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Thermodynamics of interaction of octyl glucoside with phosphatidylcholine vesicles: partitioning and solubilization as studied by high sensitivity titration calorimetry

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Abstract

The interaction of the surfactant octyl glucoside (OG) with dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), and soy bean phosphatidylcholine (soy bean PC) was studied using high-sensitivity titration calorimetry. We determined the partition coefficient of OG between water and lipid bilayers and the transfer enthalpy of the surfactant by addition of lipid vesicles to OG monomers or vice versa. Comparison with the micellization enthalpy of the surfactant gives information on differences in the hydrophobic environment of OG in a liquid-crystalline bilayer or a micelle. The average partition coefficient P in mole fraction units for $x_e \approx 0.12$–0.2 decreases slightly from 4152 at 27°C to 3479 at 70°C for DMPC and from 4260 to 3879 for soy bean PC, respectively. The transfer enthalpy ΔH^T of OG into lipid vesicles is positive at 27°C and negative at 70°C. Its temperature dependence is larger for the incorporation of OG into DMPC than into soy bean PC vesicles. It is concluded that OG in DMPC vesicles is better shielded from water than in soy bean PC vesicles or in micelles. Titration calorimetry was also used to determine the phase boundaries of the coexistence region of mixed vesicles and mixed micelles in the systems OG/DMPC, OG/DPPC, OG/DSPC, and OG/soy bean PC vesicles at 70°C in the liquid-crystalline phase. DMPC and soy bean PC solubilization was also studied at 27°C to investigate the effect of temperature. The effective surfactant to lipid ratios at saturation, R_e^{sat}, for all PCs studied are in the range between 1.33–1.72 and the ratios at complete solubilization, R_e^{sol}, are between 1.79–3.06. At 70°C, the R_e^{sat} values decrease with increasing chain length of the saturated PC. The ratios depend also slightly on temperature and the degree of unsaturation of the fatty acyl chains. For the OG/soy bean PC system, the coexistence range for mixed vesicles and mixed micelles is larger than for the corresponding PCs with saturated chains.

Keywords: Micelle; Vesicle; Octyl glucoside; Solubilization; Partition coefficient; Titration calorimetry

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1. Introduction

We have used high sensitivity isothermal titration calorimetry (hs-ITC) before to study the self assembly of amphiphiles in aqueous solutions [1,2]. This method has proven very useful, because it is very sensitive and provides the thermodynamic quantities for the micellization process in a temperature range between 5 and 80°C. Titration calorimetry is also ideally suited to determine membrane solubilization by the addition of surfactant to lipid vesicles [3,4] The phase transition of the bilayer from lipid/surfactant mixed vesicles to surfactant/lipid

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mixed micelles is described by the term ‘solubilization’ of vesicles. Lichtenberg and co-workers [5–9] developed a three-stage model to describe this process. According to this model, surfactant incorporates first into the bilayer until a characteristic saturation ratio R_e^{sat} is reached. R_e is the ratio of the surfactant concentration D_e in the aggregates (vesicles or micelles) and the lipid concentration L. Both concentrations refer to the total sample volume. In the second stage, addition of surfactant leads to a transformation of the mixed vesicles of the composition R_e^{sat} to mixed micelles of the composition R_e^{sol}. In the third stage the solubilization of the vesicles has been completed and all of the mixed vesicles have been transformed. The ratio R_e increases with further surfactant addition.

In this study, we used the hs-ITC to determine the partitioning of OG into DMPC and soy bean PC vesicles as a function of temperature. We also studied the solubilization of DMPC, DPPC, DSPC, and soy bean PC vesicles by the addition of OG and determined the phase diagrams in a concentration range up to ~6 mM phospholipid. The transfer enthalpies ΔH^T for the transfer of OG into lipid vesicles were determined at 27°C and at 70°C. From the partition coefficients we calculated the Gibbs energy changes ΔG^T and also the entropy changes ΔS^T. From the temperature dependence of the ΔH^T values the heat capacity change ΔC_p^T for the transfer of OG monomers into the vesicles could be calculated and compared with the ΔC_p value from the temperature dependence of the micellization enthalpy which describes the transfer of monomeric OG into an OG micelle. We will show that these values depend on the nature of the lipid bilayers and reflect the shielding of hydrophobic surfaces of the amphiphiles from water.

2. Materials and methods

2.1. Materials

The surfactant octyl-β-D-glucopyranoside (OG) was purchased from Bachem Biochemica, Heidelberg. The purity was > 99% as determined by TLC. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and soy bean phosphatidylcholine (soy bean PC, Phospholipon 90/90G) were gifts from Natterman Phospholipid, Köln. All phospholipids were pure as tested by TLC and were used without further purification.

2.2. Sample preparation and light scattering measurements

The surfactant solutions and the lipid dispersions were prepared by dissolving the required amount of lipid or surfactant in ultra pure water (Reinstwassersystem RS 90-4 MF, Barsbüttel). Lipid vesicular dispersions were then prepared by vortexing for 30 s followed by mild sonication for 10–20 min at 50°C in an ultrasonic bath (Elma T310/H, Germany). The size of the lipid vesicles were determined by dynamic light scattering using a Malvern Zetasizer 3 (Malvern Instruments, Herrenberg, Germany). The vesicle diameters for most of the experiments were in the range of 80–120 nm. For the solubilization experiments with light scattering detection, 0.5 ml of the vesicles suspension was filled into the light scattering cell. The temperature was kept constant at 30°C. Aliquots (μl amounts) of a concentrated OG solution was added under stirring. Several measurements of the scattering intensity and the particle radius were then performed in a time range of 15–30 min after the addition of the surfactant solution. The experimental procedure was therefore very similar to the ITC experiment (see below).

2.3. Titration calorimetry

High-sensitivity isothermal titration calorimetry (hs-ITC) measurements were performed with an OMEGA Differential Titration Calorimeter from MicroCal, Northampton, MA, USA. The data were analysed using the ORIGIN™ software (version 2.9) as provided by MicroCal.

2.3.1. Demicellization experiments

Heats of dilution and demicellization were determined as described before [2].

2.3.2. Solubilization experiments

The solubilization of lipid vesicles by addition of surfactant was achieved in principle as described before [4]. Lipid vesicles were filled into the sample cell and micellar OG solution was added. The concentrations were chosen in such a way that solubilization of the vesicles occurred during the experiment. In contrast to the POPC/$C_{12}EO_8$ system the concentration D_W of the OG monomer in water is much higher for solubilization by OG and cannot be neglected. The size of the vesicles was usually between 80 and 120 nm. In some cases, we also used larger vesicles (probably multilamellar) in the ITC solubilization experiments with diameters up to 450 nm. No significant dependence of the experimental values on the vesicle size was observed, all data were within the precision of the calorimetric method.

2.3.3. Partitioning experiments

Lipid vesicles were injected into a surfactant solution with a concentration well below the cmc or vice versa. The surfactant concentrations always remained below 10 mM and therefore also below the monomer concentration D_W in the two component system. The experimental curves were analysed and fitted on the basis of the appropriate partitioning model using a non-linear least square fitting procedure as implemented in the software SCIENTIST for Windows (MicroMath, Salt Lake City, UT, USA).

3. Results

3.1. Demicellization of OG

Fig. 1 shows a schematic phase diagram of a surfactant/lipid/water mixture at high water content. The numbered arrows represent the pathways of the different experiments carried out with the ITC. Arrow (1) describes the demicellization experiment. A highly concentrated micellar surfactant solution is titrated into the sample cell which was originally filled with pure water. The demicellization enthalpy and the cmc can be determined from one and the same experiment as described before [1,2]. The demizellization enthalpies, Gibbs free energies and entropies are shown in Table 1 and were similar as reported previously [1,2].

