# Interaction of Octyl-β-Thioglucopyranoside with Lipid Membranes

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ABSTRACT Octyl-β-thioglucopyranoside (octyl thioglucoside, OTG) is a nonionic surfactant used for the purification, reconstitution, and crystallization of membrane proteins. The thermodynamic properties of the OTG-membrane partition equilibrium are not known and have been investigated here with high-sensitivity titration calorimetry. The critical concentration for inducing the bilayer ⇄ micelle transition was determined as $c_D^* = 7.3$ mM by 90° light scattering. All thermodynamic studies were performed well below this limit. Sonified, unilamellar lipid vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with and without cholesterol were employed in the titration calorimetry experiments, and the temperature was varied between 28°C and 45°C. Depending on the surfactant concentration in the membrane, the partition enthalpy was found to be exothermic or endothermic, leading to unusual titration patterns. A quantitative interpretation of all titration curves was possible with the following model: 1) The partitioning of OTG into the membrane follows a simple partition law, i.e., $X_b = Kc_{D,f}$, where $X_b$ denotes the molar amount of detergent bound per mole of lipid and $c_{D,f}$ is the detergent concentration in bulk solution. 2) The partition enthalpy for the transfer of OTG from the aqueous phase to the membrane depends linearly on the mole fraction, $R$, of detergent in the membrane. All calorimetric OTG titration curves can be characterized quantitatively by using a composition-dependent partition enthalpy of the form $\Delta H_D(R) = -0.08 + 1.7\,R$ (kcal/mol) (at 28°C). At low OTG concentrations ($R \leq 0.05$) the reaction enthalpy is exothermic; it becomes distinctly endothermic as more and more surfactant is incorporated into the membrane. OTG has a partition constant of 240 $M^{-1}$ and is more hydrophobic than its oxygen-containing analog, octyl-β-D-glucopyranoside (OG). Including a third nonionic amphiphile, octa(ethyleneoxide) dodecylether ($C_{12}EO_8$), an empirical relation can be established between the Gibbs energies of membrane partitioning, $\Delta G_p$, and micelle formation, $\Delta G_{mic}$, with $\Delta G_p = 1.398 + 0.647\,\Delta G_{mic}$ (kcal/mol). The partition constant of OTG is practically independent of temperature and of the cholesterol content of the membrane. In contrast, the partition enthalpy shows a strong temperature dependence. The molar specific heat capacity of the transfer of OTG from the aqueous phase to the membrane is $\Delta C_p = -98$ cal/(mol·K). The OTG partition enthalpy is also dependent on the cholesterol content of the membrane. It increases by ~1 kcal/mol at 50 mol% cholesterol. As the partition constant remains unchanged, the increase in enthalpy is compensated for by a corresponding increase in entropy, presumably caused by a restructuring of the membrane hydration layer.

## INTRODUCTION

Octyl-β-thioglucopyranoside (octyl thioglucoside, OTG) is an efficient nonionic surfactant specifically designed for the solubilization of membrane proteins (Saito and Tsuchiya, 1984; Tsuchiya and Saito, 1984). It has been successfully used, for example, to purify $H^+$-ATPase from chloroplasts (Kirch and Gräber, 1992) or rat liver (Okamoto et al., 1996), to crystallize cytochrome *c* oxidase from *Thermus thermophilus* (Soulimane et al., 1995), or to develop transdermal systems for the percutaneous absorption of physiologically active peptides (Ogiso et al., 1994a,b). The thioether has a better chemical stability than its oxygen analog, octyl-β-D-glucopyranoside (octyl glucoside, OG), and is not degraded by β-D-glucosidase. The high stability of the thioether bond has induced the synthesis of thiomaltoside-containing surfactants (Izawa et al., 1993). The critical micellar concentration of OTG has been determined as ~9 mM (in water) (Saito and Tsuchiya, 1984; Brackman et al., 1988) compared to 20–25 mM for OG (Antonelli et al., 1994; Paula et al., 1995), suggesting that OTG is a more hydrophobic amphiphile than OG. Whereas the interaction of OG with lipid membranes has been studied in considerable detail (Jackson et al., 1982; Ollivon et al., 1988; Almog et al., 1990; De la Maza and Parra, 1994; Paternostre et al., 1995; Wenk et al., 1997; Wenk and Seelig, 1997; Keller et al., 1997), no thermodynamic data are available for OTG, i.e., neither the binding mechanism nor the association constant is known. OTG has no chromophore, and optical measurements are not suited to measuring the binding isotherm.

It has been demonstrated recently that high-sensitivity titration calorimetry opens a new avenue to the study of membrane-ligand equilibria. Early applications were directed toward the interaction of small amphiphilic molecules (Seelig and Ganz, 1991), drugs (Bäuerle and Seelig, 1991; Thomas and Seelig, 1993; Wenk et al., 1996), metal ions (Lehrmann and Seelig, 1994), and peptides (Beschiaschvili and Seelig, 1992; Seelig et al., 1993) with charged and noncharged lipid membranes. The thermodynamic analysis of peptide-membrane equilibria has been described in detail (Seelig, 1997). For a broad range of conditions and experimental systems, titration calorimetry allows a model-independent measurement of the ligand-to-membrane ad-

*Received for publication 3 April 1997 and in final form 13 August 1997.*
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0006-3495/97/11/2565/10 $2.00

sorption (binding) isotherm. The second step is then the analysis of the experimental data in terms of judicial models for the association process. If charged molecules are involved in the binding process, the application of the Gouy-Chapman theory becomes mandatory (Seelig, 1997; McLaughlin, 1977; McLaughlin, 1989).

