimetrically by carefully weighing the samples and by adding defined amounts of buffer. In separate experiments we have analyzed the lipid content of sonified and extruded vesicles with phosphorus analysis (Böttcher et al., 1961). For sonified lipid vesicles the phosphate analyis yielded a 4.7% (average of nine determinations) smaller lipid content than the nominal concentration, with maximum deviations between −2% and +10%. For extruded vesicles the lipid loss was in the range of 20–30% (four determinations). The thermodynamic data reported here for sonified POPC vesicles were calculated on the basis of the weighing-in concentration. For extruded vesicles the nominal lipid concentration was reduced by 30%.

If not otherwise stated, the buffer composition was 10 mM Tris (pH 7.4), 100 mM NaCl. The phospholipid dispersions as well as the OG solutions were prepared in the same buffer to avoid heats of dilution caused by the buffer.

### High-sensitivity titration calorimetry

Isothermal titration calorimetry was performed using an Omega high-sensitivity titration calorimeter from Microcal (Northampton, MA) (Wiseman et al., 1989). To avoid air bubbles, solutions were degassed under vacuum before use. The calorimeter was calibrated electrically. The data were acquired by computer software developed by MicroCal. In control experiments the corresponding detergent solution (or vesicle suspension) was injected into buffer without lipid (or detergent). At detergent concentrations below the critical micelle concentration, the heat of dilution of the detergent was small compared to the detergent-lipid reaction enthalpy. Injection of lipid suspensions into buffer alone yielded small reaction heats. Both control values were included in the final analysis.

### NMR measurements

Solid-state deuterium NMR measurements of membranes were recorded at 28°C on a Bruker MSL 400 NMR spectrometer operating at a deuterium frequency of 61.4 MHz. A quadrupole echo sequence with an echo spacing of 40 $\mu$s was used. The $\pi/2$ pulse width was 3.5–4 $\mu$s for a 10-mm solenoid coil, the spectral width was 50–125 kHz, and the recycling delay was 100 ms. Two thousand to eight thousand free induction decays were accumulated. Usually 8–10 mg of deuterium-labeled lipid was incubated with a known amount of detergent and mixed in organic solvent. The solvent was evaporated by nitrogen, and a small amount ($\sim$50 $\mu$l) of deuterium-depleted water was added. The samples were sealed, vortexed extensively, and subjected to several freeze-thaw cycles.

### Monolayer measurements

The monolayer apparatus consisted of a round Teflon trough designed by Fromherz (1975) with a total area of $A = 362$ cm$^2$ divided into eight compartments (type RMC 2-T; Mayer Feintechnik, Göttingen, Germany). For the measurement of the Gibbs adsorption isotherms, only one com-

partment, filled with 20 ml of buffer, was utilized. Small aliquots of the detergent stock solution were injected into the buffer by means of a Hamilton syringe, thus increasing the concentration stepwise. The surface pressure, $\pi = \gamma_0 - \gamma$, where $\gamma_0$ is the surface tension of pure buffer and $\gamma$ is the surface tension of the detergent solution, was monitored with a Wilhelmy balance. All measurements were done at room temperature (23°C).

For insertion experiments two compartments were used. A monolayer of pure POPC was formed by depositing a drop of lipid, dissolved in hexane/ethanol (9:1, v/v), on the buffer surface, which was then left to stabilize for about 15 min. The initial area, $A$ (typically 50 cm$^2$), contained $n_L$ lipid molecules of area $A_L$ each. Detergent was injected through the lipid monolayer into the subphase. The surface pressure was kept constant during insertion experiments by means of an electronic feedback system. The penetration of $n_D$ detergent molecules into the monolayer gave rise to an area expansion $\Delta A$.

## RESULTS

### High-sensitivity titration calorimetry

The thermodynamics of OG partitioning into lipid bilayers at detergent concentrations below the critical micellar concentration ($c_{cm}$) was investigated by means of high-sensitivity isothermal titration calorimetry (Wiseman et al., 1989). As an example, Fig. 1 displays the titration of octyl-$\beta$-D-glucopyranoside ($c_D^{\circ} = 2$ mM in buffer) with sonified POPC vesicles ($c_L^{\circ} = 36.0$ mM in buffer). Using a Hamilton syringe coupled with a stepping motor, 10-$\mu$l aliquots of the phospholipid vesicle suspension are injected into the calorimeter cell (volume $V_{cell} = 1.3353$ ml). Each injection gives rise to an endothermic heat of reaction, $h_i$, produced by the partitioning of OG molecules into the membrane. $h_i$ is given by the area of the titration peak. Fig. 1 demonstrates that the heat of reaction decreases with consecutive injections, because less and less detergent is available for binding. As a control, the same phospholipid vesicle suspension was injected into buffer without detergent. The heat of reaction, $h_c$, was small ($\leq 5$ $\mu$cal/inj) and, furthermore,
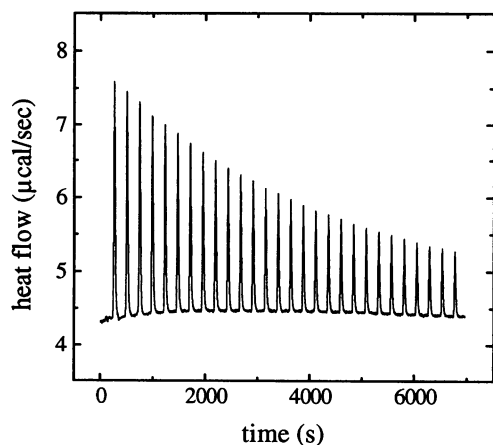


FIGURE 1   Titration calorimetry of octyl-$\beta$-D-glucopyranoside (2.0 mM) with 30 nm unilamellar POPC vesicles (36.0 mM) in buffer (10 mM Tris, pH 7.4, 100 mM NaCl, $T = 28$°C). Aliquots (10 $\mu$l) of the vesicle suspension were added to the OG solution in the reaction cell ($V = 1.3353$ ml).

constant during consecutive injections. The quantitative analysis of the data was based on the corrected heats of titrations, $\delta h_i$, given by

$$\delta h_i = h_i - h_c. \tag{1}$$

The height of the individual titration peaks is strongly correlated with the detergent concentration, whereas the steepness of the decrease depends primarily on the lipid concentration $c_L^{\circ}$ and the binding constant $K$. The cumulative heat of reaction, $\delta H_i$, is defined as

$$\delta H_i = \sum_{k=1}^{i} \delta h_k \tag{2}$$

and is shown in Fig. 6 $B$ for the data of Fig. 1.

Titration curves qualitatively similar to those of Fig. 1 were observed for OG concentrations in the range of 0.2 mM $\leq c_D^{\circ} \leq 10$ mM. In contrast, OG solutions with concentrations $c_D^{\circ} \geq 15$ mM produced distinctly more complex titration patterns. At high OG concentrations detergent partitioning was accompanied by membrane disruption and micellization, yielding additional heats of reaction. The present study was therefore restricted to OG concentrations distinctly below this critical limit. The influence of lipid composition on OG partitioning was investigated with sonified vesicles composed of POPC in mixture with a negatively charged lipid (POPG), a saturated lipid (DMPC), or a steroid (cholesterol). Although most measurements were performed with sonicated vesicles with an average size of 30 nm, we have also prepared POPC vesicles with diameters of 200 nm or 400 nm.

