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Biochimica et Biophysica Acta 1196 (1994) 114–122

BBA Biochimica et Biophysica Acta

Membrane/water partition of oligo(ethylene oxide) dodecyl ethers and its relevance for solubilization

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Received 2 May 1994; revised 31 August 1994

Abstract

Mixed aqueous dispersions containing palmitoyloleoylphosphatidylcholine and oligo(ethylene oxide) dodecyl ethers $C_{12}EO_n$ with $n = 2–8$ have been investigated. The aggregates composition has been determined as a function of the aqueous detergent concentration using Laurdan fluorescence spectroscopy. The partition of the detergent between the membranes and the aqueous phase has been analyzed on the basis of the regular solution model. Solubilization has been analyzed in terms of the limiting detergent fraction in the membrane, the minimal detergent fraction in micelles and the critical aqueous detergent concentration using thermodynamical coexistence conditions for the aqueous, bilayer and micellar pseudo-phases. A thermodynamic criterion for solubilization is presented. The standard chemical potential differences of the transfer of the detergents from water to the bilayer have been found to follow the empirical relation $\Delta\mu^o(n = 2–8) = -37.6 + 0.9n$ kJ/mol. Thus, a similar conformation and localization of the oxyethylene units within the membrane are suggested.

Keywords: Nonionic detergent; Lipid–detergent interaction; Lipid; Partition coefficient; Solubilization; Laurdan

1. Introduction

The interaction between lipids and detergents is an object of interest for several fields of biophysics and biochemistry. Incorporation of detergent molecules into membranes can modify their fluidity and their surface properties, e.g., headgroup dipole density and packing geometry. Lipid membranes modified by detergents were used to study the origin of the hydration force between the membrane surfaces [1–3]. The knowledge of the partition coefficient of the detergent between membrane and water is of fundamental interest for all experiments requiring to adjust the composition of mixed vesicles.

In this work the partition coefficients of oligo(ethylene oxide) dodecyl ethers $C_{12}EO_n$ with $n = 2–8$ between water and mixed lipid/detergent vesicles were investigated as a function of membrane composition by Laurdan steady-state fluorescence spectroscopy [4] using the dilution method introduced by Encinas and Lissi [5].

The partition coefficient and the critical micelle concentration are exact thermodynamic quantities within the phase separation model [6]. In the frame of this model the aqueous solution, membranes and micelles are considered to be separated pseudo-phases. It has been widely used [7,8] and will be applied in this work, too.

Most of the detergents investigated cause solubilization after reaching a critical fraction in the lipid membrane. That means, additional detergent molecules are no longer incorporated into the bilayer but form coexisting mixed micelles of a distinct (higher) detergent fraction. Solubilization is completed by total dissolution of the membranes leaving only mixed micelles.

Various mechanisms have been discussed regarding the driving force of solubilization [9]. Lichtenberg [10] found detergents to destabilize membranes and concluded that detergents exceeding a saturating fraction cause a destruction of lamellar structure. Schurtenberger [11] proposed solubilization to start, when the critical micelle concentration is reached. Hence, micelles would be formed spontaneously as observed in case of pure detergent systems. For mixed systems the situation is more complicated by the

Abbreviations: POPC, palmitoyloleoylphosphatidylcholine; Laurdan, 6-dodecanoyl-2-dimethylaminonaphthalene; $C_{12}EO_n$, oligo(ethylene oxide) dodecyl ether ($C_{12}H_{25}(OCH_2CH_2)_nOH$); EO-unit, oxyethylene unit ($-OCH_2CH_2-$).

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fact that detergent's CMC is affected by the presence of lipid.

In this work, a thermodynamical treatment of solubilization will be presented and proved to be consistent at the POPC/$C_{12}EO_8$ system. In the frame of this model expressions are derived linking the composition of mixed aggregates with the free energy differences of the lipid and detergent molecules between the coexisting pseudo-phases. This treatment allows an interpretation of solubilization data observed experimentally (for example by light and X-ray scattering measurements [12,9]) on a molecular level and can facilitate the discussion of various morphological solubilization models.

2. Materials and methods

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids, USA and oligo(ethylene oxide) dodecyl ether $CH_3(CH_2)_{11}(OCH_2CH_2)_nOH$ with $n = 2–8$ ($C_{12}EO_n$) from Nikko Chemicals, Japan. The fluorescent probe 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan) was obtained from Molecular Probes, Eugene, OR, USA. All substances were used without further purification.