The extension of high-sensitivity titration calorimetry to surfactant-membrane systems appears to be obvious. Nevertheless, only a very few studies have been performed. Heerklotz et al. have investigated the interaction of octa(ethyleneoxide) dodecylether ($C_{12}EO_8$) with membranes composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Heerklotz et al., 1996). The focus of their work was on the transition of lipid bilayers to micelles upon the addition of micelle-forming concentrations of surfactant. The partition equilibrium at low $C_{12}EO_8$ concentrations was examined only in a cursory manner. In contrast, the interaction of octyl-β-D-glucopyranoside (OG) with POPC membranes has been investigated systematically at subsolubilizing surfactant conditions by both titration calorimetry and structural methods (Wenk et al., 1997). The binding isotherm could be measured with sufficient precision to allow a decision between the various binding models proposed in the literature. Because of the close structural similarity of OG and OTG, it was expected that the same model would hold true also for OTG-membrane interactions. However, as will be demonstrated below, the experimental titration curves of the OTG-lipid bilayer system were surprisingly more complex than those of the OG-POPC system. In particular, an otherwise smooth titration curve could change from endothermic to exothermic without any evidence of an accompanying phase change. Although an explanation of these phenomena is of general physical-chemical interest, it has specific relevance for membrane-protein studies. Similar titration curves have been observed for small fusogenic peptides at low peptide concentrations (M. Wenk and J. Seelig, unpublished work), and their understanding could shed light on the initial step of peptide-induced membrane fusion. The present work thus has two purposes: 1) the thermodynamic characterization of the OTG-lipid membrane system and 2) the quantitative analysis of an unusual set of calorimetric titration curves that are of general significance for membrane binding equilibria.

## MATERIALS AND METHODS

### Materials

Octyl-β-D-glucopyranoside (OG) and octyl-β-thioglucopyranoside (OTG) were purchased from Fluka (purity > 99% TLC). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) dissolved in chloroform was from Avanti Polar Lipids (Birmingham, AL). Cholesterol was from Sigma. All chemicals were used without further purification. Buffers were prepared from 18 MΩ water obtained from a NANOpure A filtration system.

### Liposome preparation

Small unilamellar vesicles (SUVs) were prepared as follows. A defined amount of lipid (~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. A 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.

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 vesicles. The phosphate analysis (Böttcher et al., 1961) yielded a 4.7% (average of nine determinations) smaller lipid content than the nominal concentration, with maximum deviations between −2% and +10%. The data reported for sonified POPC vesicles were calculated on the basis of the weighing-in concentration.

If not otherwise stated, the buffer composition was 10 mM Tris, 100 mM NaCl, pH 7.25. 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 with 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.

### Right-angle light scattering

Light-scattering measurements were made with a Jasco FP 777 spectrofluorometer (Japan Spectroscopic Co., Tokyo, Japan), with both excitation and emission wavelengths set at 350-nm, 3-nm slit widths. The sample ($V$ = 1.5 ml detergent solution) was continuously stirred and thermostatted ($T$ = 28°C). Vesicles ($c_L \approx$ 30 mM lipid concentration) were added (10 μl) with a Hamilton microliter syringe. The scattering intensity at 90° was recorded as a function of time. As a reference, the same experiment was repeated, using 1.5 ml buffer without detergent.

## RESULTS

### Light scattering

Light-scattering experiments were performed to establish the phase boundaries of the OTG-POPC system, following the protocol described for the OG-phopsholipid system (Jackson et al., 1982; Ollivon et al., 1988; Almog et al., 1990; De la Maza and Parra, 1994; Paternostre et al., 1995). Addition of phospholipid vesicles to a buffer solution leads to light scattering. The scattering intensity observed at 90° with respect to the incoming beam increases linearly with the lipid content. Fig. 1 summarizes titration experiments with sonified phospholipid vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). Lipid titrations into buffer without OTG or into buffer with rather low OTG concentrations (400 μM) produce almost identical

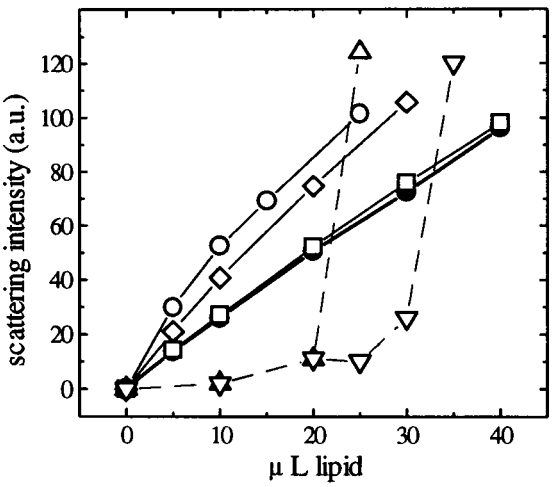


FIGURE 1 90° light scattering intensity as a function of phospholipid concentration in OTG solutions. Sonified POPC vesicles were titrated into OTG solutions of different concentrations, $c_D^\circ$. ●, No detergent; □, $c_D^\circ$ = 400 μM OTG; ◇, 5.24 mM; ○, 6.9 mM; △, 9 mM; ▽, 10 mM. Temperature, 28°C; sonified unilamellar POPC vesicles (30 nm); buffer 10 mM Tris, 100 mM NaCl, pH 7.25.

scattering curves. Raising the OTG concentration to 5.24 mM or 6.9 mM leads to a small increase in the scattering intensity, which still grows linearly, however, with the lipid concentration. The larger scattering intensity can be explained by an uptake of OTG into the lipid membrane, increasing the size of the lipid vesicles without disrupting their structure. A completely different behavior is encountered for titration experiments employing OTG concentrations of 9 or 10 mM OTG. For $c_D^\circ$ = 9 mM, the first two lipid injections produce almost no light scattering; however, the light-scattering intensity increases dramatically with the third injection. The critical lipid concentration is $c_L^* \approx 0.46$ mM. For $c_D^\circ$ = 10 mM, the light-scattering intensity remains low for the first four injections and then rises rapidly. In the latter two experiments, the initial detergent concentration is high enough to cause a complete disruption and micellization of the phospholipid vesicles. The light-scattering intensity of the micellar solution is low. As more and more lipid is added, the free OTG concentration falls below a critical limit and is no longer sufficient for micellization. The addition of further phospholipid vesicles then produces vesicle swelling but no longer produces vesicle disruption. Consequently, the light-scattering intensity shows a sudden increase, which defines the phase boundary between the micellar phase and the micelle/bilayer coexistence phase. A plot of $c_D^\circ$ versus $c_L^*$ for a number of measurements yields $c_D^\circ \approx 7.3 + 3.8c_L^*$ (mM). Of particular interest is the limiting detergent concentration $c_D^\circ$ = 7.3 mM at $c_L^*$ = 0. For thermodynamic reasons, the second phase boundary of the system, i.e., that between the pure bilayer phase and the micelle/bilayer coexistence phase, must have the same limiting value. It thus follows that stable POPC/OTG bilayers exist only up to an OTG concentration of $c_D^\circ \approx 7.3$ mM. This was confirmed with phosphorus-31 NMR (data not shown; cf. Wenk et al., 1997). All calorimetric titrations discussed in the following were performed below this limiting concentration.

