

# Correlation of Membrane/Water Partition Coefficients of Detergents with the Critical Micelle Concentration

H. Heerklotz and J. Seelig

Department of Biophysical Chemistry, Biocenter of the University of Basel, Basel, Switzerland

**ABSTRACT** The membrane/water partition coefficients,  $K$ , of 15 electrically neutral (non-charged or zwitterionic) detergents were measured with phospholipid vesicles by using isothermal titration calorimetry, and were compared to the corresponding critical micellar concentrations, cmc. The detergents measured were oligo(ethylene oxide) alkyl ethers ( $C_mEO_n$  with  $m = 10/n = 3, 7$  and  $m = 12/n = 3, \dots, 8$ ); alkylglucosides (octyl, decyl); alkylmaltosides (octyl, decyl, dodecyl); diheptanoylphosphatidylcholine; Tritons (X-100, X-114) and CHAPS. A linear relation between the free energies of partitioning into the membrane and micelle formation was found such that  $K \cdot CMC \sim 1$ . The identity  $K \cdot CMC = 1$  was used to classify detergents with respect to their membrane disruption potency. "Strong" detergents are characterized by  $K \cdot CMC < 1$  and solubilize lipid membranes at detergent-to-lipid ratios  $X_b < 1$  (alkylmaltosides, tritons, heptaethylene glycol alkyl ethers). "Weak" detergents are characterized by  $K \cdot CMC > 1$  and accumulate in the membrane- to detergent-to-lipid ratios  $X_b > 1$  before the bilayer disintegrates (alkylglucosides, pentaethylene glycol dodecyl ether).

## Introduction

The understanding of detergent-lipid interactions is of practical importance to solubilize and purify membrane proteins and membrane lipids, and to reconstitute a membrane protein in a native environment (Helenius and Simons, 1975; Helenius et al., 1981; Banerjee et al., 1995; Lasch, 1995). Knowledge of the forces governing lipid-detergent interactions may also shed light on fundamental membrane problems such as lipid clustering, domain formation, and detergent-resistant membrane patches (Brown and London, 1998; Solomon et al., 1998; Schroeder et al., 1998). Which membrane properties decide whether a membrane becomes resistant or not to a particular detergent? Which are the characteristic features of "bicelles," well-defined mixtures of lipid and detergents, which lead to an almost perfect alignment of these structures in a sufficiently strong magnetic field (Sanders and Landis, 1995; Vold and Prosser, 1996)?

To answer such questions, detailed phase diagrams have been established for a limited number of pure phospholipid-surfactant systems providing a quantitative description of the composition, structure, and coexistence range of the different mesophases. In the following we address a more specialized question which, nevertheless, appears to be of general relevance. How does the tendency of a surfactant to form micelles correlate with its potency to penetrate into membranes and to eventually disrupt membranes? To this purpose we have measured the water  $\rightarrow$  lipid bilayer partition coefficients,  $K$ , of 15 different, electrically neutral

detergents at concentrations well below their critical micellar concentration. We have determined the binding isotherm with high sensitivity isothermal titration calorimetry (ITC) and have described the experimental data with a simple partition equilibrium. The partition coefficients,  $K$ , were then correlated with the corresponding critical micellar concentration, CMC. All measurements were made with vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) with a diameter of  $\sim 100$  nm.

## Experimental

### Materials

The lipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (D7PC) were purchased from Avanti Polar Lipids, Alabaster, AL. The surfactants *n*-decyl- $\beta$ -D-glucopyranoside ( $C_{10}$ -Gluc), *n*-octyl- $\beta$ -D-maltopyranoside ( $C_8$ -Malt), *n*-decyl- $\beta$ -D-maltopyranoside ( $C_{10}$ -Malt), and *n*-dodecyl- $\beta$ -D-maltopyranoside ( $C_{12}$ -Malt) were from Anatrace, Maumee, OH in Anagrade (i.e., >99% HPLC) purity. Triton X-100, Triton X-114, and CHAPS were from Fluka BioChemika (Buchs, Switzerland). The oligo(ethylene oxide) alkyl ethers ( $C_mEO_n$  with  $m = 10/n = 3, 7$  and  $m = 12/n = 3, \dots, 8$ ) were obtained from Nikko Chemicals, Tokyo, Japan.

The dry lipid was weighed and dispersed in buffer (TRIS 10 mM + NaCl 100 mM, pH 7.4) by vortexing. Large unilamellar vesicles were formed by extrusion through two stacked Nuclepore polycarbonate membranes of 100 nm pore size (MacDonald et al., 1991).

### Isothermal titration calorimetry

Isothermal titration calorimeters of the types Omega and VP produced by MicroCal Inc. (Northampton, MA) were used. The cell (volume 1.4 ml) was filled with a detergent solution at a concentration of about one-third of the CMC or less. The reference cell contained buffer only. The injection syringe was filled with 250  $\mu$ l of a 20 mM or 40 mM POPC vesicle dispersion, and a series of typically 10  $\mu$ l injections was made. At each injection, surfactant was incorporated into the lipid membranes, leading to a characteristic heat signal. Integration of the individual calorimeter traces yielded the heat of binding,  $h_i$ , of each injection step.