Stock solutions of all substances were prepared in spectroscopic grade methanol and mixed in glass pistons. The solvent was removed under vacuum at room temperature. After addition of pure water, the samples were vortexed rapidly for 2 min and left over night under nitrogen at about 4°C. About 10–20 aqueous stock dispersions were prepared for each detergent, containing 500 μM POPC, various total mol fractions of $C_{12}EO_n$ and 1 mol Laurdan per 250 mol of lipid. Each of these stock dispersions was used to prepare a series of samples with a fixed lipid/detergent molar ratio and lipid concentrations between about 5 μM and 200 μM. The sample with the lowest lipid concentration was prepared by mixing 2 ml of water with an appropriate amount of the stock dispersion, the others by successive addition of stock dispersion. After each addition the samples were left under rapid stirring for at least 15 min to reach thermodynamic equilibrium (see below).

Steady-state emission spectra were recorded at the excitation wavelength 337 nm at 25°C at a Perkin Elmer LS50 spectrometer using quartz cuvettes.

An equilibration time of 15 min was found to be sufficient to reach thermodynamic equilibrium. No further changes were observed after longer waiting times. Le Maire et al. [13] found that $C_{12}EO_8$ binds to and is released from both sides of vesicle membranes in the millisecond timescale.

The partition coefficients were determined using the dilution method developed by Encinas and Lissi [5,14,4]. The balance equation for the total molar detergent concentration within the sample (d_t) includes the aqueous detergent concentration (d_w) and the concentration of detergent situated within aggregates (d_c):

$$d_t = d_c + d_w \tag{1}$$

We emphasize that all molar concentrations d_t, d_c, d_w and the lipid concentration (l) refer to the total volume. The aggregate composition is given by the mol fraction of detergent x_c:

$$x_c = \frac{d_c}{d_c + l} \tag{2}$$

Combinination of Eq. (1) with (2) yields:

$$d_t = \frac{x_c}{1 - x_c} \cdot l + d_w \tag{3}$$

which is equivalent to equation number 3 of Lissi et al. [14].

Eq. (3) becomes linear for constant x_c. That means, if one manages to find data pairs (d_t,l) which can be assumed to correspond to the same aggregate composition x_c, the value of x_c and the corresponding aqueous detergent concentration d_w can be calculated by linear regression.

The choice of various experimental conditions giving the same membrane composition was realized using the Laurdan generalized polarization (GP) which is related to the degree of dipolar relaxation occurring within fluorescence lifetime [15]:

$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}} \tag{4}$$

The fluorescence intensities I_λ at 440 and 490 nm correspond to the unrelaxed and relaxed states, respectively.

Laurdan is insoluble in water. A continuous shift of Laurdan GP was found upon addition of detergent, showing the sensitivity of GP to the effective detergent fraction in the membrane. For our approach the knowledge of the nature and mechanism of this correlation is without significance. However, it should be noted that the relaxation process is due to reorientations of the fluorophore and/or its environment and, therefore, it is influenced by membrane fluidity.

Summarizing we state that, in the range where addition of detergent causes a continuous shift of GP, equal GP values can be assumed to indicate equal membrane composition.

3. Theory

The partition coefficient of the detergent is defined by [7]:

$$P_D(x_c) = \frac{x_c}{x_w} \tag{5}$$

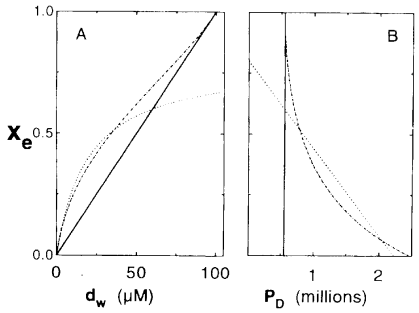

Fig. 1. Typical plots of aggregate composition (x_e) versus aqueous monomer concentration (d_w) (A) and the corresponding partition coefficient P (B) for different models (schematical): – ideal mixing, $P_D = 0.55 \cdot 10^6$; · · · · binding according to mass-action law, 4 binding sites per lipid, binding constant = 10^4 l/mol; - · – · - · regular solution model, $P_{DD} = 0.55 \cdot 10^6$, $\rho_o = -3.7$ kJ/mol.

x_e and x_w are the molar fractions of detergent monomers in the aggregates (membranes, micelles, cf. Eq. (2)) and in the aqueous phase:

$$x_w = \frac{d_w}{d_w + w} \approx \frac{d_w}{w} \tag{6}$$

where w denotes the water molar concentration within the sample. The approximation $w \gg d_w$ applied in Eq. (6) is fulfilled within the concentration range used in this work. Because of the very low aggregate content, we used $w \approx 55.5$ mol/l (molar concentration of pure water).