3.2. Partitioning of OG into bilayers

Arrows (2) and (3) in Fig. 1 describe two possible partitioning experiments. For experiment (2) a 3 mM

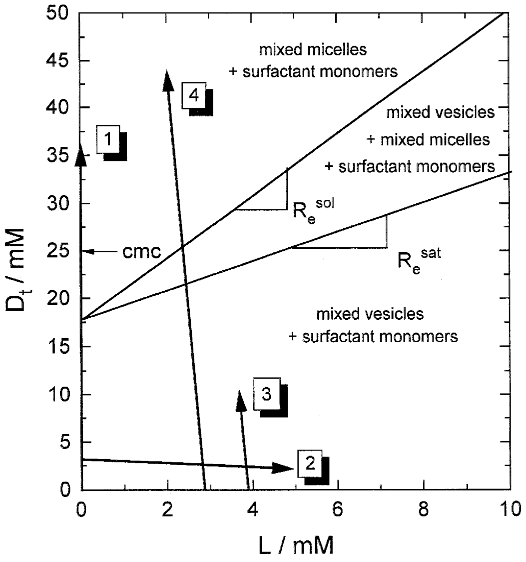

Fig. 1. Schematic phase diagram for a phospholipid/surfactant/water mixture with in excess water. The arrows designate the different calorimetric titration experiments performed in this study. Arrow (1) corresponds to the demicellization of OG as described before [1,2]. Arrows (2) and (3) are experiments to determine the partition coefficient of OG into lipid bilayers. Arrow (4) crosses the coexistence region and describes the solubilization of lipid vesicles by OG.

OG solution was filled into the sample cell and titrated with a 50 mM DMPC vesicle suspension. The resulting normalised enthalpograms at two different temperatures are shown in Fig. 2. The experimental curves can be simulated with the assumption that the surfactant partitions into the lipid bilayers. The partition coefficient P is defined as the ratio of the mole fractions of the respective component in two phases [9–11]:

$$P = \frac{x_e}{x_W} \tag{1}$$

with x_e the mole fraction of surfactant in the bilayer and x_W the mole fraction of surfactant in water:

$$x_e = \frac{D_e}{D_e + L} \tag{2}$$

$$x_W = \frac{D_W}{D_W + W} \tag{3}$$

D_e is the concentration of surfactant in aggregates and D_W the concentration of surfactant in the water phase, both based on total sample volume. L is the

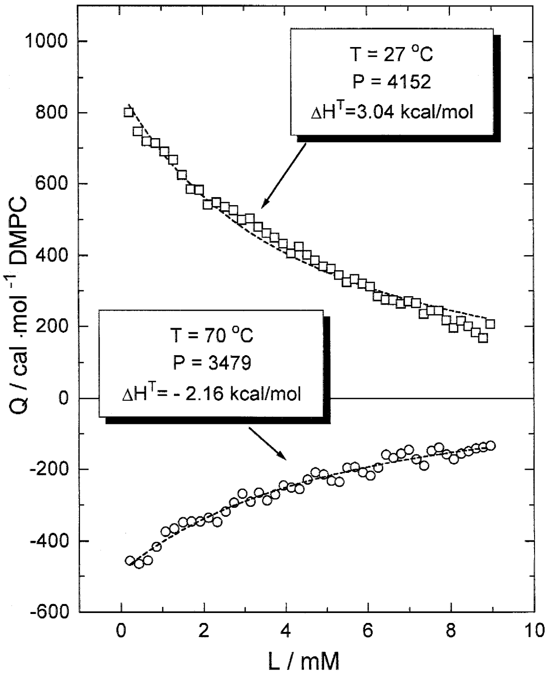

Fig. 2. Results of a partitioning experiment according to arrow (2) in Fig. 1. A 3 mM OG solution is titrated with a 50 mM DMPC vesicle suspension. The dotted lines are calculated using Eq. (7) with the parameters P and ΔH^{T} as obtained from a non-linear least square fit.

lipid concentration and W is the water concentration. Insertion of Eq. (2) and Eq. (3) into Eq. (1) yields:

$$P=\frac{D_{\mathrm{e}}\cdot(D_{\mathrm{W}}+W)}{(D_{\mathrm{e}}+L)\cdot D_{\mathrm{W}}} \tag{4}$$

With $D_{\mathrm{W}}=D_{\mathrm{t}}-D_{\mathrm{e}}$ with D_{t} being the total surfactant concentration and using the approximation $D_{\mathrm{W}}+W\approx W$ one finds [4]:

$$D_{\mathrm{e}}=\frac{1}{2P}\Big[P(D_{\mathrm{t}}-L)-W+\sqrt{P^2(D_{\mathrm{t}}+L)^2-2\cdot P\cdot W(D_{\mathrm{t}}-L)+W^2}\Big] \tag{5}$$

To determine the change of the surfactant concentration D_{e} in the lipid bilayer occurring upon injection of ΔL moles of lipid to the surfactant monomers, we have to differentiate Eq. (5) with respect to L:

$$\frac{\Delta D_{\mathrm{e}}}{\Delta L}=-\frac{1}{2}+\frac{P\cdot(D_{\mathrm{t}}+L)+W}{2\cdot\sqrt{P^2\cdot(D_{\mathrm{t}}+L)^2+2\cdot P\cdot W\cdot(L-D_{\mathrm{t}})+W^2}} \tag{6}$$

Two effects are considered to contribute to the reaction enthalpy Q. One is the incorporation of the surfactant monomers into the lipid bilayer and the other is the dilution of the vesicular dispersion. The observed reaction enthalpy Q is then related to Eq. (6) by [4]:

$$Q=\frac{\Delta D_{\mathrm{e}}}{\Delta L}\cdot\Delta H^{\mathrm{T}}+\Delta H_{\mathrm{dil}} \tag{7}$$

with ΔH^{T} being the transfer enthalpy from monomeric OG molecules into the aggregate and ΔH_{dil} the heat of dilution of lipid dispersion into pure

Table 1
Thermodynamic parameters for the transfer of the surfactant OG from water to OG micelles or to DMPC and soy bean PC bilayers, respectively, as obtained from the demicellization and partition experiments

	OG		DMPC		Soy bean PC	
T	27°C	70°C	27°C	70°C	27°C	70°C
P	2127	2099	4152	3479	4260	3879
$P(x_{\mathrm{e}}=0)$	–	–	5570	3805	–	–
ρ [cal/mol]	–	–	−630	−650	–	–
ΔG^{T}	−4568	−5214	−4967	−5558	−4982	−5632
[cal/mol]			(−5123)	(−5599)		
ΔH^{T}	+1500	−2049	+3036	−2159	+1326	−2133
[cal/mol]			(+3100)	(−2400)		
$T\Delta S^{\mathrm{T}}$	+6068	+3165	+8003	+3399	+6308	+3499
[cal/mol]			(+8223)	(+3199)		
$\Delta C_{\mathrm{p}}^{\mathrm{T}}$ [cal · mol^{-1} · K^{-1}]	−82.5		−120.8 (−128)		−80.5	

P was obtained from experiment (2), i.e. for $x_{\mathrm{e}}\approx 0.12$–$0.19$. Numbers in parenthesis refer to transfer data obtained from the partition experiment according to arrow (3) in Fig. 1, i.e. data calculated with values for $P(x_{\mathrm{e}}=0)$.

water. Combination of Eq. (6) and Eq. (7) enables a two parameter least squares fit of the experimental data with P and ΔH^{T} as parameters (see dashed lines in Fig. 2). ΔH_{dil} was determined from separate dilution experiments and turned out to be small.