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Address reprint requests to Dr. Joachim Seelig, Dept. of Biophysical Chemistry, Biocenter of the University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland. Tel.: 41-61-267-2190; Fax: 41-61-267-2189; E-mail: joachim.seelig@unibas.ch.

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## Partitioning model

A simple partition equilibrium was assumed to describe the partitioning of surfactant between the aqueous phase and the lipid membrane according to:

$$X_b = K \cdot C_{D,f} \quad (1)$$

where  $C_{D,f}$  is the equilibrium concentration of detergent D in the aqueous phase. The degree of binding,  $X_b$ , is defined as

$$X_b = \frac{n_{D,b}}{n_L^0} = \frac{C_{D,b}}{C_L^0} \quad (2)$$

where  $n_{D,b}$  denotes the molar amount of bound detergent and  $n_L^0$  that of total lipid  $C_{D,b}$  and  $C_L^0$  are the corresponding concentrations referred to the same volume  $V$  (in the present context:  $V_{\text{cell}}$ ). Mass conservation implies that  $C_D^0 = C_{D,b} + C_{D,f}$  leading to

$$C_{D,b} = \frac{K \cdot C_L^0}{1 + K \cdot C_L^0} \cdot C_D^0 \quad (3)$$

In the present experiment the concentration of detergent in the calorimeter cell is fixed (except for a small dilution effect) and the concentration of lipid,  $C_L^0$ , is increased by consecutive injections of liposomes. The variation of the bound detergent is given by the first derivative of Eq. 3:

$$\delta C_{D,b} = \frac{KC_D^0}{(1 + KC_L^0)^2} \delta C_L^0 \quad (4)$$

The molar amount of detergent transferred from the aqueous phase to the membrane is

$$\delta n_{D,b} = \delta C_{D,b} \cdot V_{\text{cell}} \quad (5)$$

where  $V_{\text{cell}}$  denotes the volume of the calorimeter cell. If the transfer reaction is characterized by a reaction enthalpy,  $\Delta H$ , each injection of lipid adds  $\delta n_L^0 = \delta C_L^0 V_{\text{cell}}$  moles of lipid and produces a heat of

$$\delta h_i = \delta C_L^0 \frac{KC_D^0}{(1 + KC_L^0)^2} \cdot V_{\text{cell}} \cdot \Delta H \quad (6)$$

Taking into account dilution effects for  $C_L^0$  and  $C_D^0$  the above results are equivalent to previous expressions derived for  $\sum \delta h_i$ , i.e., the sum of heats released or consumed during the first  $i$  injection steps (Wenk et al., 1997; Wenk and Seelig, 1997).

Another widely used model to describe detergent-lipid interactions is (cf. Lasch, 1995):

$$\frac{C_{D,b}}{C_{D,b} + C_L^0} = \frac{X_b}{1 + X_b} = K_s C_{D,f} \quad (7)$$

In this model, lipid and bound detergent together constitute the matrix for the incorporation of new detergent. A comparison of Eqs. 1 and 7 leads to

$$K = K_s(1 + X_b) \quad (8)$$

indicating a nonlinear relationship between the two binding constants. Previous studies using Eq. 7 have indeed demonstrated experimentally that  $K_s$  decreases substantially with increasing  $X_b$  (Heerklotz et al., 1994; Keller et al., 1997; Paternostre et al., 1995). Fitting the previous data of Heerklotz et al. (1994) with Eq. 1 yields a constant  $K$ . For all measurements performed in connection with this study, the partition model (1) provided the best fit to the data with a constant  $K$  for the concentration range measured. The difference between Eq. 1 and Eq. 7 is  $\sim 10\%$  at  $X_b = 0.1$ ,  $33\%$  at  $X_b = 0.25$ , and  $67\%$  at  $X_b = 0.4$ . Experimental  $X_b$  values measured in this study fall in the range  $0 < X_b \leq 0.3$ .

## Results

Fig. 1 *A* shows a titration pattern obtained by injecting  $10 \mu\text{L}$  aliquots of a POPC vesicle suspension ( $10 \text{ mM}$ ) into a  $150 \mu\text{M}$   $C_{10}E0_7$  detergent solution. Each injection produced an endothermic heat of reaction which decreased with consecutive injections as less and less surfactant was free in solution. In a separate experiment, the same phospholipid suspension was injected into pure buffer. The heat of dilution was found to be small. As a second control, buffer without lipid was injected into the detergent solution. Since the concentration of the latter was well below the critical micellar concentration ( $\text{CMC} = 850 \mu\text{M}$ ) the heats of detergent dilution were again small. Fig. 1 *B* displays the integrated titration peaks as a function of the total lipid concentration. The solid line represents the theoretical simulation using a partition coefficient of  $K = 770 \text{ M}^{-1}$ , a molar binding enthalpy of  $\Delta H = 27 \text{ kJ/mol}$ , and a constant heat of dilution of  $-13.8 \mu\text{J}$  per injection. An excellent agreement between theory and experiment is obtained. For each detergent, typically three different detergent concentrations were measured, and Fig. 1 *B* also contains the