As mentioned above, the dilution method yields the effective molar fraction of the detergent in the membrane x_e and the corresponding aqueous monomer concentration d_w. With Eqs. (5) and (6) we find both quantities to be related by means of the partition coefficient $P_D(x_e)$:

$$x_e = \frac{P_D(x_e)}{w} \cdot d_w \tag{7}$$

3.1. The partition coefficient P_D as a function of aggregate composition x_e

Generally, P_D depends on the aggregate composition x_e. There are various models which exhibit different, typical dependencies. Fig. 1 illustrates such $P_D(x_e)$ models and the corresponding partition plots $x_e(d_w)$.

For ideal mixing P_D is constant, which gives a straight line when plotting x_e versus d_w (cf. Eq. (7)). The partition coefficient is related to the difference of the standard chemical potential of the detergent upon transfer from water into the aggregate ($\Delta\mu^o$) [8]:

$$\Delta\mu^o = -RT \cdot \ln P_D \tag{8}$$

with the universal gas constant $R = 8.31$ J/mol per K and the absolute temperature T ($T = 298$ K in our study).

Nonideal binding of solute to a limited number of binding sites per lipid [16] can be explained on the basis of mass-action law leading to a linear decrease of $P(x_e)$ (cf. dotted line in Fig. 1B). Consequently, $x_e(d_w)$ approaches asymptotically the limiting fraction of sites showing a typical saturation behaviour (cf. dotted line in Fig. 1A).

Another model for nonideal behaviour is given by the theory of regular solutions [8,17]. Considering molecular pair interaction energies between nearest neighbours, nonideality is due to a net interaction between the components ρ_o. That means, that lipid/detergent interaction (u_{DL}) differs from the average of lipid/lipid (u_{LL}) and detergent/detergent interactions (u_{DD}) in the aggregate:

$$\rho_o = N \cdot \left(u_{LD} - \frac{u_{LL} + u_{DD}}{2} \right) \tag{9}$$

N is the number of interacting neighbours per molecule in the membrane. The nonideality is considered in the chemical potential of the detergent by an additional term [17]:

$$\mu = \mu^o + RT \cdot \ln x_e + \rho_o \cdot (1 - x_e)^2 \tag{10}$$

Hence, the standard chemical potential difference between the nonideal membrane and the ideally mixed water phase is:

$$\Delta\mu^o = -RT \cdot \ln P_D(x_e) - \rho_o \cdot (1 - x_e)^2 \tag{11}$$

Rearrangement of Eq. (11) yields:

$$P_D(x_e) = P_{DD} \cdot e^{-\frac{\rho_o}{RT} \cdot (1 - x_e)^2} \tag{12}$$

where:

$$P_{DD} = e^{-\frac{\Delta\mu^o}{RT}} \tag{13}$$

The constant P_{DD} corresponds to $P_D(x_e = 1)$, i.e., the limiting value due to detergent/detergent contacts. At low detergent content in the membrane ($x_e \to 0$) each detergent molecule is surrounded only by lipid molecules and we obtain the limiting partition coefficient P_{DL}:

$$P_{DL} = P_D(x_e \to 0) = P_{DD} \cdot e^{-\frac{\rho_o}{RT}} \tag{14}$$

We note that the formulae derived above apply to lamellar as well as to micellar lipid/detergent aggregates.

In the latter case, the d_w corresponding to the pure detergent aggregates ($x_e = 1$) is called the critical micelle concentration (CMC) and the micelle/water partition coefficient for pure detergent $P_{DD}^{m/w}$ is obtained according to Eq. (7)

$$P_{DD}^{m/w} = \frac{w}{CMC} \tag{15}$$

3.2. Solubilization

If the pure detergent forms micelles, the mixed system must transform from bilayer structures stabilized by the lipid to micelles preferred by the detergent upon increasing the detergent concentration. This transition, starting with the saturation of the bilayer with detergent and being