The titration curves provided the individual heats of titration, $\delta h_i$, as well as the cumulative heat, $\delta H_i$. These data were analyzed in terms of a 2-parameter partition model (described in detail below) leading to the partition constant $K$ and the standard partition enthalpy, $\Delta H_D^{\circ}$. These parameters are summarized in Table 1. For sonified POPC vesicles and for 200-nm and 400-nm vesicles, the partition constant is $K = 120 \pm 10$ M$^{-1}$ over the whole concentration range measured. The standard reaction enthalpy is also independent of the OG concentration, with $\Delta H_D^{\circ} = 1.3 \pm 0.15$ kcal/mol for sonified vesicles and 1.75–1.9 kcal/mol for 200–400-nm vesicles. Mixing of POPC with POPG, DMPC, or cholesterol reduces the partition constant by about 30% to $K \approx 100$ M$^{-1}$, but increases the partition enthalpy. This effect is most pronounced for cholesterol containing POPC membranes, where $\Delta H_D^{\circ}$ increases from 1.6 kcal/mol at 5 mol% cholesterol to 2.25 kcal/mol at 50 mol% cholesterol.

Finally, we have measured the temperature dependence of OG partitioning into sonified POPC vesicles. To this purpose, the titration experiment shown in Fig. 1 (at 28°C) was repeated at various higher temperatures up to 50°C. Within the accuracy of the measurement the partition constant $K$ remained constant at $120 \pm 10$ M$^{-1}$. In contrast, $\Delta H_D^{\circ}$ decreased linearly with increasing temperature, as

**TABLE 1 Partitioning of octyl-$\beta$-D-glucopyranoside into lipid membranes (at 28°C)**

| Lipid (mol:mol) | Size (nm) | $K^*$ (M$^{-1}$) | $\Delta H_D^{\circ}$ (kcal/mol) | $C_D^{\circ}$ (mM) | No. of measurements |
|---|---|---|---|---|---|
| POPC | 30 | 120 ± 10 | 1.30 ± 0.15 | 0.2–10 | 5 |
| | 200 | 130 ± 5 | 1.75 ± 0.15 | 1; 2 | 2 |
| | 400 | 120 ± 10 | 1.9 ± 0.1 | 2; 5 | 2 |
| POPC:POPG (75:25) | 30 | 100 | 1.45 ± 0.1 | 1–3 | 3 |
| POPC:cholesterol (95:5) | 30 | 111# | 1.6 | 2 | 1 |
| (85:15) | 30 | 106# | 1.7 | 2 | 1 |
| (80:20) | 30 | 100# | 1.8 | 2 | 1 |
| (70:30) | 30 | 103# | 1.75 | 2 | 1 |
| (50:50) | 30 | 90# | 2.25 | 2 | 1 |
| Egg-PC | 30 | 78 | 1.47 | 2 | 1 |

*The binding constants are calculated under the assumption that all phospholipid is available for binding.
#The binding constant is calculated on the basis of the POPC content only. If cholesterol is also considered as a matrix for OG partitioning, the new binding constants are given by $K_{chol} = K \cdot X_{POPC}$, where $X_{POPC}$ denotes the mole fraction of POPC.

shown in Fig. 2. At the highest temperature measured, the reaction even became exothermic, with $\Delta H_D^{\circ}$ (50°C) = $-0.4$ kcal/mol. Linear regression analysis yields a molar heat capacity of $\Delta C_p = -75$ cal/(mol $\cdot$ K) for the transfer of OG from water into the bilayer vesicle. Such large $\Delta C_p$ values are usually considered a characteristic signature of the hydrophobic effect. Similar results have been found for the binding of other amphiphilic compounds to model membranes (Privalov and Gill, 1989; Seelig and Ganz, 1991).

The integrity of the lipid vesicles under the conditions of the calorimetric titration experiments was tested by performing fluorescence dequenching experiments. Liposomes were prepared in the presence of self-quenching concentrations of fluorescent dyes (carboxy fluorescein, CF) or high-molecular-weight fluorescein isothiocyanate (FITC)-dextran (MW 19,700). The untrapped dye was removed from

the vesicles by passing the liposome suspension through a Sepharose column. Aliquots of these vesicle suspensions were injected into solutions of increasing OG concentration, and the time course of the fluorescence signal was recorded. After 15 min, Triton X-100 was added to completely disrupt the vesicles, yielding the upper limit of the fluorescence intensity. Efflux of dye molecules from the vesicle interior into the suspending medium leads to an increase in the fluorescence intensity. The fluorescence increase of both dyes was found to be proportional to the detergent concentration. At $c_D^{\circ} \approx 16$ mM, both dyes were completely liberated (data not shown). The results of these studies are in good agreement with related dye-efflux measurements (De la Maza and Parra, 1994) and phase diagrams of OG-egg lecithin mixtures (Ollivon et al., 1988; Almog et al., 1990). They indicate that for a given OG concentration the vesicles are more permeable for small dyes (CF) than for large dyes (FITC-dextran), but that total disruption occurs only at detergent concentrations $c_D^{\circ} = 16$ mM OG. In the present study all calorimetric experiments were performed at detergent concentrations well below this critical limit. The fluorescence experiments provide evidence that the vesicle structure remains essentially unchanged under these conditions.

## Deuterium NMR experiments with POPC/OG membranes

NMR studies were undertaken to elucidate the structural changes in the lipid bilayer induced by the uptake of OG and to shed light on the location of the detergent molecule within the membrane. POPC was selectively deuterated at different segments of the choline headgroup and the fatty acyl chains, and the variation of the quadrupolar splittings upon OG addition was recorded. The deuterium quadrupolar splitting, $\Delta \nu_Q$, not only provides structural information
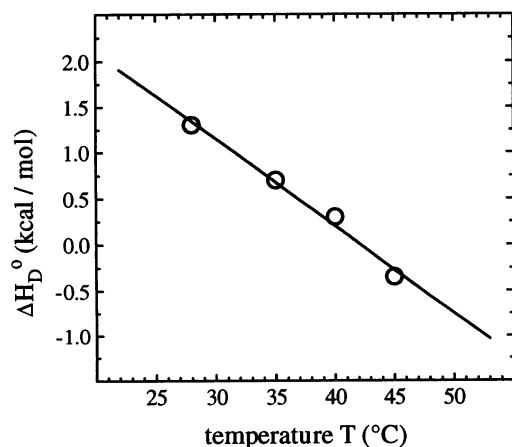


FIGURE 2 Transfer of OG from the aqueous phase to POPC membranes. Temperature dependence of the transfer enthalpy. Experimental conditions are the same as indicated in Fig. 1. The solid line is a linear least-squares fit with a slope of $\Delta C_p = -75$ cal mol$^{-1}$ K$^{-1}$.

on the deuterium labeled lipid segment but is also strongly influenced by the extent of the molecular fluctuations. An increase in molecular disorder will always decrease the quadrupole splitting; in contrast, a conformational change can either increase or decrease $\Delta\nu_Q$ (Seelig, 1977). Fig. 3 shows representative deuterium NMR spectra of POPC bilayers labeled at the choline headgroup and at the C-2 segment of the oleic acyl chain. The lineshape of the spectra corresponds to the typical "powder pattern" of liquid crystalline bilayers (Seelig, 1977). The $\alpha$- and $\beta$-segments of the choline headgroup produce just one quadrupole splitting, and the two deuterons are motionally equivalent (Fig. 3 $A$, spectra $a$ and $b$). In contrast, the oleic acid C-2 segment of POPC gives rise to a deuterium NMR spectrum consisting of two doublets (Fig. 3 $A$, spectra $c$ and $d$). This can be explained by a bend in the $sn$-2 chain at this position, leading to a motional inequivalence of the two deuterons (Seelig and Seelig, 1975). The variation in the quadrupolar splittings with the amount of bound detergent is shown in Fig. 3 $B$.