## High-sensitivity titration calorimetry

The reaction enthalpy of OTG partitioning into membrane vesicles was measured with isothermal titration calorimetry. In a first type of experiment, the calorimeter cell contained sonified POPC vesicles, and small aliquots of a dilute OTG solution were injected. As a control, a similar experiment was performed with a dilute OG solution, because the thermodynamic properties of this surfactant were known. Fig. 2 summarizes experiments with OG and OTG solutions at different temperatures. In Fig. 2 *A* a 5 mM OG solution is injected into a suspensions of POPC vesicles ($V_{inj}$ = 20 μl). The reaction is endothermic and the heat of reaction is $h_i \approx 102$ μcal/inj, as derived from an integration of the titration peaks. In a control experiment, detergent was injected into buffer without lipid, leading to an endothermic heat of $h_c$ = 8.5 μcal/inj. From the difference between the two measurements, the apparent heat of OG partitioning into POPC membranes is derived as $\Delta H_{app}$ = +0.94 kcal/mol. $H_{app}$ is calculated with the assumption that all injected OG is bound to the lipid vesicles. This is, in fact, an oversimplification. A more detailed analysis reveals that only ~75% OG is membrane bound (Wenk et al., 1997). The true partition enthalpy of OG is $\Delta H_D^\circ$ = 1.3 kcal/mol. If the same experiment is repeated with the thioether analog, much smaller titration peaks are observed, with $h_i \approx 6$ μcal. After substraction of the control value of $h_c \approx 9$ μcal, the reaction is even slightly exothermic, with $\delta h_i = h_i - h_c \approx -3$ μcal. The exothermic reaction enthalpy of OTG becomes more pronounced at higher temperatures, as demonstrated in Fig. 2, *C* and *D*, with $\delta h_i = -11.7$ and $-27.3$ μcal/inj, respectively. The apparent partition enthalpies of OTG are determined as $\Delta H_{app} \approx -0.06$ kcal/mol (−0.33 kcal/mol, −0.76 kcal/mol) at 28°C (35°C, 45°C). They constitute a minimum value for the true partition enthalpy, $|\Delta H_D^\circ|$.

In a second type of experiment, the detergent solution was contained in the calorimeter cell, and sonified lipid vesicles were injected. Fig. 3 displays four experiments at various OTG concentrations below the critical concentration, $c_D^*$, for membrane disruption. The heat of reaction is essentially endothermic in all four experiments. However, distinct exothermic titration peaks are discernible toward the end of the 2.66 mM and 5.41 mM titrations. The size of the individual titration peaks, $h_i$, decreases with consecutive injections as less and less detergent is available for binding to phospholipid vesicles. As a control, sonified phospholipid vesicles were injected into buffer without OTG. The heat of injection, $h_c$, is again small and constant, but must be included in the evaluation. The cumulative reaction en-

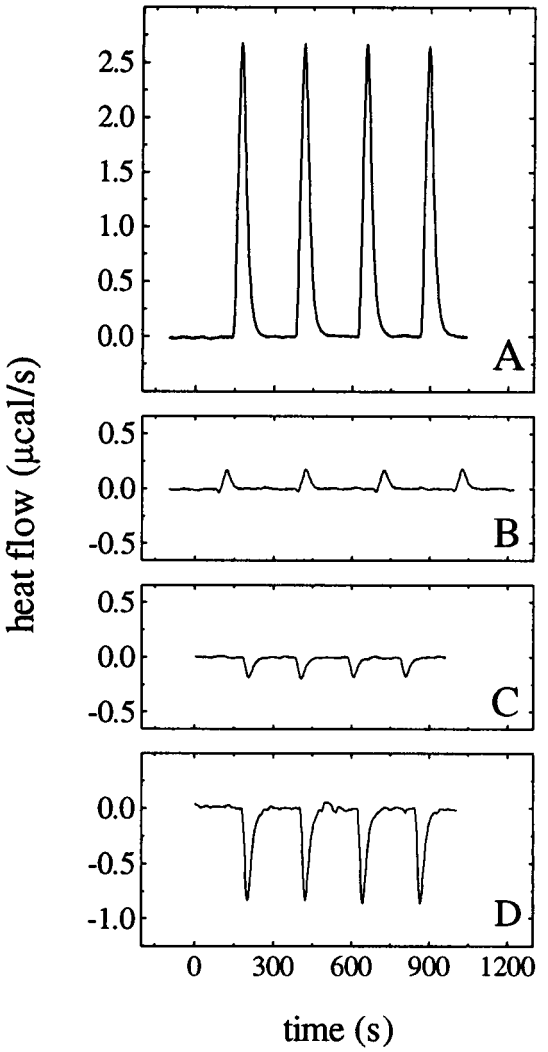


FIGURE 2 Surfactant-into-lipid titration. The calorimeter cell ($V_{cell}$ = 1.3353 ml) contained sonified unilamellar POPC vesicles (30 nm diameter), which were titrated with either OG (*A*) or OTG (*B–D*) solutions (in buffer). The temperature was 28°C for experiments *A* and *B*, 35°C for *C*, and 45°C for *D*, respectively. Note the different scales for the heat flow. (*A*) OG stock solution of concentration $c_D^\circ$ = 5 mM; injection volume $V_{inj}$ = 20 μl; lipid concentration in calorimeter cell $c_L^\circ$ = 20.0 mM; buffer 10 mM Tris, 100 mM NaCl, pH 7.25; temperature 28°C. (*B*) $c_D^\circ$ = 2.37 mM; $c_L^\circ$ = 24.0 mM. (*C*) $c_D$ = 1.8 mM; $c_L^\circ$ = 17.5 mM. Temperature 35°C. (*D*) $c_D^\circ$ = 1.8 mM, $c_L^\circ$ = 15.0 mM. Temperature 45°C.