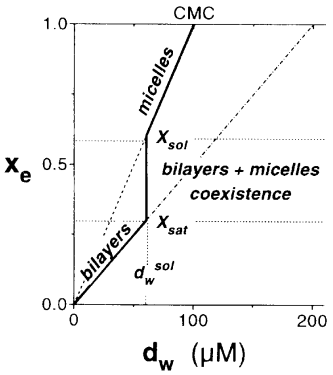

Fig. 2. Typical behaviour of $x_e(d_w)$ of an ideally mixed system undergoing solubilization: – real behaviour of the system including the vertical jump corresponding to aggregate coexistence, — - micelles, -·-·- bilayers.

completed with the total dissolution of the bilayers is called solubilization. Correspondingly, there are a maximal detergent fraction for the lamellar phase (X_{sat}) and a minimal detergent content in the micelles (X_{sol}). Within the solubilization range, $X_{sat} < x_e < X_{sol}$, membranes with constant detergent fraction X_{sat} and micelles with X_{sol} coexist and x_e is now a weighted average between these two constant values. Since both types of aggregates are in thermodynamic equilibrium with the water phase, d_w^{sol} must also remain constant [17].

Subsequently, the $x_e(d_w)$ dependence jumps from the curve for the bilayer to that for the micelle upon solubilization at d_w^{sol} (cf. Fig. 2).

The partition coefficient is generally defined as the ratio of the mol fractions of the respective component in two phases [7] (cf. e.g., Eq. (7)). For the case of coexistence of lamellae and micelles we introduce additionally the partition coefficient for the detergent between the bilayers and the micelles:

$$P_D^{b/m} = \frac{X_{sat}}{X_{sol}} \tag{16}$$

Analogously the lipid partition coefficient between bilayers and micelles $P_L^{b/m}$ is defined:

$$P_L^{b/m} = \frac{1 - X_{sat}}{1 - X_{sol}} \tag{17}$$

By use of Eqs. (16) and (17) the solubilization parameters X_{sat} and X_{sol} can be interpreted on a molecular level.

From Eq. (16) we obtain with $X_{sol} \leq 1$ the condition

$$X_{sat} \leq P_D^{b/m} \tag{18}$$

i.e., the bilayer/micelle partition coefficient of the detergent represents the upper limit of the saturation fraction X_{sat} in mixed vesicles. The limiting case $X_{sol} \to 1$ (i.e., $X_{sat} \to P_D^{b/m}$ and $P_L^{b/m} \to \infty$, cf. Eq. (17)) corresponds to the coexistence of mixed bilayers with pure detergent micelles.

With the definition of P (cf. Eq. (7)) for bilayers as well as for micelles and considering Eqs. (16) and (17), one can verify the following relations for the detergent:

$$P_D^{b/m} = \frac{P_D^{b/w}(X_{sat})}{P_D^{m/w}(X_{sol})} \tag{19}$$

and for the lipid:

$$P_L^{b/m} = \frac{P_L^{b/w}(X_{sat})}{P_L^{m/w}(X_{sol})} \tag{20}$$

We can derive two coexistence conditions, for the detergent and the lipid, respectively, by use of the regular solution model. In order to do that Eqs. (16) and (19) are combined. The aggregate/water partition coefficients $P_D^{b/w}(X_{sat})$ as well as $P_D^{m/w}(X_{sol})$ can be substituted using Eq. (12), yielding the coexistence condition for the detergent:

$$\frac{X_{sat}}{X_{sol}} = P_{DD}^{b/m} \cdot \exp\left\{ \frac{\rho_o^m}{RT} \cdot (1 - X_{sol})^2 - \frac{\rho_o^b}{RT} \cdot (1 - X_{sat})^2 \right\} \tag{21}$$

with:

$$P_{DD}^{b/m} = \frac{P_{DD}^{b/w}}{P_{DD}^{m/w}} \tag{22}$$

Analogously to the detergent the regular solution model yields the composition dependence of the lipid aggregate/water partition coefficient [17]:

$$P_L(x_e) = P_{LL} \cdot e^{-\frac{\rho_o}{RT} \cdot x_e^2} \tag{23}$$

and thus, the coexistence condition for the lipid, supplementary to Eq. (21), is:

$$\frac{1 - X_{sat}}{1 - X_{sol}} = P_{LL}^{b/m} \cdot \exp\left\{ \frac{\rho_o^m}{RT} \cdot X_{sol}^2 - \frac{\rho_o^b}{RT} \cdot X_{sat}^2 \right\} \tag{24}$$

4. Results and discussion

4.1. Membrane-water partition of $C_{12}EO_n$

Fig. 3 shows plots of the generalized polarization of Laurdan fluorescence versus total $C_{12}EO_4$ fraction in the sample x_t:

$$x_t = \frac{d_t}{d_t + l} \tag{25}$$

for various POPC concentrations l. For a lower lipid concentration a higher x_t is required to cause the same GP.