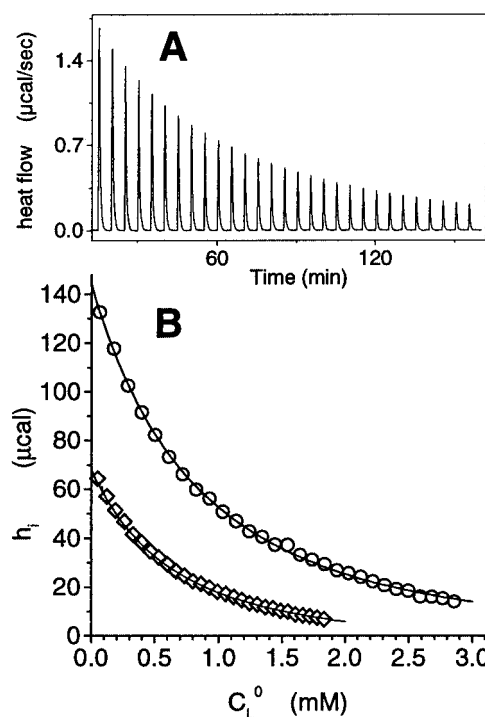


Fig. 1 Titration of  $C_{10}E0_7$  with  $100 \text{ nm}$  POPC vesicles. (*A*) Calorimetric traces (heat flow) observed upon addition of POPC vesicles ( $C_L^0 = 10 \text{ mM}$ ) into  $150 \mu\text{M}$   $C_{10}E0_7$ , both in buffer ( $100 \text{ mM NaCl}$ ,  $10 \text{ mM Tris}$ ,  $\text{pH } 7.4$ );  $10 \mu\text{L}$  injections of lipid-vesicles at  $60\text{-min}$  intervals. (*B*) Heats of reaction as obtained from the integration of the calorimetric traces. ( $\diamond$ )  $150 \mu\text{M}$   $C_{10}E0_7$  titrated with  $10.0 \text{ mM}$  POPC. Integration of the heat flow measurements shown in (*A*). ( $\circ$ )  $250 \mu\text{M}$   $C_{10}E0_7$  titrated with  $14.8 \text{ mM}$  POPC. The solid lines correspond to theoretical fits using the partition equilibrium  $X_b = K C_{D,f}$ . The fit parameters were  $K = 770 \text{ M}^{-1}$  and  $\Delta H = 27 \text{ kJ/mol}$ .

results of a second titration at a higher detergent concentration (250  $\mu\text{M}$   $\text{C}_{10}\text{EO}_7$  titrated with 14.8 mM POPC vesicles). Within the accuracy specified in Table 1 the data could be described by the same  $K$  and  $\Delta H$  values as given above.

A slightly different situation is encountered for *n*-decyl- $\beta$ -D-maltopyranoside ( $\text{C}_{10}$ -Malt). Fig. 2 *A* shows the titration of  $\text{C}_{10}$ -Malt at a concentration of 0.5 mM (CMC = 1.8 mM) with 100 nm POPC vesicles ( $C_L^0 = 39.7$  mM). The titration pattern has a similar shape as observed for  $\text{C}_{10}\text{EO}_7$ ; however, for the analysis of the data (Fig. 2 *B*) only the outer lipid layer was taken into account (50% of total lipid)

leading to  $K = 195 \text{ M}^{-1}$  and  $\Delta H = 13.8 \text{ kJ/mol}$  for the optimum fit. The choice of  $C_L^0/2$  as the relevant lipid concentration reflects an asymmetric incorporation of  $\text{C}_{10}$ -Malt into the outer vesicle monolayer only, and is based on experiments with radiolabeled  $\text{C}_{12}$ -Malt demonstrating a slow flip-flop of this detergent in bilayer membranes (Kragh-Hansen et al., 1998). We have confirmed this finding for  $\text{C}_{12}$ -Malt (data not shown) with an ITC release assay specifically developed to detect asymmetric binding and slow flip-flop (Heerklotz et al., 1999). By analogy, we assume that a half-sided incorporation and a slow flip-flop are also characteristic for  $\text{C}_{10}$ -Malt,  $\text{C}_8$ -Malt, D7PC, and

**Table 1** Micelle formation and partitioning of surfactants into POPC membranes at room temperature: Thermodynamic parameters