In the partitioning experiment according to arrow (3) in Fig. 1 a monomeric solution of OG is titrated to lipid vesicles. In this case, the mole fraction x_{e} increases with further additions. The normalised experimental data for the titration of DMPC with OG at two different temperatures are shown in Fig. 3. The signal to noise ratio is here much lower because fewer OG molecules are transferred from water to the bilayers. The experimental data could not be fitted using an approach starting from Eq. (5) with a single value for P, indicating that the partition coefficient is not constant in this type of experiment (see dashed lines in Fig. 3). This can occur, when the mixing of the surfactant with the lipid in the vesicles is non-ideal. We used the theory of athermal non-ideal mixing to account for this effect [11]. In this approach, the Gibbs free energy ΔG_{mix} of mixing has an ideal mixing term ΔG_{id} and an excess term $\Delta G^{\mathrm{E}} = \rho(1-x_{\mathrm{e}})x_{\mathrm{e}}$ with ρ being the non-ideality parameter of mixing and the non-ideality is assumed to be solely due to an excess entropy of mixing, i.e. $\Delta G^{\mathrm{E}} = -T\Delta S^{\mathrm{E}}$. The model of athermal mixing seems to be more appropriate when the shape of the two molecules in the mixture is different. Then, ΔG^{E} is dominated by the entropic contribution and ΔH^{E} can be neglected.

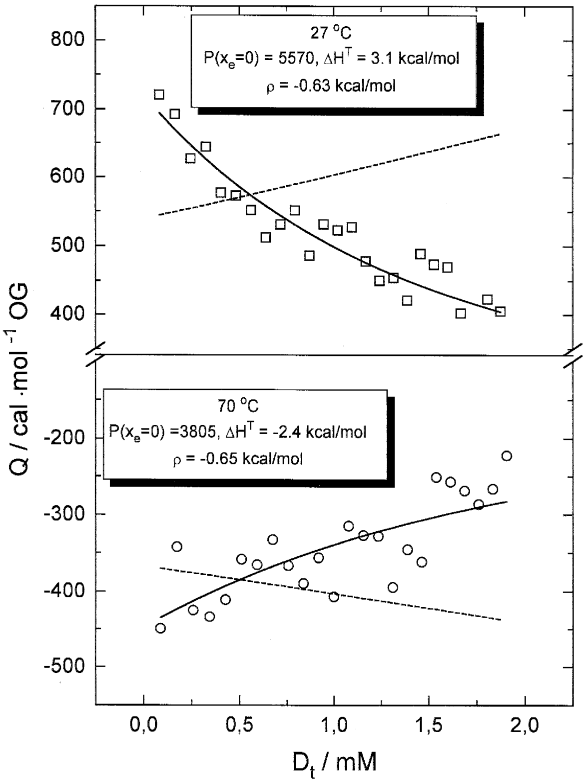

Fig. 3. Enthalpograms of a partitioning experiment according to arrow (3) in Fig. 1. A 11.02 mM OG solution is titrated to a 3.9 mM DMPC vesicle suspension. The solid lines are calculated according to Eq. (11) using the parameters $P(x_{\mathrm{e}}=0)$, ΔH^{T}, and ρ as shown. These were obtained from a non-linear least square fit of the experimental points. The dashed lines were calculated with the values for the partition coefficient P and for ΔH^{T} as determined from the partitioning experiment according to arrow (2) (see Fig. 2).

For the athermal model, the partition coefficient P can be written as [11]:

$$P = P(x_{\mathrm{e}}=1)\cdot\exp\left[-\rho\cdot(1-x_{\mathrm{e}})^2/RT\right] \tag{8}$$

with $P(x_{\mathrm{e}}=1)$ being the partition coefficient for partitioning into hypothetical pure surfactant bilayers and $P = P(x_{\mathrm{e}}=1)\cdot\exp[-\rho/RT] = P(x_{\mathrm{e}}=0)$ the coefficient for partitioning into pure PC bilayers. The incorporated surfactant concentration D_{e} is given by Eq. (5). To determine the change of D_{e} occurring upon injection of D_{t} moles of surfactant we have to differentiate Eq. (5) with respect to D_{t}. However, Eq. (5) is an implicit equation with respect to D_{e}, because P is dependent on x_{e} via Eq. (8) and x_{e} is related to D_{e} by Eq. (2). The differentiation can be achieved by setting:

$$F = 0 = -D_{\mathrm{e}} - \frac{1}{2}\left(L + \frac{W}{P} - D_{\mathrm{t}}\right) + \frac{1}{2}\sqrt{\left(L + \frac{W}{P} - D_{\mathrm{t}}\right)^2 + 4\cdot D_{\mathrm{t}}\cdot L} \tag{9}$$

and calculating the derivative $\Delta D_{\mathrm{e}}/\Delta D_{\mathrm{t}}$ from the relation:

$$\frac{\Delta D_{\mathrm{e}}}{\Delta D_{\mathrm{t}}} = -\left(\frac{\Delta F}{\Delta D_{\mathrm{t}}}\right)\Big/\left(\frac{\Delta F}{D_{\mathrm{e}}}\right) \tag{10}$$

The observed heat of reaction is now:

$$Q = \frac{\Delta D_{\mathrm{e}}}{\Delta D_{\mathrm{t}}}\cdot\Delta H^{\mathrm{T}} + \Delta H_{\mathrm{dil}} \tag{11}$$

with ΔH^{T} again the heat of transfer of OG into lipid vesicles and ΔH_{dil} in this case the heat of dilution of surfactant monomers into water. This is again determined in a separate experiment. The solid lines in Fig. 3 are the fitted curves on the basis of Eq. (11) with $P(x_e = 1)$, ΔH^{T}, and ρ as adjustable parameters. The dashed lines were calculated with values for P determined from experiment (2) as shown in Fig. 2. It is obvious that a constant partition coefficient cannot describe the results of experiment (3).

The resulting values for the transfer enthalpies ΔH^{T}, the Gibbs free energies $\Delta G^{\mathrm{T}} = -RT \ln(P)$, and the term $T\Delta S^{\mathrm{T}} = \Delta H^{\mathrm{T}} - \Delta G^{\mathrm{T}}$, as determined from the partitioning experiments for the two lipids DMPC and soy bean PC are summarised in Table 1. Values in parentheses are those for OG partitioning into DMPC bilayers at infinite dilution ($x_e = 0$) as determined from experiment (3). Whereas the partition coefficients change only slightly with temperature, the transfer enthalpies show the characteristic change of sign in the temperature range between 27 and 70°C. Assuming a linear temperature dependence of ΔH^{T} in this temperature range one can calculate the change in heat capacity ΔC_p^{T} occurring during the transfer of OG from water to a micelle or a lipid bilayer. Positive ΔC_p^{T} values are characteristic for the transfer of hydrophobic groups from a hydrocarbon solvent to water. For the reverse process they are therefore negative as shown in Table 1. The differences in ΔC_p^{T} values for OG transfer into OG micelles and DMPC bilayers show changes in the exposure of hydrophobic groups in the different amphiphilic aggregates (see Section 4 below).

3.3. Solubilization of PC bilayers by OG

Arrow (4) describes the solubilization experiment. Here, a micellar surfactant solution is added to a lipid vesicular dispersion. The concentrations are chosen in such a way, that during the experiment total solubilization occurred. Fig. 4 shows an original experimental titration curve of a 2.1 mM DMPC vesicle suspension with 35×8 μl injections of a micellar OG solution at a temperature of 27°C. It is evident that between the 16th and 20th addition the enthalpic effects suddenly decrease. For a purpose of comparison, Fig. 5 shows the heat effects observed for titration of OG micelles into water (demicellization of OG) and for the addition of OG micelles to a DMPC vesicle suspension. In the enthalpogram of a solubilization experiment one finds an intermediate extreme value. We now assumed, that the points of inflexion, i.e. the extrema of the first derivative of this curve (see lower part of Fig. 5), correspond to the OG concentrations D_t^{sat} and D_t^{sol}, i.e. points on the phase boundaries of the coexistence region between mixed vesicles and mixed micelles [6,7]. Because the number of experimental points between these two concentrations seemed to be too small for a reliable determination of D_t^{sat} and D_t^{sol} we performed experiments with higher resolution (shown in the inserts). However, no significant changes were observed. The reproducibility for the D_t^{sat} and D_t^{sol} values was ± 0.5 mM and relatively independent on the number of experimental points in this concentration regime.