Binding of OG leads to a small decrease in the quadrupole splitting of the $\alpha$-segment, $\Delta\nu_\alpha$, but to an increase in the $\beta$-splitting, $\Delta\nu_\beta$. This counterdirectional change in the two neighboring segments excludes the possibility of a more random motion of the phospholipid headgroup, because an increase in statistical disorder would reduce both quadrupole splittings simultaneously. The data of Fig. 3 can be approximated by straight lines according to

$$\Delta\nu_i = \Delta\nu_i^0 + m_i X_b, \tag{3}$$

where $\Delta\nu$ and $\Delta\nu_i^0$ are the measured quadrupolar splittings of membranes with and without detergent, respectively. The slope $m_i$ provides a measure of the efficacy of the detergent in changing the segment orientation. From Fig. 3 one calculates $m_\alpha = -2.1$ kHz and $m_\beta = 0.9$ kHz, yielding $m_\beta/m_\alpha = -0.43$. Qualitatively similar changes have been observed previously for the binding of positively charged metal ions, local anesthetics, amphiphilic detergents, and peptides to lipid membranes (Altenbach and Seelig, 1984; Seelig and Macdonald, 1989; Scherer and Seelig, 1989). In all of these cases, $\Delta\nu_\alpha$ decreased and $\Delta\nu_\beta$ increased, and the ratio $m_\beta/m_\alpha \approx -0.5$ remained constant. The conformational change corresponds to a small rotation of the $^-$P-N$^+$-dipole away from the membrane surface toward the water phase (Scherer and Seelig, 1989). OG incorporation into a lipid bilayer appears to induce a headgroup rotation similar to that induced by a positive charge, albeit to a much lesser extent. Because OG carries no net charge, the origin of the positive charge effect could be the dipole moment of the hydroxyl groups of the sugar ring. Dipole effects have been observed previously upon incorporation of phloretin into POPC membranes (Bechinger and Seelig, 1991).

At binding ratios of $X_b > 1.3$ mol/mol, isotropic peaks were observed in the $^2$H-NMR spectra, indicating the onset of bilayer disruption and the formation of micelles. For $X_b > 2.5$ mol/mol, only isotropic peaks were obtained, providing evidence for a complete micellation of the bi-
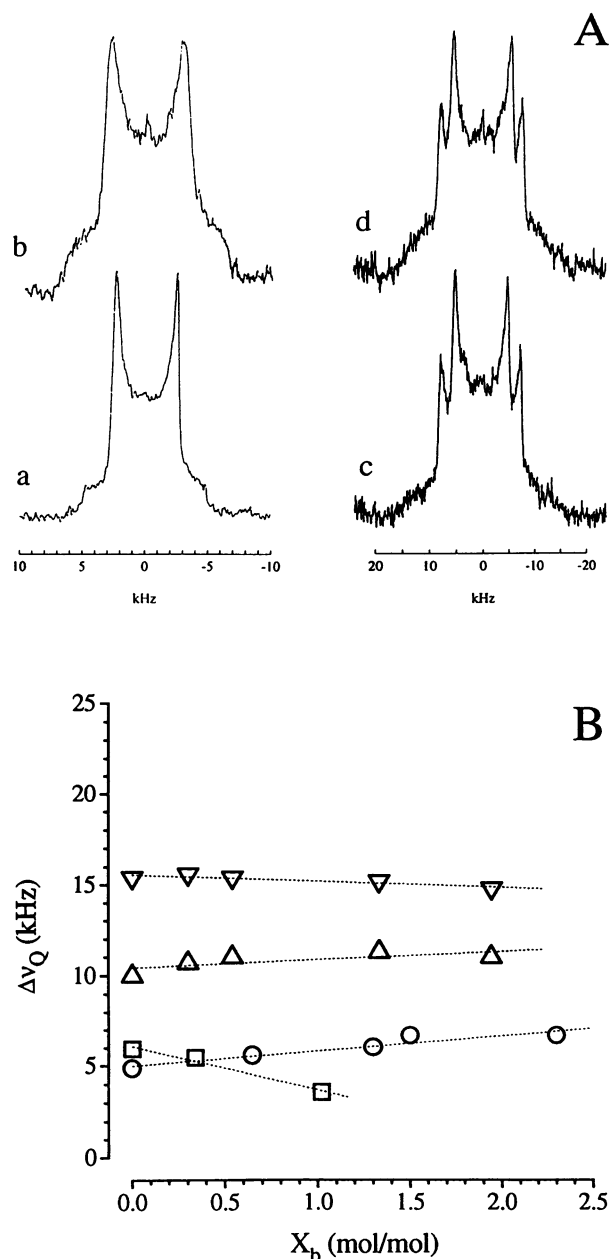


FIGURE 3 Deuterium NMR studies of deuterium-labeled 1-palmitoyl-2-oleoyl-$sn$-glycero-3-phosphocholine (POPC) in mixture with octyl-$\beta$-D-glucopyranoside (OG). The lipid headgroup region. ($A$) $^2$H-NMR spectra of multilayered lipid dispersions. The two lower spectra ($a$, $c$) correspond to pure lipid systems. The two upper spectra ($b$, $d$) correspond to lipid in mixture with OG. The specifics are: ($a$) $\beta$-CD$_2$-POPC (pure lipid), $\Delta\nu_Q = 4.9$ kHz. ($b$) $\beta$-CD$_2$-POPC plus OG, $X_b = 1.3$, $\Delta\nu_Q = 6.0$ kHz. ($c$) POPC deuterated at the C-2 segment of the oleic acyl chain (2'-D$_2$ POPC) (pure lipid), $\Delta\nu_{Q,1} = 15.4$ kHz, $\Delta\nu_{Q,2} = 10.0$ kHz. ($d$) 2'-D$_2$ POPC plus OG, $X_b = 0.54$, $\Delta\nu_{Q,1} = 15.4$ kHz, $\Delta\nu_{Q,2} = 11.0$ kHz. Deuterium frequency 61.4 MHz, 8000 transients, $T = 28$°C. ($B$) Variation of the quadrupole splittings, $\Delta\nu_Q$, of deuterated POPC lipids with $X_b$, the amount of bound OG. $\triangle$, $\triangledown$, 2'-D$_2$ POPC. $\square$, $\alpha$-CD$_2$-POPC. $\bigcirc$, $\beta$-CD$_2$-POPC.

layer. This is in excellent agreement with the phase diagram of Almog et al. (1990).