Surfactant	$\Delta H$ (KJ/mol)	$K$ ( $10^3 \text{ M}^{-1}$ )	$K$ from literature ( $10^3 \text{ M}^{-1}$ )	CMC ( $10^{-3} \text{ M}$ )	$K \cdot \text{CMC}$	$X_b^{\text{sal}}$
Oligo (ethylene oxide) alkyl ether						
$\text{C}_{10}\text{EO}_3$	8	6		0.6 <sup>    </sup>	0.36	LAM <sup>    </sup>
$\text{C}_{10}\text{EO}_7$	27	0.77		0.85 <sup>    </sup>	0.65	$0.61 \pm 0.05$ <sup>    </sup>
$\text{C}_{12}\text{EO}_3$	5.5	100	50*	0.06 <sup>+++</sup>	6	LAM <sup>++</sup>
$\text{C}_{12}\text{EO}_4$	12	35	32*	0.06 <sup>+++</sup>	2.1	LAM <sup>++</sup>
$\text{C}_{12}\text{EO}_5$	16	24	23*	0.065 <sup>+++</sup>	1.56	$3.1 \pm 0.4$ <sup>++</sup>
$\text{C}_{12}\text{EO}_6$	20	20	16*	0.065 <sup>+++</sup>	1.3	$1.2 \pm 0.1$ <sup>++</sup>
$\text{C}_{12}\text{EO}_7$	20	12	12*, 7 <sup>†</sup>	0.075 <sup>+++</sup>	0.9	$0.7 \pm 0.03$ <sup>++</sup>
$\text{C}_{12}\text{EO}_8$	32	6	8*, 6, 9 <sup>¶</sup>	0.09 <sup>+++</sup>	0.54	$0.57 \pm 0.03, 0.62$ <sup>¶</sup>
Tritons						
TX-100	15	3.0	3.4, <sup>  </sup> 2–4**	0.23 <sup>\$\$\$</sup>	0.69	$0.8, \uparrow\uparrow\uparrow 0.64$ <sup>  , **</sup>
TX-114	8	3.5		0.17 <sup>\$\$\$</sup>	0.59	$0.9$ <sup>†††</sup>
Alkyl (thio) glucosides						
$\text{C}_8$ -Gluc	5.4 <sup>††</sup>		0.12, <sup>††</sup> 0.11, <sup>  </sup> 0.09, <sup>\$\$\$</sup> 0.08–0.12 <sup>¶¶</sup>	23 <sup>¶¶¶</sup>	2.3	$1.3, \uparrow\uparrow,   , \uparrow\uparrow\uparrow 1.6$ <sup>\$\$\$</sup>
$\text{C}_8$ -Thioglug	~4–8 <sup>    </sup>		0.24 <sup>    </sup>	9 <sup>¶¶¶</sup>	2.1	1.5 <sup>¶¶</sup>
$\text{C}_{10}$ -Gluc	4.9	1.6		2.2 <sup>¶¶¶</sup>	3.5	LAM <sup>    </sup>
Alkyl maltosides						
$\text{C}_8$ -Malt	10	0.025		19.5 <sup>¶¶¶</sup>	0.487	
$\text{C}_{10}$ -Malt	14	0.2		1.8 <sup>¶¶¶</sup>	0.36	
$\text{C}_{12}$ -Malt	4	5	8–19***	0.17 <sup>¶¶¶</sup>	0.85	0.9***
Phospholipid						
D7PC	7	0.2		1.9 <sup>    </sup>	0.38	$0.08 \pm 0.03$ <sup>    </sup>
Steroid						
CHAPS	29	0.6		10	6	

The values of  $K$  measured at different detergent concentrations vary by 4 to 20%, indicating the experimental error and also possible variation of  $K$  with the detergent concentration. LAM = lamellar phase is formed at high detergent concentration. Most literature data refer to EYPC.

The binding constants for  $\text{C}_8$ -,  $\text{C}_{10}$ -,  $\text{C}_{12}$ -Malt, D7PC, and CHAPS were calculated assuming detergent incorporation into the outer halflayer only.

\* (Heerklotz et al., 1994).

† (Heerklotz et al., 1999).

‡ (Heerklotz et al., 1996).

§ (Ueno, 1989).

¶ (Edwards and Almgren, 1991).

|| Paternostre et al., 1988).

\*\* (De la Maza and Parra, 1994b).

†† (Wenk et al., 1997).

‡‡ (Heerklotz et al., 1997).

\$\$\$ (Opatowski et al., 1997).

¶¶ (de la Maza and Parra, 1994a).

|||| (Wenk and Seelig, 1997).

\*\*\* (de la Maza and Parra, 1997).

††† (Partearroyo et al., 1996).

‡‡‡ (Heerklotz, 1996).

\$\$\$ (Sigma Catalog, St. Louis, MO, USA).

¶¶¶ (Anatrace Catalog, Maumee, OH, USA, 1999).

|||| (H. Heerklotz, unpublished observations).