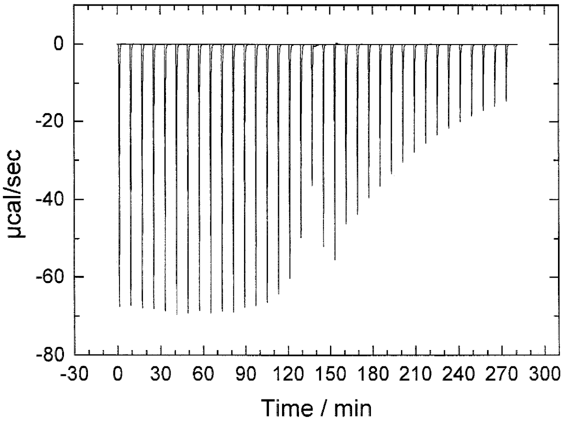

Fig. 4. Experimental curve for the solubilization of DMPC at 27°C. A 2.1 mM DMPC vesicle suspension was titrated with a 252 mM micellar OG solution in 8 μl steps. The crossing of the coexistence regime leads to a sudden decrease in heat effect.

The ratio of surfactant to lipid in the aggregates is given by:

$$R_e = \frac{D_e}{L} \qquad (12)$$

With $D_t = D_W + D_e$ one obtains:

$$D_t^{\#} = R_e^{\#} \cdot L + D_W^{\#} \qquad (13)$$

where the '#' sign now stands for 'sat' or 'sol' values [6,7]. Plotting the $D_t^{\#}/L$ data pairs of a series of experiments with varying concentrations of lipid yields the phase diagram shown in Fig. 6 for DMPC at 27 and 70°C. The overall precision for the D_t^{sat} and D_t^{sol} (± 0.5 mM) is indicated in Fig. 6 by error bars.

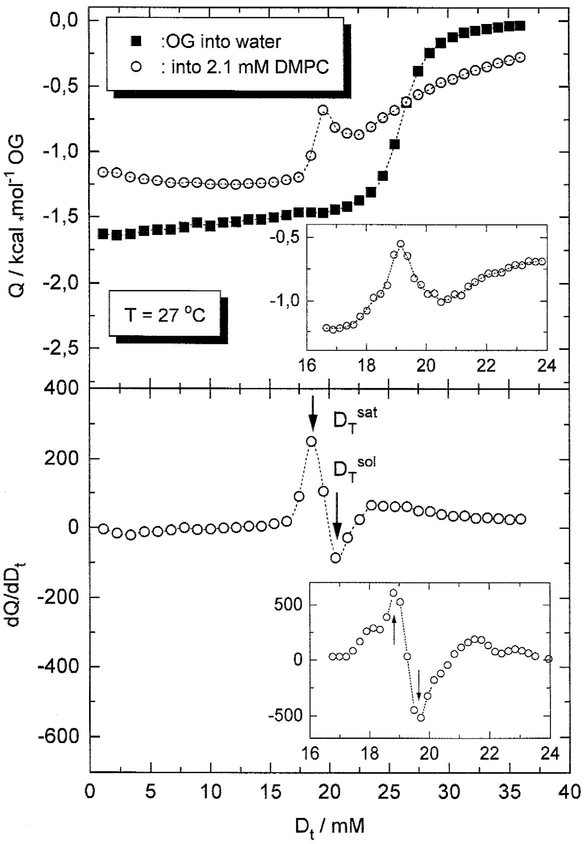

Fig. 5. Top: Enthalpograms for the demicellization of OG in comparison to the solubilization of DMPC vesicles by OG at 27°C. Bottom: first derivative of the solubilization enthalpogram for the determination of the points on the phase boundaries D_t^{sat} and D_t^{sol}. The inserts show the solubilization region in the vicinity of D_t^{sat} and D_t^{sol} obtained with a larger number of data points.

A plot of $D_t^{\#}$ vs. L according to Eq. (13) gives as the slope the values $R_e^{\#}$, i.e. the characteristic surfactant to lipid ratio R_e^{sat} in vesicles which are saturated with OG and the ratio R_e^{sol} when the vesicles are completely dissolved and only mixed micelles are present. Extrapolation of the phase boundaries to $L = 0$ yields $D_W^{\#}$, the hypothetical critical aggregation concentration of the surfactant in the presence of lipid vesicles. In the scheme proposed by Lichtenberg [6,7], the extrapolation of both lines of the phase boundaries should end at the same points on the D_t axis (see Fig. 1). In our experiment this was never the case, we always found a difference of 1–2 mM in the $D_W^{\#}$ values obtained by extrapolation of the experimental data to $D_t = 0$. This might be caused by systematic errors in the determination of the D_t^{sat} and D_t^{sol} values (see below).

With Eq. (4) and Eq. (13) one can also calculate the partition coefficients of OG at the boundary of the coexistence regions, the values P^{sat} and P^{sol}. These are shown in Table 2. It is evident that these partition coefficients are much lower than those listed in Table 1. However, in the partitioning experiment according to arrow (3) we found a decrease of the partition coefficient with increasing saturation of the bilayers. Therefore, our assumption for the explanation of this experiment using Eq. (8) was correct. Calculation of the partition coefficient as a function of D_t according to Eq. (8) using the $P(x_e = 0)$ and ρ values in Table 1 yields for P^{sat} at $x_e^{sat} = 0.61$ a value of approx. 2200. This is in good agreement with the value of $P^{sat} = 2083$ determined from Eq. (4) and Eq. (13) (see below).

As mentioned above, we assumed that the points of inflexion of our calorimetric titration curves correspond to points on the phase boundaries. Because the coexistence region is very narrow, we used an independent method to obtain D_t^{sat} and D_t^{sol} values, namely by light scattering measurements. We performed a titration under almost identical conditions as in the calorimetric experiments using dynamic light scattering (DLS) as detection method. Fig. 7 shows the scattering intensity and the particle size as determined by DLS for several samples of unilamellar vesicles as a function of total surfactant concentration for the lipid DMPC and soy bean PC. Included as dashed and dotted lines are the D_t^{sat} and D_t^{sol} values obtained from the phase diagrams determined by calorimetry. As observed before by others, in the region where solubilization starts a sudden decrease of the light scattering intensity is found whereas the particle size seems to increase even further. A meaningful analysis of the DLS data is, however, not possible, because in the coexistence region aggregates of various shape, possibly long wormlike micelles coexist with larger vesicles [5,8,12–15]. The comparison of the light scattering data with those determined from calorimetry shows, that the ITC data do indeed unequivocally show the onset and end of transformation of vesicles to micelles. The absolute values for D_t^{sat} and D_t^{sol} are very similar but not completely identical. The values determined by DLS have similar precision as those from ITC and are