The glycerol backbone constitutes the most rigid structural element of the lipid bilayer. The fluctuations of this

region are monitored by the C-2 segment of the sn-2 oleic acyl chain. Note that the quadrupolar splittings of the C-2 segment do not change substantially with $X_b$. It can be concluded that neither the orientation of the C-2 segment not its average fluctuations are changed upon detergent incorporation into the bilayer. Taken together, the data of Fig. 3 thus demonstrate that incorporation of OG does not disorder the headgroup region of the POPC membrane. Instead, the headgroup segments, including the C-2 segment of the sn-2 chain, retain their average orientation and fluctuation up to the highest detergent concentration possible for an intact bilayer.

A completely different result is obtained for the chain segments in the membrane interior, as demonstrated in Fig. 4. Fig. 4 A shows representative spectra of 1,2-dielaidoyl-sn-glycero-3-phosphocholine deuterated at the trans double bond (trans-9'-10'-$D_2$-DEPC) and of POPC deuterated at C-12 of the oleic acyl chain (12',12'-$D_2$-POPC), with and without OG. The spectra are again typical bilayer spectra characterized by a single quadrupole splitting. The addition of OG leads to a distinct reduction of both splittings by about 30–50%. The variation in $\Delta\nu_Q$ with the OG content of the membrane is shown in Fig. 4 B. Also included in Fig. 4 B are data obtained with 1-(5',5'-$D_2$)palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (5',5'-$D_2$-POPC) and 1-palmitoyl-2-(9',10'-$^2$H)oleoyl-sn-glycero-3-phosphocholine, i.e., POPC deuterated at the cis double bond of the oleic acyl chain. The deuterium NMR spectra of the latter lipid are characterized by two splittings. The large splitting can be assigned to the C-9 deuteron, the small one to the C-10 deuteron of the cis double bond (Seelig and Waespe-Sarcevic, 1978). Fig. 4 demonstrates that for almost all segments of the fatty acyl chains (i.e., C-5, trans-C-9,10, cis-C-10, and C-12), $\Delta\nu_Q$ decreases linearly with the OG content and the slopes of the straight lines are similar. This can only be explained by an increase in the extent of segmental fluctuations, producing a more disordered bilayer interior. The only exception appears to be the C-9 deuteron of the cis double bond, which exhibits only a small variation with $X_b$. This will be explained below by a reorientation of the cis double bond superimposed on an increase in statistical fluctuations.

## Surface activity and monolayer penetration of octyl-β-D-glucopyranoside

The surface area requirement of OG at the air/water interface was determined from the Gibbs adsorption isotherm, measuring the surface pressure $\pi$ as a function of the OG concentration. Up to a concentration of 40 $\mu$M OG, the surface pressure was essentially zero. Above 100 $\mu$M the surface pressure increased linearly with the logarithm of the detergent concentration (data not shown). From the slope of the Gibbs adsorption isotherm, the area of the detergent molecule at the buffer/air interface, $A_S$, was determined as $A_S = 51 \pm 3$ Å$^2$.

In a second experiment we have measured the penetration of OG into a POPC monolayer. The monolayer was kept at a constant surface pressure by means of an electronic feedback system, and the area increase of the monolayer, $\Delta A$, due to OG insertion was recorded. For each surface pressure a new monolayer was prepared. In a typical experiment 200-$\mu$l aliquots of a 2 mM OG solution were injected into the monolayer subphase ($v \approx 20$ ml), and the area increase was followed over time until a stable equilibrium value was reached. Fig. 5 A shows a semilogarithmic plot of the relative area increase, $\Delta A/A$, as a function of the preset surface pressure. From the slope of the straight line, the insertion area of OG in the monolayer, $A_I$, can be determined as $A_I = 59 \pm 10$ Å$^2$ (Seelig at al., 1996). In Fig. 5 B the relative area increase $\Delta A/A$ is plotted as a function of the equilibrium concentration of octyl-β-D-glucopyranoside. The POPC monolayer was kept at a constant surface pressure of $\pi = 32.0$ mN/m, which can be considered as bilayer-monolayer equivalence pressure (Blume, 1979; Seelig, 1987). From the relative area increases $\Delta A/A$, the degree of binding, $X_b$, can be calculated according to

$$\frac{\Delta A}{A} = X_b \cdot \frac{A_I}{A_L}, \tag{4}$$

where $A_L$ is the surface area of the lipids constituting the monolayer (Seelig, 1987; Seelig and Macdonald, 1989). The surface area of POPC is known from monolayer experiments to be $A_L = 65$ Å$^2$ at 32 mN/m (Evans et al., 1987). $X_b$ denotes the molar amount of OG inserted into 1 mol of POPC monolayer at the preset pressure. Under the present experimental conditions the free equilibrium concentration of OG is equal to the total concentration, i.e., $c_{D,f} \approx c_{D,tot}$. If POPC penetration follows a partition equilibrium according to $X_b = K \cdot c_{D,f}$ (cf. below), Eq. 4 can be used to calculate the partition coefficient for OG partitioning into POPC monolayers at $\pi = 32$ mN/m, yielding $K = 320 \pm 40$ M$^{-1}$.

## DISCUSSION

### Thermodynamic analysis

Lipid vesicles are injected into a detergent solution, causing detergent molecules to partition into the bilayer membrane. If the detergent is applied at low enough concentrations ($c_D^0 < 15$ mM), membrane partitioning is the only process to be considered. Let us denote by $\delta n_i$ the molar amount of detergent bound in the ith injection step. The associated heat of reaction is

$$\delta h_i = \delta n_i \Delta H_D^\circ, \tag{5}$$

where $\delta h_i$ is defined by Eq. 1, and $\Delta H_D^\circ$ is the standard partitioning enthalpy. After i injections the total amount of bound detergent is