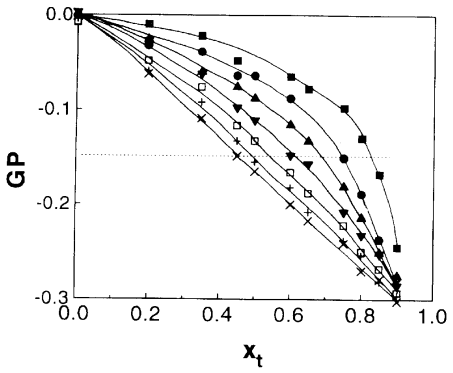

Fig. 3. The Laurdan generalized polarization (GP) in POPC/$C_{12}EO_4$ mixtures versus the total mole fraction (x_t) of the detergent. The symbols correspond to the dilution steps characterized by the lipid concentrations $l = 5$ μM (■), 10 μM (●), 15 μM (▲), 25 μM (▼), 45 μM (□), 100 μM (+), 200 μM (×). A horizontal cut at a definite GP yields pairs of experimental conditions x_t and l, which can be assumed to cause the same detergent aggregate fraction x_e. For example, for GP = −0.14 (· · · · · · · · ·) one obtains $x_e = 0.44$ and $d_w = 22$ μM.

Data pairs (x_t,l) which correspond to a constant GP are transformed to a set of data (d_t,l) by use of Eq. (25). Linear regression according to Eq. (3) yields x_e and the corresponding d_w for every chosen GP.

Fig. 4 shows the $x_e(d_w)$ plots for $C_{12}EO_n$ with $n = 2$–6. The slopes, i.e., the partition coefficients are observed to decrease with increasing x_e.

The binding model fails to explain this behaviour, because no saturation of the membrane with detergent was detected. The corresponding fit of the experimental data yields a negative number of binding sites per lipid, which is without any physical sense.

But, using $P_D(x_e)$ derived from the regular solution model (cf. Eq. (12)), Eq. (7) fits the data quite well. In a first attempt both the limiting partition coefficient P_{DD}

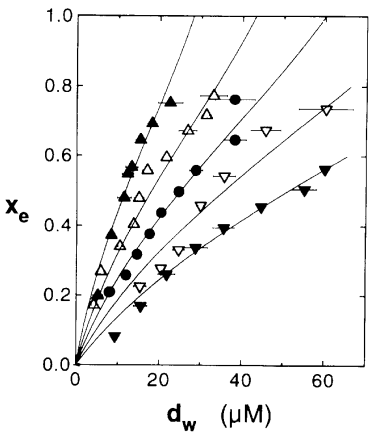

Fig. 4. Partition plots $x_e(d_w)$ for $C_{12}EO_n$/POPC membranes for $n = 2$ (▲), 3 (△), 4 (●), 5 (▽), 6 (▼). The lines are according to the regular solution model using $\rho_0 = -1.7$ kJ/mol.

Table 1
Membrane-water partition coefficients of $C_{12}EO_n$ for the detergent membrane fractions: $x_e = 1$ (P_{DD}); $x_e = 0.35$ ($P_D^{b/w}(0.35)$) and $x_e \to 0$ (P_{DL})

n	Partition coefficients (millions)		
	$P_{DD}^{b/w}$	$P_D^{b/w}$ (0.35)	$P_{DL}^{b/w}$
2	2.0	2.7	4.0
3	1.3	1.7	2.6
4	0.92	1.2	1.8
5	0.65	0.87	1.3
6	0.45	0.60	0.91
7	0.33	0.44	0.66
8	0.22	0.30	0.44

The values were obtained on the basis of the regular solution model using $\rho_o^b = -1.7$ kJ/mol.

and the nonideality parameter ρ_0 have been varied to get the best fit. However, no significant variation of ρ_0 with the detergent series was detected. Therefore, a fixed average value of $\rho_0 = -1.7$ kJ/mol was used to fit the P_{DD} for the different $C_{12}EO_n$. The P_{DD} obtained and the corresponding P_{DL} are summarized in Table 1.