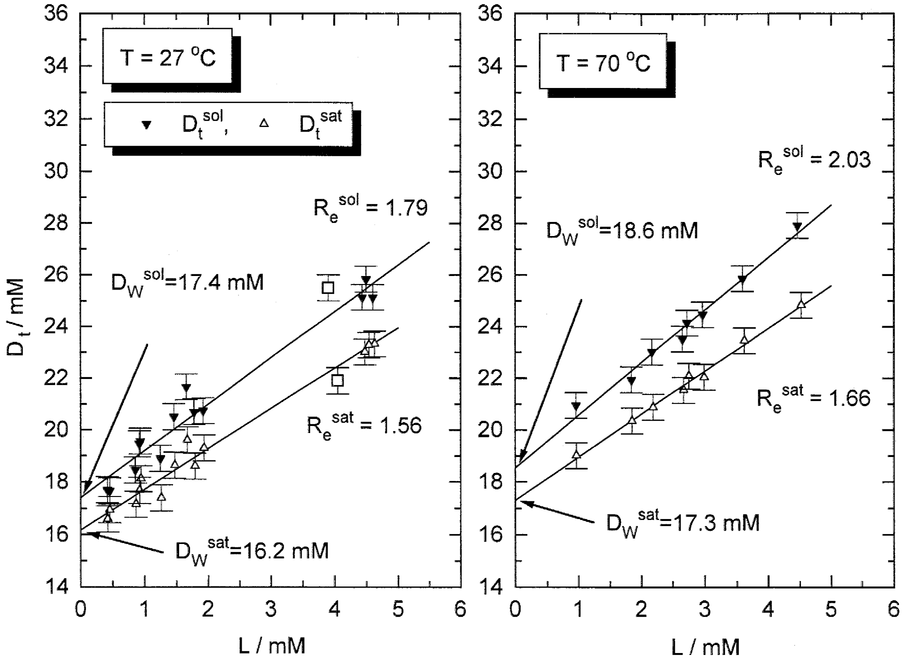

Fig. 6. Phase diagram for the DMPC/OG system at two different temperatures as obtained from titration calorimetry. The points on the phase boundaries were obtained from the D_t^{sat} and D_t^{sol} values as indicated in Fig. 5. Error bars of ±0.5 mM are estimated from several experiments at one particular lipid concentration using different resolutions. The R_e^{sat} and R_e^{sol} values were obtained from a linear least square fits of the experimental points. The two open squares are values determined by light scattering measurements, they have similar precision (see Fig. 7).

shown in Fig. 6 as open squares. The values determined by calorimetry seem to indicate a slightly narrower coexistence region.

As already shown in Fig. 7, we performed a second control experiment with a lipid system where enough experimental data are published, namely the solubilization of a PC with mixed unsaturated chains. We used soy bean PC instead of egg PC, the fatty acid composition of soy bean PC being 15% saturated and 85% unsaturated chains and therefore having slightly more unsaturated fatty acids compared to egg PC. The light scattering data are shown in Fig. 7. The calorimetric data gave very similar results (indicated as vertical dashed lines) and showed that for the solubilization of soy bean PC higher surfactant concentrations are needed and that the two points of inflexion are farther apart indicating a wider coexistence region.

The phase diagram determined by series of calorimetric titration experiments (not shown) indicates that the upper phase boundary is considerably steeper for OG/soy bean PC than for OG/DMPC. In good

Table 2

Values for the surfactant/lipid ratios at saturation R_e^{sat} and solubilization R_e^{sol}, the surfactant concentration in water, and the partition coefficients P^{sat} and P^{sol} at the saturation and solubilization limits, respectively

	DMPC		Soy bean PC		DPPC	DSPC
T [°C]	27	70	27	70	70	70
P^{sat}	2083	1873	1777	1794	1892	1694
P^{sol}	2037	1791	2137	1823	1840	2025
R_e^{sat}/R_e^{sol}	1.56/1.79	1.66/2.03	1.55/3.06	1.72/2.55	1.50/1.94	1.33/2.47
D_W^{sat}/D_W^{sol} [mM]	16.2/17.4	17.3/18.6	18.9/19.5	19.3/21.5	17.6/19.9	18.7/19.5

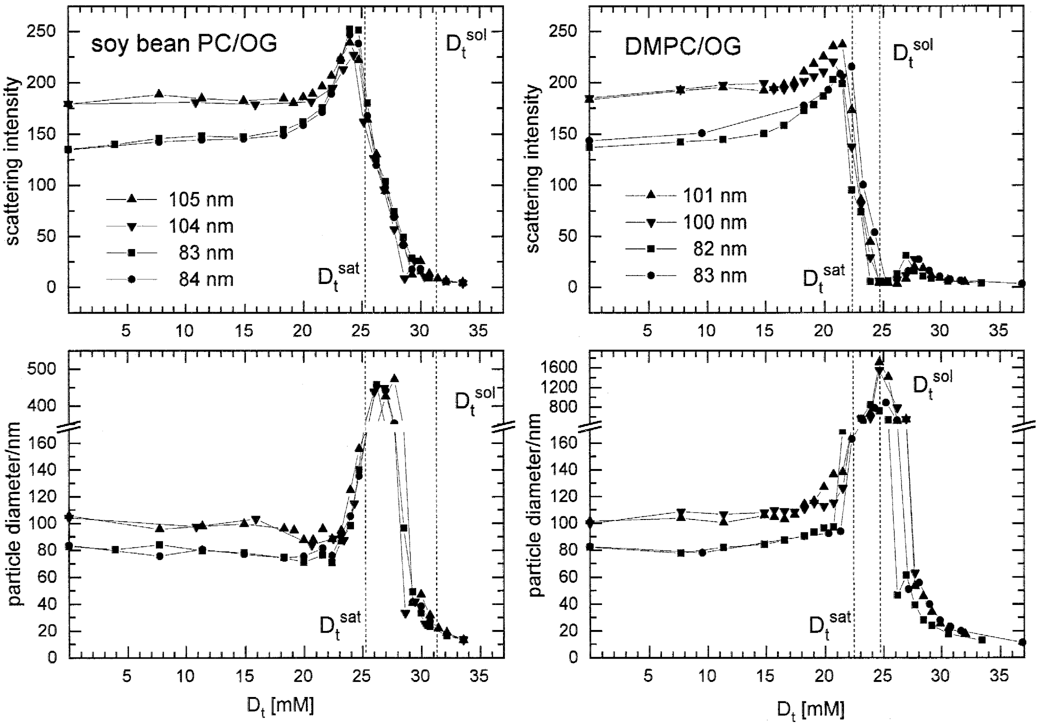

Fig. 7. Solubilization of soy bean PC (4.45 mM, left) and DMPC vesicle suspensions (4.35 mM, right) with 200 mM OG as observed by dynamic light scattering. The top diagrams show the light scattering intensity, the bottom the apparent particle diameters. The solubilization region as obtained by calorimetry is indicated by vertical dashed lines. The values for the particle diameter in the region where the light scattering intensity is low (below 15 U), is uncertain. This is the region where mainly micelles are existent.

agreement with previous observation on the egg PC/OG system the R_e^{sat} value is 1.55 and the R_e^{sol} value 3.06 at 27°C compared to 1.4 and 3.2 reported by Almog et al. [8] but lower than those published by da Graca Miguel et al. [12] (see Table 2).

For a test of the dependence of the solubilization values on the chain length of saturated PCs we also studied the systems DPPC/OG and DSPC/OG. At 27°C both of these lipids are in the gel phase. Therefore, only data at 70°C were collected, where these lipids are in the liquid-crystalline phase to facilitate comparison with the data obtained for DMPC and soy bean PC. The R_e^{sat} and R_e^{sol} values for all four systems are listed in Table 2.

4. Discussion

Isothermal titration calorimetry is a suitable method for the determination of thermodynamic parameters of aggregation processes in water/amphiphile systems. We have shown that before for the process of demicellization [1,2] and recently for the solubilization of PC vesicles by non-ionic surfactants of the type C_nEO_m, i.e. oligoethyleneoxide alkyl ethers [3,4]. In a systematic study of the solubilization of POPC by $C_{12}EO_8$ it was shown that not only the phase boundaries could be determined, but that also the thermodynamic quantities ΔH, ΔG, and ΔS for the transfer of surfactant and lipid between water and the various aggregates could be extracted from the experimental data [4].

4.1. Partition coefficients of OG

The OG/PC systems studied here differ significantly from the POPC/$C_{12}EO_8$ system. OG has a much higher cmc than $C_{12}EO_8$, namely ~25 mM vs. ~90 μM. The demicellization enthalpies are also significantly different with a value of -3.8 kcal/mol for $C_{12}EO_8$ compared to -1.5 kcal/mol for OG [2,4]. This has also consequences for the partition coefficients of OG into PC vesicles below the saturation limits. We determined the partition coefficients using two different approaches. In the experiment according to arrow (2) in Fig. 1, a 3 mM surfactant solution is titrated with a lipid vesicle suspension. The surfactant concentration decreases slightly during this experiment due to the dilution of surfactant with vesicle solution but the lipid concentration increases up to ~ 8 mM. When the effective mole fraction of surfactant x_e in the bilayer is calculated for this type of experiment, it changes from ~ 0.19 to 0.12 with increasing lipid concentration. The experimental curves in Fig. 2 can therefore be simulated using an average constant partition coefficient. This is different for the experiment according to arrow (3) in Fig. 1. Here a ~ 4 mM DMPC dispersion is titrated with OG up to $D_t = 1.8$ mM. In this case, the effective mole fraction x_e increases from 0 to ~ 0.11. Because the partition coefficient changes much more in this range, a simulation of experimental data as shown in Fig. 3 has to take into account a x_e dependent partition coefficient.