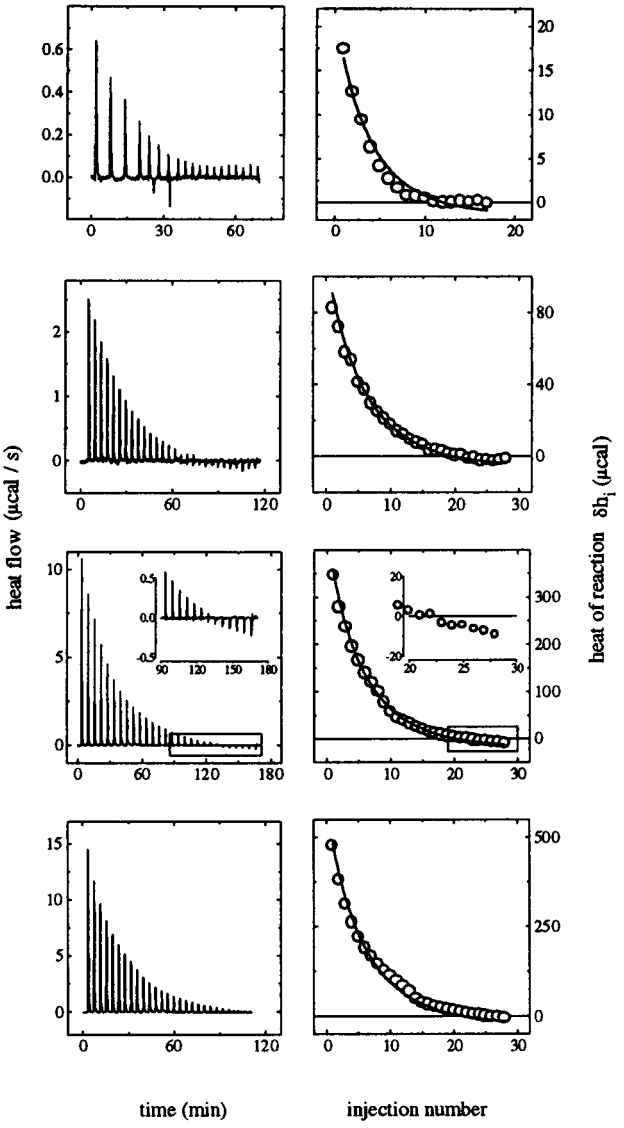


FIGURE 3 Lipid-into-surfactant titration. The calorimeter cell ($V_{cell}$ = 1.3353 ml) contained OTG solutions (in buffer). Ten-microliter aliquots of a phospholipid stock solution (in buffer) were injected. Buffer composition as in Fig. 2, 28°C. The specific measuring conditions were (from top to bottom):
$c_D^\circ$ = 0.86 mM OTG, $c_L^\circ$ = 35.2 mM POPC
$c_D^\circ$ = 2.66 mM OTG, $c_L^\circ$ = 26.7 mM POPC
$c_D^\circ$ = 5.41 mM OTG, $c_L^\circ$ = 34.4 mM POPC
$c_D^\circ$ = 6.9 mM OTG, $c_L^\circ$ = 35 mM POPC
The panels on the left side show the original calorimeter traces. The panels on the right side contain the experimental heats of reaction (○). The solid lines represents the simulation of the titration curves under the initial conditions described above. The partition constant was $K$ = 240 $M^{-1}$, and the partition enthalpy $\Delta H_D(R) = -0.08 + 1.7\,R$ (kcal/mol) in all simulations.

thalpy, $\delta H_i$, is then given by

$$\delta H_i = \sum_i (h_i - h_c) = \sum_i \delta h_i \qquad (1)$$

The molar amount of detergent, $n_D^\circ$, in the calorimeter cell is constant and is given by

$$n_D^\circ = c_D^\circ V_0 \qquad (2)$$

where $c_D^\circ$ is the total detergent concentration and $V_0$ is the volume of the calorimeter cell. Fig. 3 suggests that all detergent might be bound to the added lipid vesicles after 10–20 lipid injections. For each titration experiment it is thus possible to calculate an apparent reaction enthalpy $\Delta H_{app} = \delta H_N / n_D^\circ$, where $N$ denotes the total number of injections. Evaluation of Fig. 3 shows that $\Delta H_{app}$ is not constant, but increases almost linearly with the OTG concentration from 0.05 kcal/mol at $c_D^\circ$ = 0.86 mM to 0.31 kcal/mol at 6.9 mM. Taken together, Figs. 2 and 3 then

provide experimental evidence for a variation of the OTG partition enthalpy with the membrane composition. Most conspicously, even the sign of the partition enthalpy changes with the experimental conditions.

The latter aspect was investigated in more detail by extending the titrations shown in Fig. 3 to even higher lipid/detergent ratios (Fig. 4 *A*). The calorimeter cell contained both POPC vesicles ($c_L^\circ$ = 3.6 mM) and OTG ($c_D^\circ$ = 6.0 mM), corresponding approximately to the situation of the 6.9 mM OTG titration (Fig. 3) in the last third of the experiment. Injection of phospholipid vesicles ($c_L^\circ$ = 30.1 mM) produced first an exothermic and then an endothermic heat of reaction, as shown in Fig. 4 *A*. Again, another pattern is observed in Fig. 4 *B*. The reaction enthalpy is negative, becomes even more negative during the first injection steps, and then decreases slowly in magnitude. At the start of the experiment the lipid concentration in the calorimeter cell was $c_L^\circ$ = 5.6 mM and that of the OTG detergent $c_D^\circ$ = 4.6 mM. The lipid/surfactant ratio, $c_L^\circ/c_D^\circ$, was thus 1.2 at the beginning of the titration and increased to ~4 after ~30 injections of 10-μl aliquots of a 55 mM phospholipid suspension.