The observed slightly negative ρ_0 points to a detergent/detergent attraction being less stable than expected for ideal mixing (cf. Eq. (9)). This fact has consequences for the structure of the system. In one respect, lateral separation of the lipid and detergent molecules will not occur, but the mixture tends to form detergent/lipid contacts. On the other hand, the number of DD-contacts has to increase with increasing x_e, leading to a loss of membrane stability.

The destabilization of the membrane caused by the different $C_{12}EO_n$ has been differentiated by analyzing the GP-values. Fig. 5 shows the interpolated effective detergent fractions x_e corresponding to an arbitrarily chosen GP-value of −0.14 as a function of n. With the exception of $C_{12}EO_2$, that requires a higher fraction for the same disturbance of the membrane, all other detergents investigated affect the bilayer stability in a similar way. It should be noted that the GP shift from 0 to −0.14 corresponds to

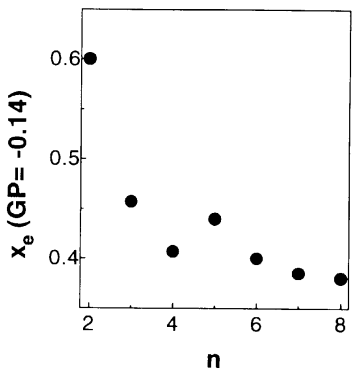

Fig. 5. Detergent fraction in the POPC membrane causing a Laurdan GP-shift from 0 (pure POPC) to −0.14 versus EO-chain length, n.

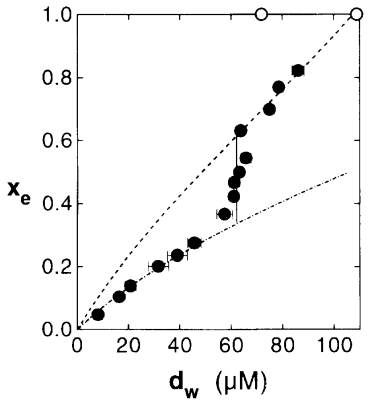

Fig. 6. x_e versus d_w for $C_{12}EO_8$/POPC mixtures (●). Lines are calculated according to the regular solution model for bilayers (-·-·-·-) with $P_{DD}^{b/w} = 0.22 \cdot 10^6$, $\rho_o^b = -1.7$ kJ/mol and micelles (— -) with $P_{DD}^{m/w} = 0.51 \cdot 10^6$, $\rho_o^m = 1$ kJ/mol. Solubilization occurs at $d_w^{sol} = 63$ μM. CMC values (○) are shown for comparison (see Table 2).

the fluidization of pure POPC achieved by raising the temperature from 25°C to 35°C.

A drastic increase of membrane fluidity and ion permeability [16,9], changes in vesicle size [16,9,12] and, possibly, transient membrane holes or ruptures [9] have been reported for lipid membranes upon addition of detergents. Destabilization may result in the total destruction of the membrane structure at detergent fractions exceeding a critical value [9].

Ueno [18] found that the partition coefficient for octyl glucoside between water and egg yolk lecithin decreases at about $X_e > 0.3$. He showed qualitatively that in this region detergent/detergent contacts become inevitable and concluded like us that these contacts are less attractive. Schubert [19] found decreasing membrane/water partition coefficients for bile salts to be due to changes in the aggregate structure. This is not in contrast to the above model, because even less stable detergent/detergent interactions might cause structural changes.

4.2. Solubilization of the membrane by $C_{12}EO_8$

The $x_e(d_w)$ plot for $C_{12}EO_8$ (cf. Fig. 6) differs qualitatively from that obtained for the detergents with $n = 2$–6. It is characterized by two separate parts, which are connected by a vertical line at a distinct aqueous detergent concentration. We suggested that this behaviour characterizes the occurrence of solubilization (cf. Fig. 2). Indeed, Edwards and Almgren [12] give a solubilization range from $X_{sat} \approx 0.35$ to $X_{sol} \approx 0.65$, i.e., micelles and lamellae coexist at $X_{sat} < x_e < X_{sol}$, whereas at $x_e < X_{sat}$ and $x_e > X_{sol}$ only bilayer structures or only micelles are found, respectively.