For clarification of this effect we have calculated P as a function of x_e using the experimentally determined value $P(x_e = 0) = 5570$ with a non-ideality parameters $\rho = -0.63$ kcal/mol as determined for $T = 27°C$ for partitioning into DMPC bilayers (see Fig. 3). A plot of P as a function of x_e is shown in Fig. 8 (top). The average partition coefficient P determined from experiment (2) and the value for P^{sat} at saturation ($x_e^{sat} = 0.61$) are indicated by the arrows. The calculated curves based on experiment (3), which was performed at low x_e values, give a surprisingly good estimate for P^{sat} compared to the experimentally determined value from the value of R_e^{sat} (see Fig. 8).

We also calculated x_e as a function of D_t with the lipid concentration L as a parameter (Fig. 8 (bottom)). The arrows (2) and (3) in the figure indicate the two different partitioning experiments. The experiment (2) only covers a range of x_e values between 0.12 and 0.19. The change in partition coefficient P in this range is relatively small and the experimental curves of this experiment can be simulated using only one average value for P. For the partitioning experiment according to arrow (3) the x_e value changes from 0 to ~ 0.11 and the change in the partition coefficient is also much larger. Therefore, the experimental curves of this experiment give only reasonable fits with a composition dependent partition coefficient P.

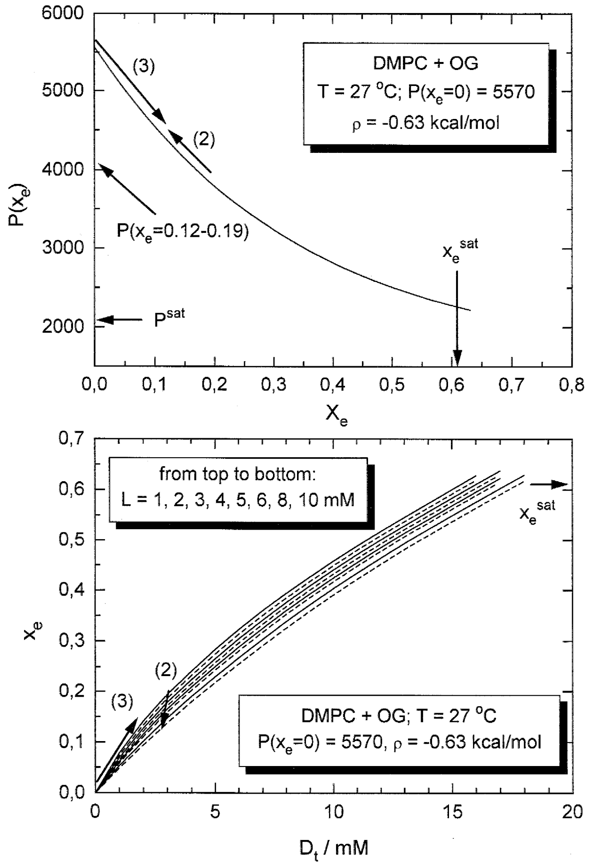

Fig. 8. Top: Calculation of the partition coefficient P for the partitioning of OG into DMPC bilayers at 27°C using the values for $P(x_e = 0)$ and the non-ideality parameter ρ obtained from experiment (3) as shown in Fig. 3. Bottom: Calculation of x_e, the mole fraction of surfactant in the lipid bilayer, as a function of the total surfactant concentration D_t for different lipid concentrations L. The arrows (2) and (3) designate the change of the partition coefficient occurring in the partitioning experiment according to arrows (2) and (3) shown in Fig. 1.

We used a model with athermal mixing to calculate these curves. This means that $\Delta G^E = -T\Delta S^E$; i.e. excess mixing enthalpies are not considered. The regular solution model with $\Delta G^E = \Delta H^E$ with $T\Delta S^E = 0$ was also calculated. It leads to an extra term of the form $\rho\ (1 - x_e)^2$ in Eq. (11) with ΔH^T now the transfer enthalpy of surfactant to a hypothetical surfactant bilayer ($x_e = 1$) [4]. The simulation yielded unreasonable high partition coefficients for all values of x_e, which were not consistent with our other experiments and those described in the literature. Therefore, we believe that the athermal model is more appropriate. An additional justification for this approach is the fact that in binary systems where the two molecules have vastly different sizes and shapes the entropic contribution to the non-ideality is normally much larger than the enthalpic one. In reality, neither of these two border line cases is appropriate. Excess enthalpies of mixing are always connected with excess mixing entropies resulting from different interaction energies between like and unlike pairs of molecules. A more detailed analysis of the experimental data is therefore clearly necessary but not warranted at the present time, because high precision data are not available. In addition, the proposed model with a term $\Delta G^{\mathrm{E}} = \rho x_{\mathrm{e}}\ (1 - x_{\mathrm{e}})$ is the simplest model based on symmetric non-ideal mixing behaviour. A more complicated dependence of ΔG^{E} on x_{e} is also highly likely, because of the different molecular volumes of the two molecules. Systematic experiments similar to the partitioning experiment according to arrow (3), but starting from different D_{t} concentrations, or experiments according to arrow (2), but with different surfactant concentrations in the cell, should give information on the x_{e} dependence of the partitioning coefficient and also of the transfer enthalpy ΔH^{T}. These will be performed in the future.

Partition coefficients for the partitioning of OG into phosphatidylcholines have been measured before using various different methods. Jackson et al. [5] report a value of $K = 0.059$ mM^{-1} ($P = 3274$) for OG partitioning into egg-PC. Almog et al. [8] measured a constant value of $K = 0.033$ mM^{-1} ($P = 1830$). Bayerl et al. [16] used DSC measurements to estimate the partition coefficient of OG into DMPC vesicles and reported values between 0.041 and 0.057 mM^{-1} ($P = 2275$–3163). Paternostre et al. [17] measured $K = 0.09$ mM^{-1} ($P = 4995$) for the egg PC/OG system. Ueno [13] and also Paternostre et al. [18] report decreasing partition coefficients with increasing saturation of the PC bilayers with OG. Ueno measured a value of $K = 0.075$ mM^{-1} ($P = 4162$) at OG concentrations below 4 mM. At higher OG content the partition coefficient decreases by a factor of 2–4. Similar results were obtained recently by Paternostre et al. [18], again for OG partitioning into egg-PC vesicles. A continuously changing K from $x_{\mathrm{e}} = 0$ to $x_{\mathrm{e}} = 0.5$ was observed, with values $K \approx 0.125$ mM^{-1} ($P \approx 7000$) for $x_{\mathrm{e}} \to 0$ and $K \approx 0.018$ mM^{-1} ($P \approx 1000$) for $x_{\mathrm{e}} \approx 0.5 \to x_{\mathrm{e}}^{\mathrm{sat}}$.

These results are qualitatively similar to our own for OG partitioning into DMPC bilayers in the liquid-crystalline phase. Differences in quantitative aspects can be related to the different chain length of the lipid and/or the presence of unsaturated chains in egg-PC. On the other hand, the experimental values reported by Ueno and Paternostre et al. for the same system also show some differences, which are in the range of our experimentally determined P values.