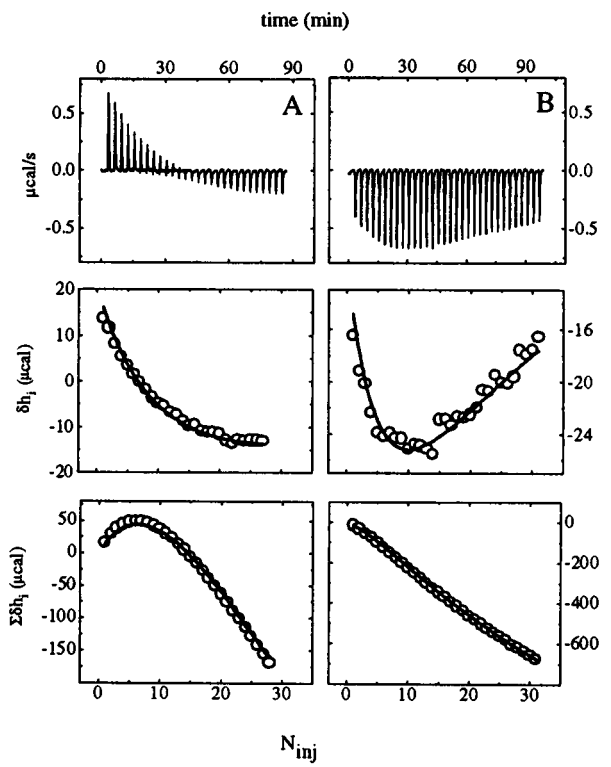


FIGURE 4 Lipid-into-surfactant titration. (*A*) The calorimeter cell contained both POPC vesicles ($c_L^\circ$ = 3.6 mM) and OTG solution ($c_D^\circ$ = 6.0 mM). Phospholipid vesicles from a stock solution ($c_{Lipid}$ = 30.1 mM) were injected in 10-μl aliquots. $K$ = 205 $M^{-1}$; $\Delta H_D(R) = -0.08 + 1.4\,R$ (kcal/mol). (*B*) The calorimeter cell contained POPC vesicles ($c_L^\circ$ = 5.6 mM) and OTG ($c_D^\circ$ = 4.56 mM). Phospholipid vesicles ($c_{Lipid}$ = 55.1 mM) were injected in 10-ml aliquots. $K$ = 240 $M^{-1}$; $\Delta H_D(R) = -0.08 + 1.7\,R$ (kcal/mol). *Top panels*: Original traces. *Middle panels*: Heat of titration (corrected). *Bottom panels*: Cumulative heat of reaction. Open symbols are experimental results. The solid line represents the theoretical analysis, calculated essentially according to Eq. 7 (cf. also Wenk and Seelig, 1997).

As discussed in more detail below, ~1.5 moles OTG per mole phospholipid can be accommodated in a lipid bilayer before membrane disruption occurs. At low surfactant concentrations, the OTG molecule hence behaves much like an integral building block of the lipid bilayer. However, most biological membranes contain not only phospholipids but also cholesterol. Because of its rigid steroid frame, cholesterol decreases the flexing motions of the hydrocarbon chains, increasing the packing density of the hydrophobic region. At the same time, cholesterol also increases the spacing between the lipid headgroups (Yeagle, 1985; Sankaram and Thompson, 1990). The question then arises whether surfactant partitioning into a lipid membrane is modified by the presence of cholesterol. We have therefore prepared lipid vesicles containing cholesterol at molar concentrations of 5–50% and have injected them into OTG solutions (0.5–5.41 mM OTG). All titration curves were characterized by endothermic heats of reaction, and no change in sign was observed. However, the reaction enthalpy varied with both the cholesterol content and the amount of OTG in the membrane. The quantitative evaluation of all titration curves is summarized in Table 1 and will be discussed below.

## DISCUSSION

The light-scattering data provide evidence that the minimum OTG concentration needed to induce the bilayer/micelle transition is $c_D^* \approx 7.3$ mM, which is well below the critical micellar concentration of a pure OTG solution (~9 mM) (Saito and Tsuchiya, 1984; Brackman et al., 1988). All calorimetric titrations presented here were performed below this critical limit. The OTG/POPC system remained in the bilayer phase throughout the whole titration experiment, as was confirmed with $^{31}P$ NMR. From a thermodynamic point of view, the most conspicuous results were the change in the sign of the reaction enthalpy and the unusual shape of some of the titration curves. In particular, the titration curves shown in Fig. 4 could easily be mistaken for a superposition of two competing processes. In the following we shall demonstrate that the partitioning of OTG into lipid membrane follows the same partition law as deduced for its oxygen-containing analog OG (Wenk et al., 1997). However, OTG partitioning is characterized by an unusual behavior of the partition enthalpy, which varies with the mole fraction of detergent in the membrane.

### Thermodynamic model

The concentration of a surfactant in a membrane can be defined according to

$$X_b = \frac{n_{D,b}}{n_L^\circ} = \frac{c_{D,b}}{c_L^\circ} \qquad (3)$$

**TABLE 1 Partitioning of octyl-β-thioglucopyranoside into lipid membranes**

| Lipid (mol:mol) | Temperature (°C) | K* ($M^{-1}$) | $\Delta H_o$ (kcal/mol) | $\Delta H_i$ (kcal/mol) | $C_D^\circ$ (mM) | No. of measurements |
|---|---|---|---|---|---|---|
| POPC | 28 | 240 ± 25 | −0.08 ± 0.02 | 1.7 ± 0.2 | 0.9–6.9 | 5 |
| | 35 | 240 | −0.55 | 0.98 | 5.41 | 1 |
| | 40 | 240 | −1.2 | 0.8 | 5.41 | 1 |
| | 45 | 225 ± 10 | −1.7 ± 0.2 | 0 | 2; 5.41 | 2 |
| POPC:cholesterol | 28 | 240# | 0.1 | 1.6 | 5.41 | 1 |
| (95:5) | | | | | | |
| (90:10) | | 240# | 0.35 | 1.0 | 5.41 | 1 |
| (85:15) | | 240# | 0.31 | 1.4 | 2.71 | 1 |
| (80:20) | | 240 ± 20# | 0.50 ± 0.1 | 0.8 ± 0.4 | 0.5–5.41 | 3 |
| (75:25) | | 260# | 1.0 | 0 | 5.41 | 1 |
| (70:30) | | 250 ± 20# | 1.0 ± 0.1 | 0 ± 0.1 | 0.5–5.41 | 3 |
| (65:35) | | 240 ± 20# | 1.0 ± 0.1 | 0 ± 0.1 | 0.5–5.41 | 3 |
| (60:40) | | 270# | 1.2 | 0 | 5.41 | 1 |
| (55:45) | | 250 | 1.15 | 0 | 5.41 | 1 |

*The binding constants are calculated with the assumption that all phospholipid is available for binding (rapid flip-flop of OTG between vesicle inside and outside).

#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.

$X_b$ is the degree of binding, also called effective surfactant-to-lipid ratio. $n_{D,b}$ is the molar amount of bound detergent, and $n_L^\circ$ is the total lipid. $c_{D,b}$ and $c_L^\circ$ are the corresponding concentrations. The free (equilibrium) concentration of surfactant in the aqueous phase is $c_{D,f}$. Different models have been proposed to describe the distribution of a surfactant between a membrane and the aqueous phase (Lasch, 1995). The simplest model is that of a partition equilibrium:

$$X_b = K\, c_{D,f} \tag{4}$$

A modification is the model of Schurtenberger et al. (1985):

$$\frac{n_{D,b}}{n_{D,b} + n_L^\circ} = \frac{X_b}{1 + X_b} = K_s c_{D,f} \tag{5}$$

which for a given $K$ predicts a stronger binding than the partition model (Eq. 4). Both models allow an unlimited swelling of the membrane with detergent. We have also investigated a Langmuir model with a limited number of surfactant-binding sites. The latter could not provide a satisfactory simulation of the titration curves and was immediately discarded.