For coexistence of different aggregates the experimentally obtained x_e is a weighted average of X_{sat} and X_{sol}. We have to note that the weights depend not only on the ratio between the lamellae and the micelles but also on the distribution of the Laurdan between them. For an uneven distribution the composition of the preferred phase (with higher Laurdan concentration) would be overestimated in the effective fraction x_e obtained, and thus the resulting apparent d_w would differ from the true value, which is a constant within the coexistence range. Comparing the experimental data with the theoretical curve deduced by use of $X_{sat} = 0.35$ [12] we note good agreement and conclude that the distribution of the Laurdan between the lamellae and micelles is essentially homogeneous.

We applied the regular solution model to both the lamellar and the micellar phases yielding the two separate data sets $P_{DD}^{b/w}$; ρ_o^b and $P_{DD}^{m/w}$; ρ_o^m, respectively. For the lamellae ρ_o^b was set to -1.7 kJ/mol, equal to that obtained for $C_{12}EO_n$ with $n = 2$–7. The calculated partition coefficients P_{DD} and P_{DL} are given in Table 1.

With $x_e = 0.35$ Eq. (12) yields $P_D^{b/w}$ (0.35) $= 0.3 \cdot 10^6$, the same value as reported by Edwards and Almgren [12]. At $x_e > 0.65$ (micellar range) the fit yields the nonideality parameter $\rho_o^m \approx -1$ kJ/mol and the micelle/water partition coefficient $P_{DD}^{m/w} = 0.51 \cdot 10^6$ which corresponds to a CMC of 108 μM according to Eq. (15). This value is quite consistent with 109 μM measured by Rosen et al. [20] and by Corti et al. [21], but a value of 72 μM has also been reported [22,23].

Taking account of our data (cf. Table 1) and $X_{sat} = 0.35$ [12] the lipid partition coefficient $P_{LL}^{b/m}$ and X_{sol} are the only quantities left unknown in both coexistence conditions, Eqs. (21) and (24). We determined $X_{sol} = 0.65$ and $P_{LL}^{b/m} = 2.0$ by a trial and error procedure. The resulting X_{sol} is in agreement with that in [12] supporting the validity of our model. Upon solubilization the lipid prefers the lamellar structures with $P_L^{b/m}$ (X_{sat}, X_{sol}) $= 1.8$ relatively to the coexisting micelles (cf. Eq. (17)). This value corresponds to a transfer free energy of about -1.5 kJ/mol according to Eq. (8). The value for the detergent $P_D^{b/m} = 0.55$ yields $+1.5$ kJ/mol, i.e., the detergent favours micelles.

4.3. Can solubilization be predicted for $n = 2$–7?

We could assume Laurdan to be distributed homogeneously between lamellae and micelles also for $n = 2$–7, as found for $C_{12}EO_8$. Consequently, the $x_e(d_w)$ plots (cf. Fig. 4) would suggest no solubilization to occur.

But we have to emphasize that the x_e range accessible by the experimental method used is restricted to intermediate mole fractions x_e. Our method for the detection of detergent in the membrane uses the Laurdan GP, which is correlated to membrane fluidity. A reasonable fluidization of the membrane, and therefore significant changes of GP, occur only with about $x_e > 0.25$, when detergent detergent contacts become more and more pronounced. At about $x_e \geq 0.75$ no further fluidization is detectable by Laurdan,

when almost all fluorophores have already completed relaxation. Therefore, we cannot detect solubilization at large x_e.

Whether solubilization occurs at all can be unambiguously decided using the phase diagrams published for the pure detergents [24]. $C_{12}EO_3$ and $C_{12}EO_4$ are known to form lamellar phases. If one mixes them with POPC that also prefers lamellae, there is no reason to induce non-lamellar structures. But in contrast to these detergents, those with $n = 5–7$ form micelles [24] and therefore solubilization must occur at some fraction, obviously beyond the sensitivity range of our experimental method.

In order to estimate the solubilization range for $C_{12}EO_n$ with $n = 5–7$ we employ the theory outlined for the coexistence of lamellae and micelles. The two independent coexistence conditions, Eqs. (21) and (24), contain the characteristic constants X_{sat}, X_{sol}, $P_{DD}^{b/w}$, ρ_o^b, $P_{DD}^{m/w}$, ρ_o^m and $P_L^{b/m}$. The bilayer data $P_{DD}^{b/w}$ and ρ_o^b have been determined above for all the detergents with $n = 2–8$ (see Table 1). The $P_{DD}^{m/w}$ were deduced from the CMC (cf. Table 2) by use of Eq. (15). The unknown micelle nonideality parameter ρ_o^m and the initial lipid partition $P_{LL}^{b/m}$ are assumed to be the same as found for $C_{12}EO_8$. These two assumptions seem to be the more critical the more n differs from 8. The remaining parameters X_{sat} and X_{sol} have been computed giving rough estimates of the coexistence range expected (cf. Fig. 7).