$$n_i = \sum_i \delta n_i, \tag{6}$$

FIGURE 4 $^2$H-NMR of POPC labeled at various segments of the hydrocarbon chains with and without octyl-$\beta$-D-glucopyranoside (OG). (A) $^2$H-NMR spectra of multilayered POPC dispersions without (a, c) and with (b, d) OG. The specifics are: (a) 1,2-Dielaidoyl-sn-glycero-3-phosphocholine deuterated at both *trans* double bonds (*trans*-9′-10′-D$_4$ DEPC) (pure lipid), $\Delta\nu_Q$ = 20.1 kHz. (b) *trans*-9′-10′-D$_4$-DEPC with OG, $X_b$ = 1.22, $\Delta\nu_Q$ = 12.4 kHz. (c) 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) deuterated at the C-12 of the oleic acyl chain (12′-D$_2$ POPC) (pure lipid), $\Delta\nu_Q$ = 12.6 kHz. (d) 12′-D$_2$ POPC with OG, $X_b$ = 1.02, $\Delta\nu_Q$ = 6.4 kHz. Deuterium frequency 61.4 MHz, 10,000 transients, $T$ = 28°C. (B) Variation of the quadrupole splittings, $\Delta\nu_Q$, of deuterated phospholipids with $X_b$, the amount of bound OG. ◇, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) deuterated at the C-5 of the palmitic acyl chain (5′-D$_2$ POPC). ○, *trans*-9′-10′-D$_4$-DEPC. □, POPC deuterated at C-12 of the oleic acyl chain (12′-D$_2$ POPC). △, ▽ 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine deuterated at the *cis* double bond (*cis*-9′-10′-D$_2$ POPC).
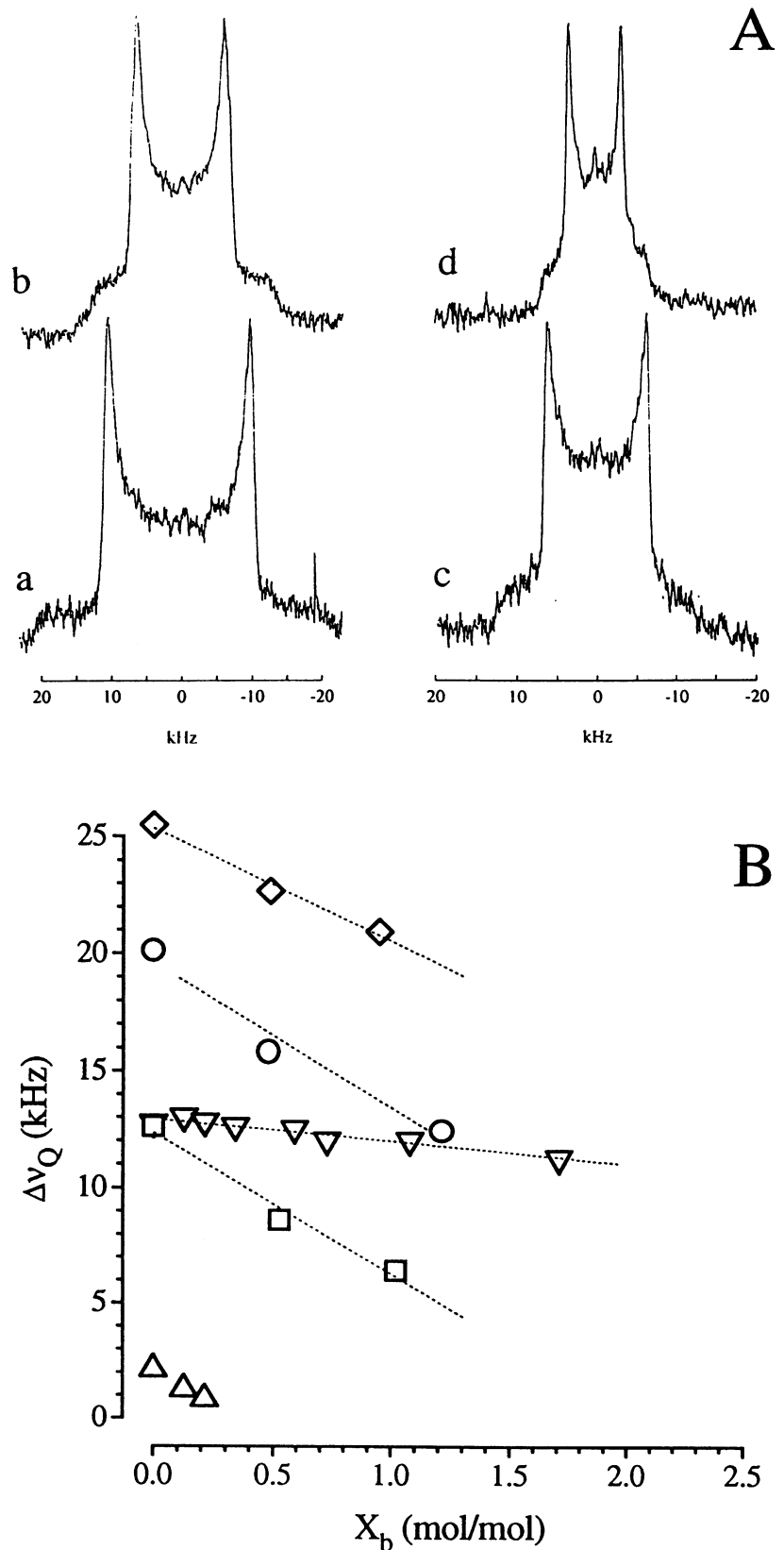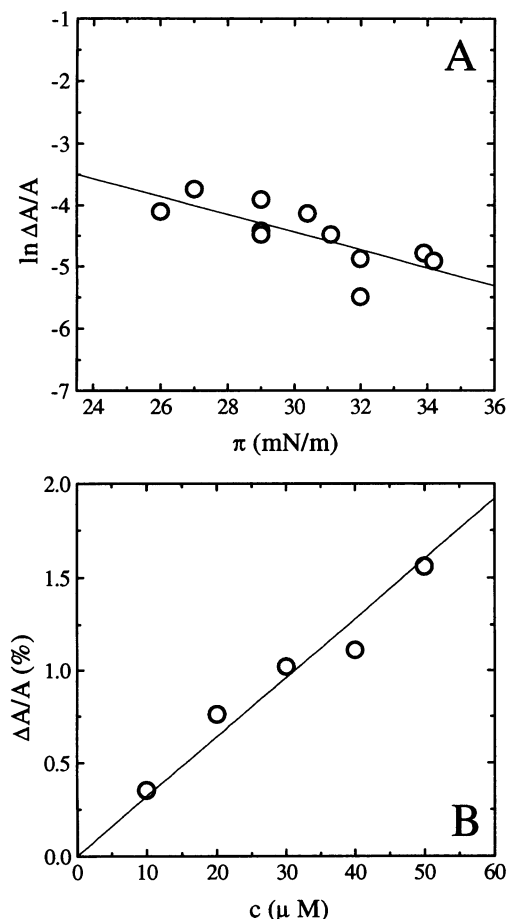
FIGURE 5 Monolayer studies with octyl-$\beta$-D-glucopyranoside. (A) Relative area increase (ln $\Delta A/A$) of a POPC monolayer due to insertion of octyl-$\beta$-D-glucopyranoside ($c = 20$ $\mu$M) as a function of surface pressure. (B) Relative area increase $\Delta A/A$ (in %) of a POPC monolayer as a function of the equilibrium concentration of octyl-$\beta$-D-glucopyranoside. The monolayer pressure is kept constant by an electronic feedback system at $\pi = 32$ mN·m$^{-1}$.

and the cumulative heat of reaction is

$$\delta H_i = \sum_i \delta h_i = n_i \Delta H_D^o. \qquad (7)$$

The reaction takes place in a calorimeter cell of defined volume $V_{cell}$. The concentration of bound detergent, $c_{D,b}$, is thus given by

$$c_{D,b} = n_i/V_{cell} = \sum_i \delta h_i/(\Delta H_D^o V_{cell}). \qquad (8)$$

$c_{D,b}$ can thus be evaluated experimentally from the cumulative heat of reaction if $\Delta H_D^o$ is known.

In principle, $\Delta H_D^o$ could be determined by injecting a small amount of detergent, $\delta n_D$, into an excess of lipid suspension. If the added detergent partitions completely (>95%) into the membrane, the measured heat of reaction would yield $\Delta H_D^o$ according to $\Delta H_D^o = \delta h/\delta n_D$. For the OG/POPC system this approach is not accurate enough, because the OG partition constant is rather small. Under the

present experimental conditions, only ~80% of the injected OG would partition into the membrane. However, a straightforward and precise evaluation of both $\Delta H_D^o$ and $K$ is possible by a complete simulation of lipid-into-detergent titration curves (Fig. 1).