Titration calorimetry can measure the surfactant binding isotherm in a model-independent manner. The experimental parameters are the titration enthalpies, $\delta h_i$, and the cumulative titration enthalpy, $\delta H_i$, as defined by Eq. 1. The concentration of membrane-bound surfactant after $i$ injections is (Wenk et al., 1997; Wenk and Seelig, 1997)

$$c_{D,b}^{(i)} = \delta H_i/(\Delta H_D^\circ V) \tag{6}$$

where $\Delta H_D^\circ$ is the molar reaction enthalpy for the transfer of detergent into the lipid phase. $V$ is the total volume of the solution. Equation 6 can then be combined with different binding models. In the case of the partition model (Eq. 4), this leads to the final expression:

$$\delta H_i = \Delta H_D^\circ c_D^\circ V_0 V_{inj} \frac{iKc_L^\circ}{V_0 + iV_{inj}(1 + Kc_L^\circ)} \tag{7}$$

$c_D^\circ$ and $c_L^\circ$ are the total surfactant and lipid concentration, respectively. In deriving Eq. 7, dilution effects were included. The initial volume $V_0$ of the reaction solution will increase with each injection by $V_{inj}$, and $V$ must be replaced by

$$V \rightarrow V_0 + iV_{inj} \tag{8}$$

This leads to dilution effects for both the lipid and the sufactant concentration, requiring the further substitutions

$$c_D^\circ \rightarrow c_D^\circ V_0/(V_0 + iV_{inj}) \tag{9}$$

$$c_L^{(i)} \rightarrow iV_{inj}c_L^\circ/(V_0 + iV_{inj}) \tag{10}$$

The two free parameters of Eq. 7 are the partition enthalpy, $\Delta H_D^\circ$, and the partition constant, $K$. $\Delta H_D^\circ$ and $K$ influence different aspects of the titration curve: $K$ (or, more precisely, $Kc_L^\circ$) determines essentially the steepness of the titration curve, whereas $\Delta H_D^\circ$ influences the height of the titration peaks. This allows an unambiguous evaluation of $K$ and $\Delta H_D^\circ$ under most experimental conditions. A corresponding analysis was developed for the Schurtenberger model, again including dilution effects.

## Composition-dependent partition enthalpy

The unusual feature of some of the titration curves shown in Figs. 3 and 4 is the transition from an endothermic to an exothermic reaction (at 28°C). As derived above, neither the partition model nor the Schurtenberger model predicts a change in the sign of the partition enthalpy. For a given $\Delta H_D^\circ$, the measured $\delta h_i$ or $\delta H_i$ can only be exothermic or

endothermic over the whole titration range. Because the membrane remains in the bilayer phase and structural changes can thus be excluded, the origin of this effect must be traced back to a variation in $\Delta H^\circ_D$ with the membrane composition. This is in contrast to the OG-POPC system, where $\Delta H^\circ_D$ is constant up to $X_b \approx 1.4$. Inspection of the experimental results suggests two extreme values for $\Delta H^\circ_D$. At low OTG concentrations the partition enthalpy is negative, with $\Delta H^\circ_D \approx -0.08$ kcal/mol; at large OTG concentrations $\Delta H^\circ_D$ is positive and approaches ~1.0 kcal/mol (at 28°C). The latter value is close to the enthalpy of OTG micelle formation, $\Delta H_{mic} = 1.1$ kcal/mol (Brackman et al., 1988). As a first approximation, we therefore suggest that the reaction enthalpy varies linearly with the mole fraction, $R$, of OTG in the lipid membrane. We introduce a composition-dependent enthalpy $\Delta H_D(R)$ according to

$$\Delta H_D(R) = \Delta H_0 + R\Delta H_1 \tag{11}$$

where $R$ is defined as

$$R = \frac{n_{D,b}}{n_{D,b} + n^\circ_L} = \frac{c_{D,b}}{c_{D,b} + c_L} \tag{12}$$

$R$ must be calculated for each injection step. Equation 11 is the key for the understanding of the present titration curves.

Fig. 3 shows a comparison between experimental and theoretical titration curves calculated with the above model (i.e., Eqs. 7–12; cf. also Wenk and Seelig, 1997; Wenk et al., 1997). All theoretical curves (*solid lines*) were calculated with the same set of parameters, namely $K = 240\ M^{-1}$ and $\Delta H(R) = -0.08 + 1.7\ R$ (kcal/mol). In these simulations the $R$ parameter covers a range of $0 < R \leq 0.6$, and the effective partition enthalpy, $\Delta H_D(R)$, varies accordingly between −0.08 kcal/mol and +1.0 kcal/mol.

The same set of parameters also explains the apparently more complex titration curves of Fig. 4. The unusual shape of the titration curves is determined not by a change in the thermodynamic properties, but by the different initial conditions for $c^\circ_L$ and $c^\circ_D$. The simulation shows the differential heats $\delta h_i$ as well as the cumulative heat of reaction $\delta H_i$. Excellent agreement between theory and experiment is obtained in all cases.

About 30 lipid-into-detergent titrations were analyzed, and in all cases an unambiguous evaluation of the three parameters $K$, $\Delta H_o$, and $\Delta H_1$ was possible. They are summarized in the binding isotherm, $X_b = Kc_{D,f}$ (Fig. 5) and in Table 1. The degree of binding increases up to $X_b \approx 1.5$ before membrane disruption occurs. This is close to the limit of $X_b \approx 1.3$–1.4 for the OG/POPC system. Based on the number of hydrocarbon chains, the POPC membrane can accommodate ~75% foreign octyl chains before disintegration. The solid line in Fig. 5 represents the theoretical binding isotherm calculated with $K = 240\ M^{-1}$.

We have also performed corresponding simulations with the Schurtenberger model (Eq. 5). Reasonable fits were obtained for some but not all of the titration curves. The parameter set used for the simulations was not consistent. It can be concluded that Eq. 5 does not provide a good description of OTG partitioning into POPC membranes.