The temperature dependence of the partition coefficients and of the transfer enthalpies ΔH^{T} follows the previously observed behaviour for micellization processes of surfactants. The partition coefficients decrease slightly with temperature but the ΔH^{T} values show the familiar change of sign at high temperatures. When the quantitative data for OG transfer into DMPC and soy bean PC bilayers are compared to the transfer of OG into pure OG micelles some interesting aspects are observed. From the values shown in Table 1 the thermodynamic functions for transfer of OG from a pure OG-micelle to a bilayer at low x_{e}-values can be calculated (see Table 3).

The ΔG^{T} values for OG transfer into PC vesicles at low values of x_{e} are always more negative than for transfer into pure OG micelles, i.e. the partitioning into bilayers is more favourable than into micelles, i.e. the ΔG^{T} value for transfer from micelle to bilayer is slightly negative with a negligible temperature dependence (see Tables 1 and 3). The transfer enthalpies ΔH^{T} for transfer from water into DMPC bilayers at 27°C is more positive than for the transfer

Table 3
Thermodynamic data for the transfer of OG from OG micelles to lipid bilayers of DMPC and soy bean PC

	DMPC		Soy bean PC	
T	27°C	70°C	27°C	70°C
ΔG^{T}	−400	−344	−414	−418
[cal/mol]	(−555)	(−385)		
ΔH^{T}	+1536	−110	−174	−84
[cal/mol]	(+1600)	(−351)		
$T\Delta S^{\mathrm{T}}$	+1935	+234	+240	−334
[cal/mol]	(+2155)	(+34)		
$\Delta C_{\mathrm{p}}^{\mathrm{T}}$ [cal·mol^{-1}·K^{-1}]	−38.3 (−45.5)		+2.0	

Numbers in parenthesis refer to transfer data obtained from the partition experiment according to arrow (3) in Fig. 1.

into micelles resulting in a positive transfer enthalpy from micelle into bilayers (see Table 3) The temperature dependence for transfer of OG from water into DMPC above T_m, i.e. into liquid-crystalline DMPC bilayers is stronger than into soy bean PC bilayers or into OG micelles. The resulting ΔC_p^T values are very similar for OG transfer from water into soy bean-PC or OG micelles but considerably larger for transfer into DMPC bilayers. This leads to ΔC_p^T values, which are almost zero for transfer from a micelle to soy bean PC but -40 cal · mol^{-1} · K^{-1} for transfer from an OG micelle into DMPC bilayers.

The change in heat capacity is linearly related to the change in hydrophobic surface area exposed to water or hydrocarbon solvent during the transfer process [2,19]. For the transfer from water to hydrocarbon solvent it was found:

$$\Delta C_p^T = -33 n_H [\mathrm{J \cdot mol^{-1} \cdot K^{-1}}] = -7.88 n_H [\mathrm{cal \cdot mol^{-1} \cdot K^{-1}}] \quad (14)$$

with n_H the number of hydrogen atoms [19]. The ΔC_p^T value of ~ -40 cal · mol^{-1} · K^{-1} corresponds to ~ 5 H atoms or 2.5 methylene groups. Therefore, one can conclude that in DMPC bilayers of low OG content more CH_2 groups of OG are shielded from water than in an OG micelle. This is not so in a soy bean PC bilayer. A plausible explanation would be that a soy bean PC bilayer is considerable more disordered than a DMPC bilayer because of the unsaturated fatty acyl chains in soy bean PC. ΔC_p^T values are a reliable measure for the existence of hydrophobic effects, i.e. the change in exposure of hydrophobic groups to water. When similar systems are compared, i.e. the transfer of one particular molecule into different environments, one can also use the entropy of transfer or the Gibbs free energy of transfer, although these quantities can also contain effects from rearrangement of solvation shells around polar groups and changes in order of the bilayer. For the transfer from water to hydrocarbon the $T\Delta S^T$ term increases by ~ 700–800 cal · mol^{-1} · K^{-1} per CH_2 group at $T = 298$ K with the assumption that the transfer enthalpy is zero [10]. The $T\Delta S^T$-values at 27°C in Table 3 for transfer from OG micelles into DMPC bilayers are ~ 2000 cal · mol^{-1} · K^{-1} corresponding again to ~ 2.5 CH_2 groups, for transfer into soy bean PC they are negligible. Therefore, the ΔC_p^T and the $T\Delta S^T$-values are consistent with each other. This indicates that the contributions from changes in head group interactions or changes in order in the hydrocarbon chain region can be neglected when the transfer from micelle to bilayer is considered.

This is different for the transfer of OG from water to micelle or bilayer. The $T\Delta S^T$ terms (see Table 1) would indicate the removal of 8 and 10.5 CH_2 groups from water, respectively. This is clearly not realistic for a surfactant with 7 CH_2 and 1 CH_3 group. It shows that the $T\Delta S^T$ terms for the transfer from water to micelle or bilayer contain contributions from changes in head group interactions. The ΔC_p^T values are here reliable and show that ~ 5 and 7.5 CH_2 groups are shielded from water when OG is transferred into a micelle or into a DMPC bilayer, respectively.

4.2. Solubilization of PC bilayers by OG

Titration of PC vesicles with increasing amounts of surfactant leads to solubilization of vesicles until only mixed micelles are present. The partition coefficient decreases up to x_e^{sat} to a value of 1700–2000, depending on temperature and on the phospholipid that is solubilised (see Table 2). When the effect of temperature on the solubilization parameters of DMPC and soy bean PC is considered, one can see that the P^{sat} values decrease slightly with temperature for DMPC but stay almost constant for soy bean PC. There is also a slight increase in $D_W^{\#}$ at saturation by 1 mM for DMPC but a negligible one for soy bean PC. $D_W^{\#}$ is the critical surfactant concentration in water in equilibrium with the bilayer. The critical micellar concentration (cmc) changes with temperature and has a minimum at ~ 47°C for OG [2]. At 70°C the cmc is almost the same. For $D_W^{\#}$ a similar temperature dependence is to be expected. However, the minimum does not necessarily have to occur at the same temperature. The values of $D_W^{\#}$ are slightly higher at 70°C, indicating that the minimum is possibly at slightly lower temperature. Da Graça Miguel et al. measured the temperature dependence of $D_W^{\#}$ in the temperature range between 5 and 35°C and found a continuous decrease [12]. However, the temperature dependence of $D_W^{\#}$ is not linear, but curved, and would indicate a minimum between 40 and 50°C in agreement with the suggestions.

At 70°C the R_e^{sat} values are increased from 1.56 at 27°C to 1.66 for DMPC and from 1.55 at 27°C to 1.72 for soy bean PC. At high temperature, the bilayers are more expanded and can obviously take up more OG before becoming unstable. The corresponding x_e^{sat} values are 0.608 at 27°C and 0.624–0.63 at 70°C.

The P^{sol} values show a decrease with temperature, in this case for both systems. The R_e^{sol} values are considerably different for DMPC and soy bean PC at 27°C with 1.79 and 3.06, respectively. Therefore, the coexistence region for mixed vesicles and mixed micelles is much wider for soy bean PC compared to DMPC. This difference is decreased at 70°C (see Fig. 6 and Table 2). Still, the coexistence region remains wider for the soy bean PC system than for OG/DMPC.

The model proposed by Lichtenberg et al. [6–9] requires that the concentrations D_W^{sat} and D_W^{sol} are the same because the chemical potential of the OG monomer in the two-phase region has to stay constant. However, the extrapolations of the experimental points on the phase boundaries to $D_t = 0$ do not lead to the same ordinate values. The reason for the difference of 1–2 mM between D_W^{sat} and D_W^{sol} is not clear. One possible explanation is that the precision of the data is not sufficient, particularly in samples with low PC content. A second explanation, which we believe is more likely, is the possibility that we make a systematic error, when determining points on the coexistence lines from the first derivatives of our experimental curves. As evident from Fig. 7, the D_t^{sat} values determined by titration calorimetry are approximately 1 mM higher than those determined in the usual way from the maximum of the light scattering intensity. For the D_t^{sol} values this difference is not so clear.