The simplest model to describe the OG/POPC equilibrium is a partition model of the form

$$\frac{c_{D,b}}{c_L^o} = Kc_{D,f}. \qquad (9)$$

$c_L^o$ is the total lipid concentration, and $c_{D,b}$ and $c_{D,f}$ are the bound and free detergent concentrations, respectively. Because the total detergent concentration, $c_D^o$, is given by $c_D^o = c_{D,b} + c_{D,f}$, Eq. 9 can also be written as

$$c_{D,b} = c_D^o \frac{Kc_L^o}{1 + Kc_L^o}. \qquad (10)$$

As an alternative model, Schurtenberger et al. (1985) proposed the following relation:

$$\frac{c_{D,b}}{c_{D,b} + c_L^o} = K_S c_{D,f}. \qquad (11)$$

We have analyzed our measurements in terms of both models.

In the experiment shown in Fig. 1, the lipid concentration in the calorimeter cell changes in a stepwise fashion. Each lipid injection increases the lipid concentration by $\delta c_L$ (neglecting dilution effects). After $i$ injections the total lipid concentration is given by

$$c_L^o = i\delta c_L = iV_{inj}c_L^o/V_{cell}, \qquad (12)$$

where $V_{inj}$ is the volume of lipid suspension injected per step. Combining Eqs. 7, 8, 10, and 12 leads to

$$\delta H_i = \Delta H_D^o V_{cell}c_D^o \frac{iV_{inj}Kc_L^o}{1 + iV_{inj}Kc_L^o}. \qquad (13)$$

Next, Eq. 13 may be used to calculate $\delta h_i = \delta H_i - \delta H_{i-1}$. The experimentally measured cumulative heat, $\delta H_i$, and the individual titration steps, $\delta h_i$, are thus explained in terms of two parameters, the standard reaction enthalpy, $\Delta H_D^o$, and the partition constant $K$. A similar expression can be derived for the Schurtenberger model.

It should be noted that during the course of the titration the volume of the solution increases from an initial value $V_{cell}$ to $V_{cell} + iV_{inj}$ after $i$ injection steps. This leads to a dilution of the detergent and the lipid concentration. Although this effect is small ($V_{cell} = 1.3353$ ml; $V_{inj} = 10$ $\mu$l), it was included in the simulation.

Fig. 6 shows a comparison of the experimental data of Fig. 1 (open circles) and the theoretical simulation with $K = 115$ M$^{-1}$ and $\Delta H_D^o = 1.25$ kcal/mol (solid lines). Excellent agreement is obtained between theory and experiment for both $\delta h_i$ (Fig. 6 A) and the cumulative heat (Fig. 6 B). Simulations of corresponding quality were possible for al-
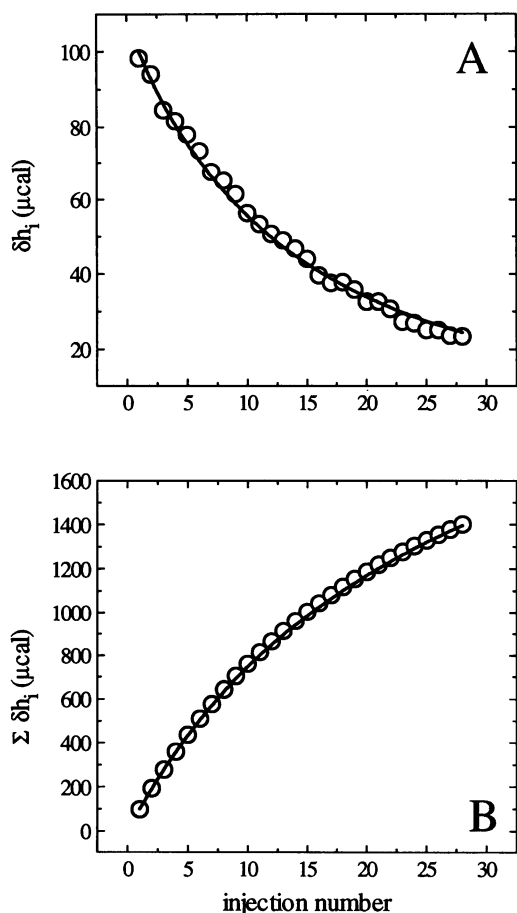
FIGURE 6 Simulation of the experimental data shown in Fig. 1 with the partition model using $K = 115$ M$^{-1}$ and $\Delta H_{D}^{o} = 1.25$ kcal/mol. The experimental data are shown as open circles. The solid line respresents the theoretical simulation. (A) Individual titration peaks. (B) Cumulative heat of reaction.

most all other experiments. The resulting parameters are summarized in Table 1.

From a single lipid-into-detergent titration, both $K$ and $\Delta H_{D}^{o}$ can be determined with high precision. This can be traced back to the fact that $K$ and $\Delta H_{D}^{o}$ influence different aspects of the titration curve. $K$ determines essentially the gradient between consecutive titration steps, e.g., large $K$ values produce a fast decrease in the titration peaks. In contrast, $\Delta H_{D}^{o}$ determines the height of the apparent plateau level of the cumulative heat of reaction. $K$ and $\Delta H_{D}^{o}$ are not strongly coupled, and no ambiguities were encountered in determining the two parameters. This was also confirmed in test runs with theoretical data sets.

## Rapid flip-flop or asymmetrical OG partitioning?

Inspection of Eq. 13 demonstrates that it is, in fact, the product $Kc_{L}^{o}$ that determines the steepness of the titration curve. Any pair $(c_{L}^{o}/a)$; $(aK)$ with $(ac_{L}^{o}) \cdot (K/a) = $ const. will yield the same good fit to the experimental results. The data summarized in Table 1 were calculated under the assump-

tion that the total phospholipid, $c_{L}^{o}$, was available for OG partitioning $(a = 1)$, implying a rapid lipid flip-flop between the vesicle outside and inside. It could be argued, however, that only the membrane outside is available for OG partitioning. Under these conditions the effective lipid concentration is $c_{out} = 0.6\ c_{L}^{o}$, and the asymmetrical binding constant will be found as $K_{out} = (K/0.6)$. The reaction enthalpy $H_{D}^{o}$ is not affected.

To allow a decision between these two modes of binding, Fig. 7 displays the variation of $c_{D,b}/c_{L}^{o}$ as a function of the free OG concentration, $c_{D,f}$ (cf. Eq. 7). The ratio $c_{D,b}/c_{L}^{o}$ is sometimes denoted as the degree of binding, $X_{b}$, or the effective surfactant/lipid ratio, $R_{e}$. The experimental data points (symbols) correspond to measurements with OG concentrations up to 10 mM. The solid line represents the theoretical fit with $K = 120$ M$^{-1}$. The figure demonstrates 1) that the partition model (Eq. 9) provides a highly accurate description of the experimental data over the whole concentration range and 2) that the maximum degree of binding at the highest OG concentration is $X_{b} \approx 1.1$, which is still below the ciritical limit of $X_{b} \approx 1.3$ for membrane disruption. In contrast, if OG would bind to the outer monolayer only, the partition constant would increase to $K_{out} = K/0.6$. The $X_{b}$ value at the highest OG concentration measured ($\sim 10$ mM) would reach $X_{b} \approx 1.83$, which would lead to bilayer disruption. This was not observed in the fluorescence quenching experiments, however. The calorimetric data hence suggest a rapid flip-flop of OG molecules within the time scale of the experiment.