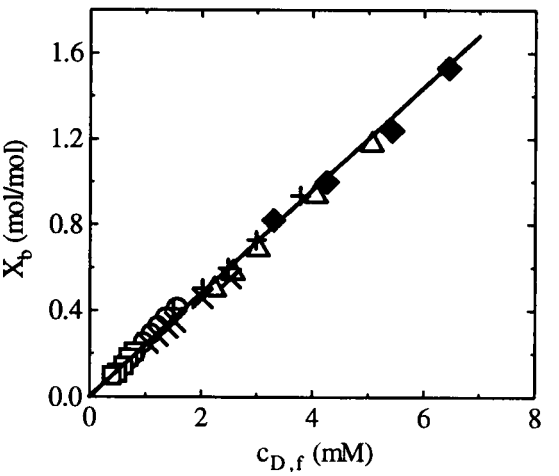


FIGURE 5 Binding isotherm. The degree of binding, $X_b$, is plotted against the concentration of free OTG. The symbols refer to experimental results obtained with titration calorimetry at different OTG concentrations. The solid line is the theoretical binding isotherm $X_b = Kc_{D,f}$ calculated with $K = 240\ M^{-1}$.

## Rapid flip-flop

All simulations were based on the assumption that the surfactant partitions sufficiently fast into both half-layers of the lipid vesicles. It could be argued that OTG binding is asymmetrical, involving the outer monolayer only (60% of total lipid for small sonified POPC vesicles). Inspection of Eq. 7 reveals that the shape of the titration curves is essentially determined by the product $Kc^\circ_L$. If $Kc^\circ_L$ provides a good fit to the data, $(K/a) \cdot (ac^\circ_L)$ will be equally well suited. Indeed, simulations show that asymmetrical binding requires a binding constant $K_{asym} = 400\ M^{-1}$. Based on the quality of the simulations alone, no choice can be made between the two alternatives. However, the larger binding constant entails larger $X_b$ values. Asymmetrical binding predicts $X_b$ values of ~2.5 before the membrane is disrupted, i.e., the outer monolayer would accommodate more than its own number of hydrocarbon chains. This appears to be unlikely, and not only for steric reasons. After all, OTG acts as a detergent, and membrane micellation must involve both monolayers of the vesicle. It can be concluded that OTG undergoes a flip-flop between the two half-layers, which is rapid on the time scale of the titration experiment.

## Membrane partitioning versus micelle formation

The Gibbs energy of membrane partitioning is $\Delta G_p = -RT \ln K$ (neglecting cratic contributions), and that of micelle formation is $\Delta G_{mic} = +RT \ln c_{CMC}$. The partition constants of the three nonionic surfactants OG, OTG, and $C_{12}EO_8$ for POPC membranes have been determined to be $120\ M^{-1}$ (Wenk et al., 1997), $240\ M^{-1}$ (this work), and $3900\ M^{-1}$

(Heerklotz et al., 1996). (The partition coefficient of $C_{12}EO_8$ as reported by the latter authors refers to a different binding model and is a dimensionless quantity, $p = 2.1 \times 10^5$. It can be related approximately to the partition constant $K$ according to $K \approx P/55.5$.) The critical micellar concentrations of the three detergents are ~23 mM (OG; Antonelli et al., 1994; Paula et al., 1995), 9 mM (OTG; Saito and Tsuchiya, 1984; Brackman et al., 1988), and 0.11 mM ($C_{12}EO_8$; Olofsson, 1985; Heerklotz et al., 1996). A plot of $\Delta G_p$ versus $\Delta G_{mic}$ yields a linear relationship between the two energies according to

$$\Delta G_p = 1.41 + 0.645\ \Delta G_{mic}\ \text{(kcal/mol)} \qquad (13)$$

(correlation coefficient of $r = 0.9996$). To the best of our knowledge, Eq. 13 is the first empirical relationship connecting a micellization equilibrium with a membrane partition equilibrium. It can be used to predict the membrane partition constants of related nonionic surfactants if the critical micellar concentrations are known.

Equation 13 also demonstrates that the self-association of detergent is favored over penetration into a lipid membrane. Micelles are liquid-like, fluctuating aggregates in which the hydrocarbon chains remain highly flexible. In contrast, membranes are liquid-crystalline structures with only a limited flexibility of the hydrocarbon chains. Hence penetration into a lipid membrane leads to a loss in chain flexibility and, in turn, to a loss in conformational entropy, which could explain the differences in $\Delta G$. The distinctly larger $K$ (smaller CMC) of $C_{12}EO_8$ compared to OG and OTG is due to the longer hydrocarbon chain ($C_{12}$ versus $C_8$).

An analogous comparison can be made for the reaction enthalpies. The enthalpies of micelle formation of OG, OTG, and $C_{12}EO_8$ are 1.5 kcal/mol (Antonelli et al., 1994; Paula et al., 1995), 1.1 kcal/mol (Brackman et al., 1988), and 3.8 kcal/mol (Paula et al., 1995; Olofsson, 1985), respectively, at 28°C. The corresponding enthalpies for the partitioning into the membrane are 1.3 kcal/mol for OG (Wenk et al., 1997), −0.08 to +0.6 kcal/mol for OTG (this work), and +7.5 kcal for $C_{12}EO_8$ (Heerklotz et al., 1996). Almost all enthalpies are endothermic, but the correlation between the enthalpies of micellization and membrane partitioning is poor. No simple linear relationship can be derived.