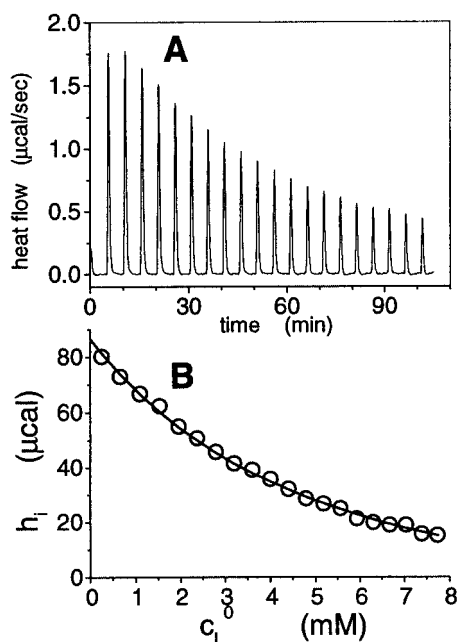


Fig. 2 Titration of  $C_{10}$ -Malt with 100 nm POPC vesicles. (A) Injection of POPC vesicles (39.7 mM) into a 0.5 mM  $C_{10}$ -Malt solution. The injection volumes were 2  $\mu$ l, 13  $\mu$ l, and 15  $\mu$ l for all further injections. (B) Heats of reaction as a function of total lipid concentration. The solid line corresponds to the best theoretical fit using the partition equilibrium (Eq. 1) with  $K = 195 \text{ M}^{-1}$  and  $\Delta H = 13.8 \text{ kJ/mol}$ . For the analysis it was assumed that  $C_{10}$ -Malt cannot translocate to the inner monolayer during the time of the ITC experiment, and that the lipid available for surfactant binding is only the outer monolayer (effective lipid concentration of  $C_{L,\text{eff}} = C_L^0/2$ ).

CHAPS. For all other detergents used in this study, a fast flip-flop and a symmetric partitioning have been established (Keller et al., 1997; leMaire et al., 1987; Wenk et al., 1997; Heerklotz et al., 1999).

It should be noted that the choice of the lipid concentration has no influence on the quality of the fit or on the enthalpy  $\Delta H$ . Equation 3 shows that the steepness of the binding isotherm is determined by the product  $K C_L^0$  and that  $(aC_L^0) \cdot (K/a)$  must yield an equally good fit. In Fig. 2 B the assumption of  $a = 0.5$  was made and the binding constant for asymmetric distribution is thus by a factor of  $1/a = 2$  larger than for symmetric distribution.

The results of all detergent titrations are summarized in Table 1. The table also contains the CMC of the detergents as given in the literature.

## Discussion

A detergent molecule in the aqueous phase has two possibilities, namely 1) to associate with other monomers to form a micelle or 2) to penetrate into the membrane forming a mixed detergent-lipid bilayer. The standard free energy of detergent binding to the lipid membrane is given by

$$\Delta G_{\text{binding}}^0 = -RT \ln(55.5 K) \quad (9)$$

that of micelle formation by

$$\Delta G_{\text{mic}}^0 = RT \ln(\text{CMC}/55.5) \quad (10)$$

The factor 55.5 corresponds to the molar concentration of water and accounts for the fact that the concentration of detergent in solution should be given by its mole fraction rather than by mole/l (Tanford, 1980; Cantor and Schimmel, 1980). At  $K \cdot \text{CMC} = 1$  both processes have equal standard free energies. Lichtenberg (1985) has provided arguments that there should be an intimate correlation between micelle formation, membrane partitioning, and membrane disruption. Fig. 3 then shows a double logarithmic plot of  $K$  vs. CMC. The solid line in the diagram has a slope of  $-1$  and corresponds to  $K \cdot \text{CMC} = 1$ . Inspection of Fig. 3 reveals that the detergents can be grouped in two classes such that the product  $K \cdot \text{CMC}$  is either larger or smaller than unity. To simplify the discussion, we denote detergents with  $K \cdot \text{CMC} < 1$  as “strong” detergents (lower part of the diagram) and those with  $K \cdot \text{CMC} > 1$  as “weak” detergents (upper part of diagram). According to this classification, the tritons,  $C_m\text{EO}_n$  with  $n = 7, 8$ , and the alkyl maltosides are “strong” detergents, whereas the alkyl glucosides and  $C_{12}\text{EO}_n$  with  $n = 3-6$  are “weak” detergents. Within each class, the  $\log K$  vs.  $\log C$  plot runs approximately parallel to the  $K \cdot \text{CMC} = 1$  line, but is displaced along the ordinate. The upper dashed line in Fig. 3 corresponds to  $K \cdot \text{CMC} = 2.2$  (“weak” detergents), the lower to  $K \cdot \text{CMC} = 0.45$  (“strong” detergents), reflecting a shift in free energy of  $\pm 2 \text{ kJ/mol}$  with respect to  $K \cdot \text{CMC} = 1$ .

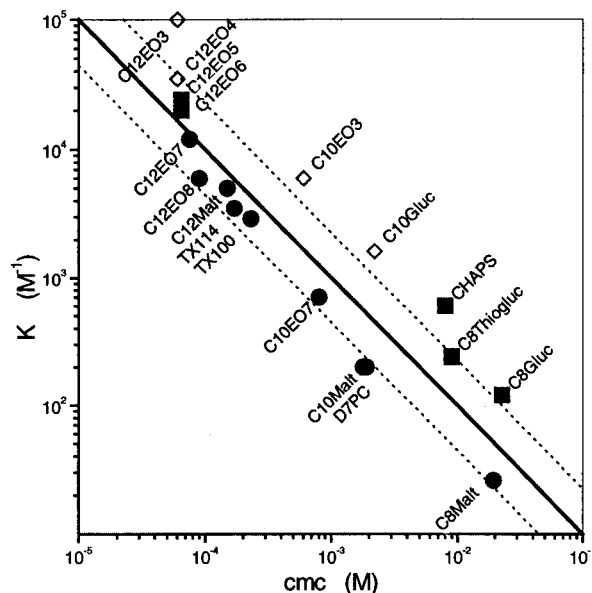


Fig. 3 Double logarithmic plot of the partition constant  $K$  (Eq. 3) for detergent partitioning into membranes versus the corresponding critical micellar concentration, CMC. The solid line represents the relationship  $K \cdot \text{CMC} = 1$ . The dashed lines correspond to free energies which deviate by  $\pm 2 \text{ kJ/mol}$  from the diagonal.