## Enthalpy, $\Delta H_{D}^{o}$, and specific heat capacity, $\Delta C_{p}$, of octyl-$\beta$-D-glucopyranoside partitioning into lipid blayers

The present data are the first measurements of the reaction enthalpy, $\Delta H_{D}^{o}$, and of the specific heat capacity, $\Delta C_{p}$, of the
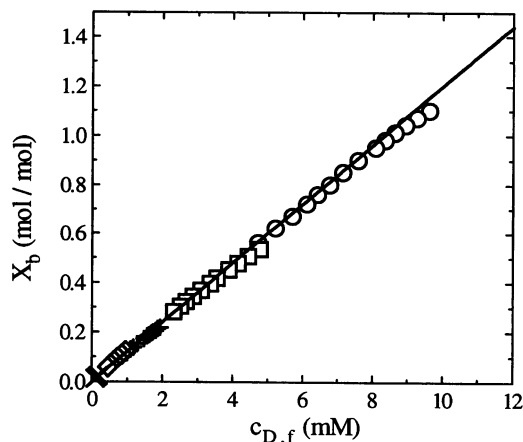


FIGURE 7 Binding of octyl-$\beta$-D-glucopyranoside to 30-nm unilamellar vesicles composed of POPC as derived by titration calorimetry. The degree of binding, $X_{b}$ (i.e., the molar amount of OG bound per mole of lipid) is plotted against the free detergent concentration in bulk solution $c_{D,f}$ $(T = 28°C)$. The straight line was calculated assuming a partition equilibrium, $X_{b} = K \cdot C_{D,f}$, and an average partition coefficient of $K = 120$ M$^{-1}$.

water $\rightleftarrows$ membrane partition equilibrium of octyl-$\beta$-D-glucopyranoside. The OG partitioning into POPC lipid vesicles is energetically unfavorable, because it entails an endothermic heat of reaction with $\Delta H_D^\circ = 1.3 \pm 0.15$ kcal/mol at 28°C. In addition, $\Delta H_D^\circ$ is strongly temperature-dependent and decreases linearly with increasing temperature. Above 41°C, $\Delta H_D^\circ$ even becomes negative (cf. Fig. 2). The specific heat capacity for the transfer of OG from water into the lipid membrane is negative, with $\Delta C_p = -75$ cal/(mol · K).

OG partitioning can be compared to the water-membrane equilibrium of free fatty acids. Richieri et al. have measured the partitioning of various long-chain fatty acids into 100-nm vesicles composed of egg yolk phosphatidylcholine (Richieri et al., 1995). From the temperature dependence of the partition coefficients, binding enthalpies in the range of $+1.1$ kcal/mol $\geq \Delta H^\circ \geq -0.4 \pm 0.8$ kcal/mol were derived. Analogous to the OG partitioning, no significant net enthalpy is involved in the water-to-membrane transfer of these fatty acids. Similar results were reported by Peitzsch and McLaughlin (1993, cf. footnote 2).

Reaction enthalpies close to zero and large negative heat capacities are typical for the transfer of hydrophobic molecules from the aqueous phase into an organic environment. For example, the transfer of hexane from water to the organic phase is characterized by $\Delta H^\circ \approx 0$ kcal mol$^{-1}$ (at 25°C) and $\Delta C_p = -105$ cal mol$^{-1}$ K$^{-1}$ (Privalov and Gill, 1989). The partitioning of OG and of the long-chain fatty acids into the lipid membrane are further examples of this conventional hydrophobic effect. However, titration calorimetry has also provided examples where hydrophobic molecules enter the membrane in an enthalpy-driven reaction, characterized by large negative $\Delta H^\circ$ values (Seelig and Ganz, 1991; Wenk et al., 1996).

## Equilibrium constant, $K$, and free energy, $\Delta G_D^\circ$, of OG partitioning into membranes

Fig. 7 summmarizes titration experiments at quite different OG concentrations. Nevertheless, all data can be fit by a single straight line through the origin with a slope of $K = 120$ M$^{-1}$. This means that the amount of detergent bound to the membrane varies linearly with the OG concentration in bulk solution. In the concentration range measured (0–10 mM OG) it suffices to describe OG binding with a simple partition equilibrium (Eq. 9), even under conditions where the amount of detergent in the membrane is high.

Schurtenberger et al. (1985) have proposed an interesting model (Eq. 11) in which the membrane-bound detergent is considered a constituent of the membrane matrix, facilitating further binding. The model degenerates into the partition model (Eq. 9) at low $X_b$ values. For $X_b > 0.1$ the model predicts a nonlinear relationship between $X_b$ and the bulk detergent concentration. The calorimetric data for OG partitioning into POPC vesicles are not in agreement with this model.

Different definitions of lipid-water partition coefficients are found in the literature and are summarized in a recent review (Lasch, 1995). $K$ as defined in the present work is identical to $K_L$ in the latter reference. Partition coefficients reported for OG fall in the range of 12 M$^{-1}$ to 75 M$^{-1}$, clustering around 60 M$^{-1}$ (Jackson et al., 1982; Ollivon et al., 1988; Almog et al., 1990; De la Maza and Parra, 1994). The present analysis yields a somewhat larger partition constant of $K = 120$ M$^{-1}$ (at 28°C) in the concentration range 0–10 mM OG. However, it should be realized that the experimental conditions such as ionic strength, chemical nature of the lipids employed, and the polydispersity of the lipid vesicle size could all influence the outcome of the equilibrium measurement. We have performed a titration of a 2 mM OG solution with sonified egg PC vesicles and obtained $K = 78$ M$^{-1}$, which is in good agreement with the above data. The titration curve could be described by the partition equilibrium (Eq. 9), and the reaction enthalpy was $\Delta H_D^\circ = 1.47$ kcal/mol.

Knowledge of $K$ allows the calculation of the free energy of binding, $\Delta G_D^\circ$, according to $\Delta G_D^\circ = -RT \ln(55.5K)$, where $R$ is the gas constant and $T$ is the absolute temperature. The factor 55.5 corrects for the cratic contribution (the water molar concentration) (Cantor and Schimmel, 1980). The free energy change upon OG binding to the membrane amounts to $\Delta G_D^\circ \approx -5.2$ kcal/mol. Knowledge of $\Delta G_D^\circ$ and $\Delta H_D^\circ$ allows the calculation of $T\Delta S_D^\circ$, yielding $T\Delta S_D^\circ \approx 6.5$ kcal/mol. The driving force for the binding reaction is therefore the large increase in entropy.

The cholesterol content of POPC membrane vesicles was varied between 0 and 50 mol% cholesterol. The reaction enthalpy, $\Delta H_D^\circ$, increased almost linearly from 1.3 kcal/mol at 0% cholesterol content to 2.25 kcal/mol at 50% cholesterol, whereas the partition constant decreased from 120 M$^{-1}$ to 90 M$^{-1}$ (cf. Table 1). The partition constant $K$ was evaluated under the assumption that only POPC was available for OG partitioning. If the cholesterol is also included in the calculation, the effective partition constant decreases according to $K_{chol} = K \cdot (1 - X_{chol})$, where $X_{chol}$ is the mole fraction of cholesterol. For 50% cholesterol $K_{chol}$ is 45 M$^{-1}$ instead of 90 M$^{-1}$. The present data suggest that OG molecules preferentially partition between POPC molecules.