The partitioning of a surfactant into a lipid membrane is an entropy-driven reaction and constitutes a classical example of the so-called hydrophobic effect. In this model the surfactant in the aqueous phase is surrounded by structured water, which is released as soon as the amphiphile enters the membrane. According to recent thermodynamic discussions, the characteristic signature of the hydrophobic effect is not so much a close-to-zero enthalpy, but a large heat capacity (Privalov and Gill, 1989). We have therefore examined the temperature dependence of the OTG partition enthalpy, $\Delta H_D(R)$. Fig. 6 shows plots of the two parameters $\Delta H_0$ and $\Delta H_1$ versus the temperature, yielding straight lines with virtually identical slopes, demonstrating again the high

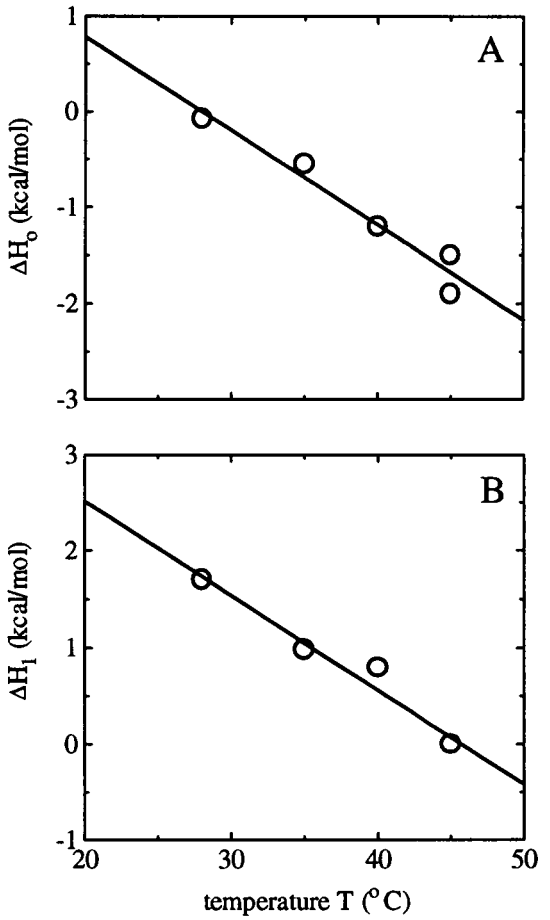


FIGURE 6 Temperature dependence of the partition enthalpy $\Delta H_D(R) = \Delta H° + \Delta H_1 \cdot R$, where $R$ is the mole fraction of detergent in the lipid phase. The figure shows the temperature dependence of the $\Delta H°$ (*A*) and the $\Delta H_1$ (*B*) parameters. The slope is identical for both parameters, with $\Delta C_P = -98$ cal/(mol · K).

precision of the simulations. Linear regression analysis leads to a molar heat capacity for the transfer of OTG from water to the lipid membrane of $\Delta C_p = -98$ cal/(mol · K) for $\Delta H°$ and −97.6 cal/(mol · K) for $\Delta H_1$. These values are similar to $\Delta C_p = -75$ cal/(mol · K), obtained for the OG/POPC partition equilibrium (Wenk et al., 1997), and are on the same order of magnitude as the heat capacities of transfer of typical nonpolar substances from the aeqeuous phase to the pure organic liquid (Privalov and Gill, 1989).

Finally, the influence of cholesterol on the surfactant partitioning needs to be discussed. Cholesterol is freely miscible with lipid, and its structural effects on the lipid molecules have been extensively investigated (Yeagle, 1985). On the other hand, only few data are available on the role of cholesterol in surfactant-membrane partition equilibria. The only calorimetric studies to date are those of the OG/POPC membrane (Wenk et al., 1997). Inspection of Table 1 reveals that the addition of cholesterol does not affect the OTG partition constant up to the highest cholesterol concentration measured ($K = 240\ M^{-1}$ for 0–50 mol % cholesterol). Likewise, the partition constant of its oxy-

gen-containing analog OG changes very little (from $K$ = 120 $M^{-1}$ at 0% cholesterol to 90 $M^{-1}$ at 50 mol% cholesterol). In contrast, the partition enthalpies of both surfactants increase approximately linearly with the cholesterol content of the membrane (by ~1.0–1.3 kcal/mol at 50 mol% cholesterol). The OTG partition enthalpy becomes independent of the membrane composition above 20 mol% cholesterol, and $\Delta H_D(R)$ (Eq. 11) is replaced by a constant $\Delta H^\circ_D$. Because $K$ and $\Delta G_p$ remain constant, the increase in $\Delta H_D$ requires a corresponding increase in $T\Delta S$. A molecular interpretation of this enthalpy-entropy compensation mechanism is difficult. From neutron diffraction studies with selectively deuterated cholesterol, it is known that the hydroxyl group of cholesterol is located in the vicinity of the lipid glycerol backbone (Worcester and Franks, 1976). Likewise, the sugar moiety of OG (and presumably that of OTG as well) penetrates into the POPC membrane up to the same level as the cholesterol OH, as deduced from deuterium and phosphorus solid-state NMR measurements (Wenk et al., 1997). The headgroups of two molecules are thus sufficiently close together to form intermolecular hydrogen bonds, releasing, in turn, water molecules from their respective hydration spheres. The entropy increase could thus be explained by a change in the hydration layer of the membrane.

## CONCLUSIONS

The interaction of OTG with POPC membrane vesicles follows a partition equilibrium of the form $X_b = Kc_{D,f}$. The partition constant is $K$ = 240 ± 10 $M^{-1}$ for membranes with and without cholesterol in the temperature range of 28–45°C. The partition enthalpy exhibits a more complex behavior, because it varies with the composition of the membrane phase and is temperature-dependent. $\Delta H_D$ is exothermic for POPC membranes with little OTG content and becomes endothermic as more and more OTG enters the membrane. $\Delta H_D$ varies linearly with the mole fraction, $R$, of OTG in the membrane according to $\Delta H_D(R) = -0.08 + 1.7\ R$. The partition enthalpy, but not the partition constant, is temperature dependent, with a molar heat capacity of $\Delta C_p = -98$ cal/(mol · K) for the transfer of OTG from the aqueous phase to the membrane. The combination of a partition equilibrium, $X_b = Kc_{D,f}$, with the composition-dependent partition enthalpy, $\Delta H_D(R)$, allows an almost perfect simulation of the calorimetric titration curves over a wide range of experimental conditions. The critical OTG concentration for membrane micellation is $c^*_D \approx 7.3$ mM, and up to 1.5 OTG molecules per POPC can be incorporated into the bilayer before disruption occurs. Compared to the related OG-POPC system, OTG exhibits a higher affinity toward the POPC membrane ($K_{OTG}$ = 240 $M^{-1}$ versus $K_{OG}$ = 130 $M^{-1}$) and a lower critical micellar concentration ($cmc_{OTG}$ = 9 mM versus $cmc_{OG}$ = 19 mM). Membrane binding and micelle formation are correlated by a linear free energy relationship. Upon the addition of cholesterol, an enthalpy-entropy compensation mechanism is observed. The thermodynamic analysis presented here is of general relevance to the interaction of fusogenic peptides with membranes.

This work was supported by Swiss National Science Foundation grant 31.42058.94.

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