Let us assume, for the sake of the argument, that the lipid bilayer remains stable upon detergent incorporation up to the CMC, i.e., up to the highest monomer concentration possible, and that  $K$  is also constant over the whole concentration range. (This is clearly a fictitious situation since it is known experimentally that the bilayer disintegrates at a detergent concentration  $C_{D,f}^{\text{sat}} < \text{CMC}$ .) Using the partition model (Eq. 1) we thus predict  $X_b^{\text{sat}} \leq K \cdot \text{CMC}$  as the limiting detergent-to-lipid ratio. In Table 1 we have calculated these hypothetical limits (penultimate column) and have compared them with the experimentally determined saturation limits,  $X_b^{\text{sat}}$  (last column). For “strong” detergents the average  $K \cdot \text{CMC}$  is  $0.58 \pm 0.19$  ( $n = 10$ ) and  $X_b^{\text{sat}} = 0.63 \pm 0.23$  ( $n = 9$ ). For “weak” detergents the scatter of the data is larger, with  $K \cdot \text{CMC} = 1.81 \pm 0.7$  ( $n = 4$ ) and  $X_b^{\text{sat}} = 1.76 \pm 0.7$  ( $n = 4$ ). The analysis demonstrates a semi-quantitative agreement between the prediction of the partition model and the experimental results, and also provides a rational basis for the classification scheme used. “Strong” detergents initiate membrane disintegration at a detergent-to-lipid ratio  $< 1$ , “weak” detergents require a detergent-to-lipid ratio  $> 1$ .

If only the CMC is known, the relationship  $K \sim 1/\text{CMC}$  may be used to obtain a first estimate of the membrane binding constant. If the saturating detergent concentration has also been measured, an even better estimate of  $K$  is given by  $K \sim X_b^{\text{sat}}/\text{CMC}$ . Alternatively, if  $K$  and CMC can be determined independently, the limiting detergent concentration in the membrane can be calculated using again  $X_b^{\text{sat}} \sim K \cdot \text{CMC}$ . For “strong” detergents the saturation limits cluster around  $X_b^{\text{sat}} \sim 0.6$ . “Weak” detergents are tolerated in membrane to a much larger extent.

The experimentally observed saturation limit of “strong” detergents can be made plausible by a thermodynamic argument. Since  $K < 1/\text{CMC}$ , micelle formation is favored over bilayer insertion if standard free energies are compared. However, at low detergent concentrations the gain in mixing entropy upon membrane insertion counteracts micelle formation. The additional contribution of the mixing process to the free energy,  $\Delta G_{\text{mix}}$ , can be estimated as

$$\begin{aligned} \Delta G_{\text{mix}} &\approx RT \ln[n_{D,b}/(n_{D,b} + n_l^0)] \\ &= -RT \ln[(1 + X_b)/X_b] \end{aligned} \quad (11)$$

$\Delta G_{\text{mix}}$  is a particularly strong driving force at low  $X_b$  values, but decreases with increasing  $X_b$ . At the saturation limit of  $X_b^{\text{sat}} \sim 0.6$  the free energy of mixing is  $-2.4$  kJ/mol, and is now of the same order as the difference  $RT \ln K - RT \ln \text{CMC} \sim 2$  kJ/mol (Fig. 3). At  $X_b^{\text{sat}} > 0.6$ , the mixing of the detergent with the bilayer is no longer advantageous compared to micelle formation. The above argument assumes ideal mixing and neglects the fact that the properties of the mixed detergent-lipid bilayer undergo gradual and finally abrupt changes near and at  $X_b^{\text{sat}}$ , respectively.

A commonly used model to predict the liquid-crystalline phases formed by an amphiphile is the geometric curvature model. In brief, molecules with a cone shape (large head-group with small hydrocarbon cross-section) form micelles, with an inverted cone shape form inverted structures (e.g., hexagonal phase), and rod-like molecules that have similar headgroup and hydrocarbon chain cross-sections form bilayers (cf. Israelachvili, 1991; Gruner, 1985).