## Area requirement of OG at the air/water interface and in the lipid monolayer

The surface area requirement of OG at the air/water inferface, $A_S$, was determined by means of the Gibbs adsorption isotherm as $A_S = 51 \pm 3$ Å$^2$, in excellent agreement with estimates from molecular models of octyl-$\beta$-D-glucopyranoside (cf. Fig. 7). $A_S$ corresponds essentially to the cross-sectional area of the sugar ring, because that of a hydrocarbon chain is only 20–25 Å (Boguslavsky et al., 1994). As a second parameter the insertion area of OG was derived from monolayer expansion experiments yielding $A_I = 58 \pm 10$ Å$^2$. The extent of penetration into the lipid monolayer can be estimated by comparing $A_I$ and $A_S$ (Boguslavsky et al., 1994; Seelig et al., 1996). Partial insertion is anticipated for

$A_I < A_S$. However, complete penetration can be assumed if $A_I \approx A_S$. Hence the area measurements provide the first evidence that OG penetrates into the lipid monolayer so that the sugar headgroup is fully integrated into the lipid headgroup region.

## Structure and statistical order of phospholipid-octyl-β-D-glucopyranoside bilayers

The uptake of detergent into a bilayer membrane leads to a disruption of the bilayer structure at sufficiently high detergent concentrations. A detailed understanding of the structural and dynamic properties of lipid-detergent bilayers and of intermediate aggregates, however, has not been achieved. It can be expected that the insertion of detergent molecules softens the packing constraints, producing changes in the average conformation of the phospholipids and in the extent of their statistical fluctuations. Deuterium NMR in combination with selectively deuterated lipids provides a convenient means of quantitatively analyzing this problem. In the present study we have used this method to study the influence of OG on both the hydrocarbon chains and the polar headgroup region. Related studies have been reported recently for mixed phosphatidylcholine-octaethyleneglycol mono-$n$-dodecylether ($C_{12}E_8$) membranes (Otten et al., 1995; Thurmond et al., 1994).

The perturbation of the hydrocarbon chains is summarized in Fig. 4. Increasing amounts of detergent lead to a decrease in the quadrupole splittings. Four segments, namely C-5 and C-12 of POPC and C-9 and C-10 of the *trans* double bond of DEPC, show a linear decrease in the quadrupole splitting with the amount of bound detergent. The interpretation of this phenomenon is straightforward. The deuterium quadrupole splittings reflect the statistical order, i.e., the fluctuations of the hydrocarbon chain segments. Even in the nonperturbed bilayer these fluctuations are larger in the inner hydrophobic region than near the lipid/water interface (Seelig and Seelig, 1974). The present data demonstrate that the addition of octyl-β-D-glucopyranoside increases the segmental fluctuations of the hydrocarbon chains. At a POPC/OG molar ratio of 1:1, the quadrupole splitting of the C-5 segment decreases by ~18%, that of the *trans*-C-9,10-DEPC by ~38%, and that of C-12 by ~50%. The thickness of the bilayer will hence decrease by roughly 15–20% if the statistical model of Schindler and Seelig is applied (Schindler and Seelig, 1975). For DMPC/$C_{12}E_8$ mixtures (2:1, mol:mol) a general decrease in the hydrocarbon chain ordering of about 20–30% was observed (Otten et al., 1995).

A unique situation is encountered for the *cis* double bond. The C-9 deuteron gives rise to a large quadrupole splitting of about $\Delta\nu_Q \approx 12.7$ kHz; that of the C-10 deuteron is much smaller, with $\Delta\nu_Q \approx 2.2$ kHz. Because the two deuterons are attached to the same rigid segment, the difference in the two quadrupole splittings cannot arise from differences in statistical disorder, but must be explained by the geometry

and the unique orientation of the *cis* double bond in the lipid bilayer. The C=C bond vector is tilted with respect to the bilayer normal $n$ such that the C-D bond vector of the C-10 deuteron is close to the magic angle and $\Delta\nu_Q$ is small; in contrast, C-9 is farther away from the magic angle and $\Delta\nu_Q$ is large (Seelig and Waespe-Sarcevic, 1978). Obviously, the addition of OG changes the tilt angle in such a way that the increase in statistical disorder at C-9 is at least partially compensated by a change in orientation. At C-10 the two effects enhance each other.

A completely different molecular picture is encountered at the lipid interface, comprising the phosphocholine headgroup, the glycerol backbone, and the beginning of the sn-2 hydrocarbon chain. The corresponding $^2H$-NMR data are summarized in Fig. 3. The $\alpha$-$CD_2$ group of the sn-2 oleic acid chains gives rise to two different quadrupole splittings for the individual deuterons, reflecting the rather rigid chain conformation at this segment position. Surprisingly, the addition of OG leaves both quadrupole splittings practically constant up to the highest concentration measured. Likewise, the addition of octyl-β-D-glucopyranoside has only a small effect on the choline headgroup, introducing a small reorientation toward the water phase.

The constancy of the headgroup region is in sharp contrast to the perturbation of the hydrocarbon chains. It is also different from earlier studies employing $C_{12}E_8$ as a detergent (Otten et al., 1995). Using selectively deuterated 1,2-dimyristoyl-sn-glycero-3-phosphocholine as a membrane matrix, the addition of $C_{12}E_8$ at a molar lipid/detergent ratio of 2:1 decreases the quadrupole splitting at the C-2 segment and at both choline segments by 20–60%. It can be concluded that $C_{12}E_8$ acts as a rather homogeneous detergent, disrupting the membrane at all levels to about the same
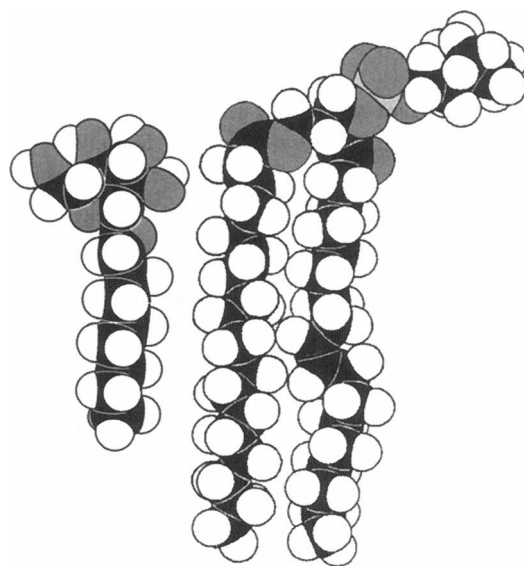


FIGURE 8    Diagram (to scale) of octyl-β-D-glucopyranosid (OG, *left*) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC, *right*), showing the approximate location of the two molecules in the membrane, as suggested by the monolayer and $^2H$-NMR measurements.

extent. In contrast, octyl-β-D-glucopyranoside acts specifically on the hydrocarbon region, leaving the headgroup region pratically unaltered up to the point of micellation.

In summary, the present results suggest an alignment of OG and POPC, as indicated schematically in Fig. 8. The sugar moiety penetrates approximately up to the level of the glycerol backbone, with the headgroup region tightly packed and the conformation of the $^-PN^+$ dipole almost unchanged. The large cross-sectional area of the OG headgroup, as well as the short C-8 chain of the detergent, produces packing defects in the central part of the membrane, leading to a distinct increase in the segmental fluctuation. Increasing the OG concentration increases only the hydrocarbon chain fluctuations but does not affect the polar headgroup.

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