One has to keep in mind that micelles and bilayers in equilibrium cannot be viewed as real 'phases' with constant composition. It is possible, that shape changes of the micelles occur in the 'coexistence region' which then lead to changes in the chemical potential of OG in the aggregates. These effects could also be dependent on the total lipid concentration. The equilibrium conditions for the chemical potential then require that the chemical potential of the OG monomer in water and also for OG in the vesicles changes in a similar way. Therefore, it is possible that the OG monomer concentration increases slightly in the 'coexistence region'. Similar effects are observed for surfactant water systems, where monomer concentration also increases slightly above the cmc. Only for the case of an aggregate with an infinite number of molecules (the true phase separation case) would it stay constant [10].

In principle, the complete enthalpogram of a solubilization experiment can be simulated using the appropriate thermodynamic models. This has been shown before for the solubilization of POPC by $C_{12}EO_8$ [4]. In the case of PC solubilization by OG the situation is more complicated, because the monomer concentration of OC can no longer be neglected in the calculations. Nevertheless, we calculated an enthalpogram for the solubilization of DMPC at 27°C based on the previously determined parameters ΔH^T, $P(x_e = 0)$, ΔH_{demic}, and ρ for the range $x_e < x^{sat}$ (see Fig. 9). The calculated curve shows significant deviations from the experimental points. Obviously, our assumptions that the concentration

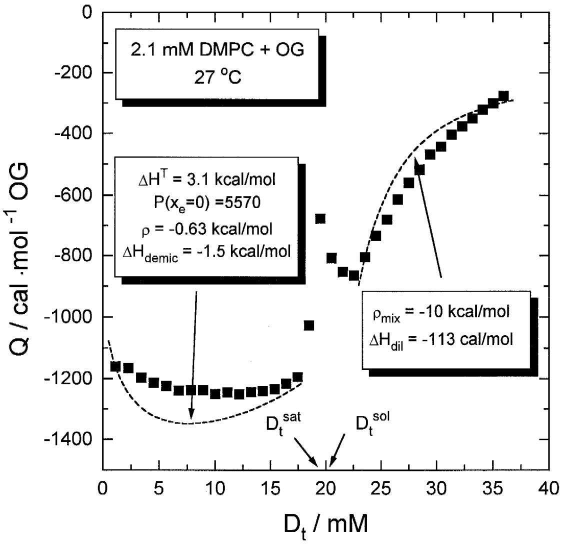

Fig. 9. Experimental calorimetric solubilization curve of a 2.1 mM DMPC vesicle suspension with OG from Fig. 5. The dashed curve for $x_e < x_e^{sat}$ was calculated with the indicated parameters. For $x_e > x_e^{sol}$ the dashed curve was calculated using non-ideal mixing of micelles with a non-ideality parameter ρ as indicated (see text for further explanation).

dependence of P can be described by a simple symmetric mixing model and that the excess mixing enthalpy $\Delta H^{E} = 0$ are too simple.

For surfactant concentrations larger than D_t^{sol}, the observed experimental heats are due to mixing effects of pure surfactant micelles with OG/DMPC mixed micelles. We have tried to calculate the enthalpogram in this concentration range using the approach presented before [4]. We assumed non-ideal mixing using regular solution theory with a non-ideality parameter ρ_{mix} and incorporated a dilution term ΔH_{dil} for the micelles, which was experimentally determined. In addition, the change in monomer concentration from $D_W = \text{cmc}$ for pure micelles to D_W^{sol} was also considered. The non-ideality parameter turned out to be strongly negative but the fit to the experimental curve is far from being satisfactory. For solubilization enthalpograms at 70°C the same problems arise. Here, the non-ideality parameter ρ for the mixing of pure OG micelles with DMPC/OG micelles becomes strongly positive. This change of sign in the non-ideality parameters would indicate that hydrophobic effects are involved in the process of equilibration of pure and mixed micelles. The non-ideality parameter is negative at 27°C and positive at 70°C. It has therefore the same sign as the transfer enthalpy of OG from a micelle to water. However, equilibration of pure and mixed micelles leads to a decrease of the monomer concentration in water, i.e. D_W^{sol} is lower than the cmc. This corresponds to a removal of hydrophobic surfaces from water. Therefore, the observed effects can only be explained by the assumption that in the mixed micelles more hydrophobic surfaces are exposed to water than in a pure micelle. This assumption is not unreasonable, because the molecular shapes of the surfactant and the lipid are very different. This shape difference could lead to packing problems in the mixed micelles so that more methylene groups can come into contact with water.

In principle, the titration curves in the intermediate regime between x_e^{sat} and x_e^{sol} can also be simulated [4]. In the case of OG/PC mixtures the coexistence range is relatively narrow, particularly for the saturated lipids. In addition, the system is very complex, as has been shown above. We have therefore refrained from trying to simulate the experimental data in this region until we have a better understanding of the thermodynamics of these systems.

Differences in molecular shape are also the reason for the phenomenon of solubilization of lipid bilayers by surfactants. Bilayer forming molecules have a cylindrical shape whereas surfactant molecules have a more cone-like shape due to the fact that the amphiphile head group requires more space than the cross-sectional area of the tail can afford. The critical packing parameter $P = V/(s \cdot l) < 1$ for surfactant molecules ($V = \text{vol}$ of surfactant, $s =$ effective surface area of the head group at the interface, $l =$ effective length of the molecule) leads to the tendency to form curved surfaces [20]. When cone shaped molecules such as surfactants are introduced into lipid bilayers strong perturbations in packing are the result. With increasing surfactant content of the bilayers the tendency to form curved surfaces increases and the bilayers finally become unstable. Fattal et al. [21] have recently developed a model for the vesicle-micelle transition based on these considerations of the different packing requirements of phospholipids and surfactants. Depending on the chain length mismatch between phospholipid and surfactant, different R_e^{sat} values are to be expected. For increasing mismatch they calculated for constant surface area of the surfactant a decrease in R_e^{sat} and also a decrease in R_e^{sol}. In their calculations the lipid chain length was constant but the surfactant chain length was changed. We did the opposite, we used OG and increased the chain length of the lipid from DMPC to DSPC, thus increasing the mismatch. Our data for solubilization at 70°C indicate a decrease for R_e^{sat} from 1.66 (DMPC) over 1.5 (DPPC) to 1.33 (DSPC) with increasing mismatch, in accordance with the calculations of Fattal et al. [21]. The predicted decrease in R_e^{sol} with increasing mismatch is not found. Here, the R_e^{sol} values for DMPC and DPPC are relatively similar with 2.03 and 1.94, respectively. However, for DSPC R_e^{sol} increases to 2.47, in contradiction to the theory.

5. Summary and conclusions

We have shown that isothermal titration calorimetry is a suitable method to determine the position of the coexistence lines in lipid/surfactant systems and have applied this method to study the interaction of

OG with the saturated phospholipids DMPC, DPPC, and DSPC. As a control we also investigated mixtures of OG with soy bean PC, a lipid with unsaturated chains. Different calorimetric experiments can be performed to determine the partition coefficient of OG between water and bilayers below the solubilization of the vesicles. The partition coefficient turned out to be strongly dependent on the effective mole fraction x_e of the surfactant in the bilayer, decreasing with increasing x_e. Comparison of the thermodynamic transfer data of OG from water to PC bilayers showed that the ΔG^T values were slightly more negative for this process than for the transfer from water to a micelle. In the case of DMPC at 27°C the ΔH^T value for transfer into bilayers was more positive than for transfer into a micelle, indicating that there is an enthalpy difference between micelles and bilayers. This was not so for unsaturated soy bean PC and for DMPC at higher temperature. Obviously, the properties of bilayers with unsaturated chains or with saturated chains at higher temperature are relatively similar to micelles.

The solubilization of saturated PCs with different chain length showed that the mole fraction at saturation x_e^{sat} decreases with increasing chain length difference between OG and PC. For soy bean PC the width of the coexistence region between mixed micelles and mixed vesicles is considerably wider than for the short chain DMPC

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