In the present study, the geometry of the detergent molecules varies from almost rod-like (“weak” detergents) to cone-shaped (“strong” detergents). For the  $C_{12}EO_n$  series the cross-sectional area changes from  $0.29 \text{ nm}^2$  for  $n = 3$  to  $1.16 \text{ nm}^2$  for  $n = 8$  (Lantzsch et al., 1996), while the cross-sectional area of a single hydrocarbon chain in a fluid-like membrane is  $0.25\text{--}0.3 \text{ nm}^2$ . If we consider comparable pairs such as  $C_{10}EO_3$  and  $C_{10}EO_7$ ,  $C_8\text{Gluc}$  and  $C_8\text{Malt}$ ,  $C_{10}\text{Gluc}$  and  $C_{10}\text{Malt}$ , and the series  $C_{12}EO_n$  with  $n = 3\text{--}8$ , i.e., the CMCs within the same group are very similar, whereas the  $K$  values differ by a factor of 4–8. In all cases, the detergent with the larger headgroup has the lower  $K$  value. The large headgroups put an additional strain on the lipid packing, leading to an early disruption. In contrast, “weak” detergents with small headgroups induce less tension and are incorporated to larger  $X_b$  values. For the formation of micelles the size of the headgroup appears to be irrelevant because the variable size and shape of the micelles allows an easy adjustment to the constraints imposed by the molecular shape.

The present correlation of  $K \sim 1/\text{CMC}$  (within one order of magnitude) has been shown to hold true so far for POPC membranes. Since POPC is one of the most common natural lipids in mammalian cells, the binding constants summarized in Table 1 can be considered as a guideline in estimating the detergent affinity also with respect to biological membranes. Nevertheless, the binding/partition constant of a detergent is definitely modulated by the actual membrane composition. Unfortunately, experimental data are rather limited. We have previously investigated the partition coefficient of  $C_8\text{-Gluc}$  and octyl- $\beta$ -thioglucopyranoside for mixed POPC/cholesterol membranes (Wenk et al., 1997; Wenk and Seelig, 1997). If POPC and cholesterol together were considered as the matrix for detergent partitioning, the partition constant decreased with increasing cholesterol content. However, if the partition equilibrium was based on POPC alone, the partition constants for both detergents became independent of the cholesterol content and remained constant up to 50 mol % cholesterol. These findings provided evidence for a preferential association of octyl- $\beta$ -d-glucopyranoside and octyl- $\beta$ -thioglucopyranoside with POPC, avoiding the interaction with cholesterol. A related situation could be encountered with “lipid rafts” or “detergent-resistant membranes.” These structures appear to be enriched in sphingomyelin and do not easily incorporate Triton X-100 at  $4^\circ\text{C}$ . By using titration calorimetry the preferential interaction of Triton X-100 with the individual

“lipid raft” components can be elucidated in an analogous manner as demonstrated for the POPC-cholesterol model membranes.

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## References

- Banerjee, P., J. B. Joo, J. T. Buse, and G. Dawson. 1995. Differential solubilization of lipids along with membrane proteins by different classes of detergents. *Chem. Phys. Lipids*. 77:65–78.
- Brown, D. A., and E. London. 1998. Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* 14:111–136.
- Cantor, C. R., and P. R. Schimmel. 1980. *Biophysical Chemistry*. Freeman, San Francisco.
- de la Maza, A., and J. L. Parra. 1994a. Structural phase transitions involved in the interaction of phospholipid bilayers with octyl glucoside. *Eur. J. Biochem.* 226:1029–1038.
- de la Maza, A., and J. L. Parra. 1994b. Vesicle-micelle structural transition of phosphatidylcholine bilayers and Triton X-100. *Biochem. J.* 303:907–914.
- de la Maza, A., and J. L. Parra. 1997. Solubilizing effects caused by the nonionic surfactant dodecylmaltoside in phosphatidylcholine liposomes. *Biophys. J.* 72:1668–75.
- Edwards, K., and M. Almgren. 1991. Solubilization of lecithin vesicles by C12E8. *J. Colloid Interface Sci.* 147:1–21.
- Gruner, S. M. 1985. Intrinsic curvature hypothesis for biomembrane lipid composition: a role for nonbilayer lipids. *Proc. Natl. Acad. Sci. USA*. 82:3665–3669.
- Heerklotz, H. 1996. *Thermodynamische Charakterisierung verdünnter wässriger Lipid-Tensid-Mischungen*. Universität Leipzig, Leipzig.
- Heerklotz, H., H. Binder, G. Lantzsch, and G. Klose. 1994. Membrane/water partition of oligo(ethylene oxide) dodecyl ethers and its relevance for solubilization. *Biochim. Biophys. Acta*. 1196:114–122.
- Heerklotz, H., H. Binder, G. Lantzsch, G. Klose, and A. Blume. 1997. Lipid/detergent interaction thermodynamics as a function of molecular shape. *J. Phys. Chem. B*. 101:639–645.
- Heerklotz, H., G. Lantzsch, H. Binder, G. Klose, and A. Blume. 1996. Thermodynamic characterization of dilute aqueous lipid/detergent mixtures of POPC and C12EO8 by means of isothermal titration calorimetry. *J. Phys. Chem.* 100:6764–6774.
- Heerklotz, H. H., H. Binder, and R. M. Epand. 1999. A “release” protocol for isothermal titration calorimetry. *Biophys. J.* 76:2606–2613.
- Helenius, A., M. Sarvas, and K. Simons. 1981. Asymmetric and symmetric membrane reconstitution by detergent elimination. Studies with Semliki-Forest-virus spike glycoprotein and penicillinase from the membrane of *Bacillus licheniformis*. *Eur. J. Biochem.* 116:27–35.
- Helenius, A., and K. Simons. 1975. Solubilization of membranes by detergents. *Biochim. Biophys. Acta*. 415:29–79.
- Israelachvili, J. N. 1991. *Intermolecular and Surface Forces*. Academic Press, London.
- Keller, M., A. Kerth, and A. Blume. 1997. Thermodynamics of interaction of octyl glucoside with phosphatidylcholine vesicles: partitioning and solubilization as studied by high sensitivity titration calorimetry. *Biochim. Biophys. Acta*. 1326:178–192.
- Kragh-Hansen, U., M. le Maire, and J. V. Moller. 1998. The mechanism of detergent solubilization of liposomes and protein-containing membranes. *Biophys. J.* 75:2932–2946.
- Lantzsch, G., H. Binder, H. Heerklotz, M. Wendling, and G. Klose. 1996. Surface areas and packing constraints in POPC/C<sub>12</sub>EO<sub>n</sub> membranes. A time-resolved fluorescence study. *Biophys. Chem.* 58:289–302.
- Lasch, J. 1995. Interaction of detergents with lipid vesicles. *Biochim. Biophys. Acta*. 1241:269–292.
- leMaire, M., J. V. Moller, and P. Champeil. 1987. Binding of a nonionic detergent to membranes: flip-flop rate and location on the bilayer. *Biochemistry*. 26:4803–4810.
- Lichtenberg, D. 1985. Characterization of the solubilization of lipid bilayers by surfactants. *Biochim. Biophys. Acta*. 821:470–478.
- MacDoanld, R. C., R. I. MacDonald, B. P. Menco, K. Takeshita, N. K. Subbarao, and L. R. Hu. 1991. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. *Biochim. Biophys. Acta*. 1061:297–303.
- Opatowski, E., M. M. Kozlov, and D. Lichtenberg. 1997. Partitioning of octyl glucoside between octyl glucoside/phosphatidylcholine mixed aggregates and aqueous media as studied by isothermal titration calorimetry. *Biophys. J.* 73:1448–1457.
- Partearroyo, M. A., A. Alonso, F. M. Goni, M. Tribout, and S. Paredes. 1996. Solubilization of phospholipid bilayers by surfactants belonging to the triton X series: effect of polar group size. *J. Colloid Interface. Sci.* 178:156–159.
- Paternostre, M., O. Meyer, C. Grabielle-Madelmont, S. Lesieur, M. Ghanam, and M. Ollivon. 1995. Partition coefficient of a surfactant between aggregates and solution: application to the micelle-vesicle transition of egg phosphatidylcholine and octyl  $\beta$ -D-glucopyranoside. *Biophys. J.* 69:2476–2488.
- Paternostre, M. T., M. Roux, and J. L. Rigaud. 1988. Mechanisms of membrane protein insertion into liposomes during reconstitution procedures involving the use of detergents. 1. Solubilization of large unilamellar liposomes (prepared by reverse-phase evaporation) by triton X-100, octyl glucoside, and sodium cholate. *Biochemistry*. 27:2668–2677.
- Sanders, C. R., and G. C. Landis. 1995. Reconstitution of membrane proteins into lipid-rich bilayered mixed micelles for NMR studies. *Biochemistry*. 34:4030–4040.
- Schroeder, R. J., S. N. Ahmed, Y. Zhu, E. London, and D. A. Brown. 1998. Cholesterol and sphingolipid enhance the Triton X-100 insolubility of glycosylphosphatidylinositol-anchored proteins by promoting the formation of detergent-insoluble ordered membrane domains. *J. Biol. Chem.* 273:1150–1157.
- Solomon, K. R., M. A. Mallory, and R. W. Finberg. 1998. Determination of the non-ionic detergent insolubility and phosphoprotein associations of glycosylphosphatidylinositol-anchored proteins expressed on T cells. *Biochem. J.* 334:325–333.
- Tanford, C. 1980. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*. Wiley, New York.
- Ueno, M. 1989. Partition behavior of a nonionic detergent, octyl glucoside, between membrane and water phases, and its effect on membrane permeability. *Biochemistry*. 28:5631–5634.
- Vold, R. R., and R. S. Prosser. 1996. Magnetically oriented phospholipid bilayered micelles for structural studies of polypeptides. Does the ideal bicelle exist? *Cryobiology*. 113:267–271.
- Wenk, M. R., T. Alt, A. Seelig, and J. Seelig. 1997. Octyl- $\beta$ -D-glucopyranoside partitioning into lipid bilayers: thermodynamics of binding and structural changes of the bilayer. *Biophys. J.* 72:1719–1731.
- Wenk, M. R., and J. Seelig. 1997. Interaction of octyl- $\beta$ -thioglucopyranoside with lipid membranes. *Biophys. J.* 73:2565–2574.