Pure $C_{12}EO_3$ and $C_{12}EO_4$ form bilayers and, therefore, no solubilization occurs. Indeed, the partition coefficients determined for the pure detergent bilayers $P_{DD}^{b/w}$ correspond to values of $d_w(x_e = 1)$ lower than the CMC published by Rosen et al. (cf. Table 2). That means, bilayers should possess the lower free energy and, consequently, are preferred. For these systems there is no solution for X_{sat}, X_{sol} of the bilayer micelle coexistence conditions, Eqs. (21) and (24).

Table 2
Aqueous monomer concentrations of pure $C_{12}EO_n$, $d_w(1) = W/P_{DD}$ as computed for bilayers and micelles applying the regular solution model to lipid-detergent mixtures

n	Bilayer (L_α)	Phase [24]	Micelle (L_1)	
	$\frac{W}{P_{DD}^{b/w}}$ (μM)		$\frac{W}{P_{DD}^{m/w}}$ (μM)	CMC (μM)
2	28	L_2		33 [a]
3	43	L_α		52 [a]
4	60	L_α		64 [a], 46 [d], 40 [e]
5	85	L_1		64 [a], 65 [c]
6	122	L_1		68 [b,c], 87 [d]
7	170	L_1		82 [a], 69 [b,c], 50 [e]
8	256	L_1	108	109 [a,d], 72 [b,c]

For comparison the phase of the respective detergent/water dispersion observed experimentally and various CMC values are cited from literature: [a] [20], [b] [22], [c] [23], [d] [21], [e] [29]. The symbols L_α, L_1 and L_2 denote lamellar, micellar and reverse micellar phases, respectively [17].

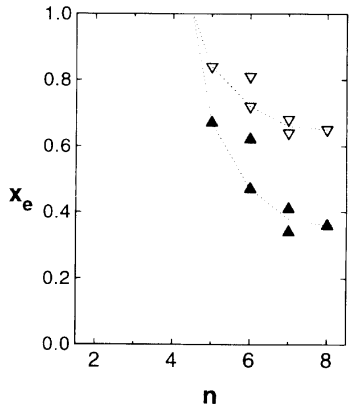

Fig. 7. Solubilization, i.e., bilayer/micelle coexistence range between the limiting detergent fraction for bilayers X_{sat} (▲) and the minimal detergent fraction for micelles X_{sol} (▽) versus detergent's EO-chain length n as predicted by aggregate coexistence conditions. For $n = 3$, 4 no solubilization occurs. Competing points are due to various CMC data (see Table 2). Lines are only for clarity.

For $n = 5–7$ decreasing saturation fractions for bilayers X_{sat} are obtained with increasing n, which are at the most beyond the experimentally accessible range reflected by Fig. 4.

4.4. Thermodynamic criterion for solubilization

Solubilization is considered in terms of the thermodynamic equilibrium of separate aqueous, bilayer and micellar pseudo-phases according to the phase separation model [6].

The thermodynamic criterion for phase coexistence is the equality of the chemical potential of the lipid as well as of the detergent in the three phases:

$$\mu_D^b(x_b) = \mu_D^m(x_m) = \mu_D^w(d_w) \quad (26)$$

$$\mu_L^b(1 - x_b) = \mu_L^m(1 - x_m) = \mu_L^w(l_w) \quad (27)$$

The coupled Eqs. (26) and (27) possess just one solution for the detergent fractions in bilayers (x_b) and in micelles (x_m), which is $x_b = X_{sat}$ and $x_m = X_{sol}$. We emphasize that the thermodynamic criterion for solubilization is just the phase coexistence of bilayers and micelles, irrespectively of the aqueous concentrations. It should be noted that the aqueous detergent concentration d_w is just the actual detergent CMC in presence of lipid [9].

Nevertheless, the mechanism of solubilization can be considered to be the formation of mixed micelles in the water phase at a critical concentration d_w^{sol} as well as the destruction of membranes at a limiting fraction X_{sat}. Namely, the saturation of the bilayer with detergent and the reaching of the stable mixed micelles CMC in the aqueous medium occur exactly simultaneously. Both quantities, d_w^{sol} and X_{sat} are interrelated by the thermodynamic equilibrium.

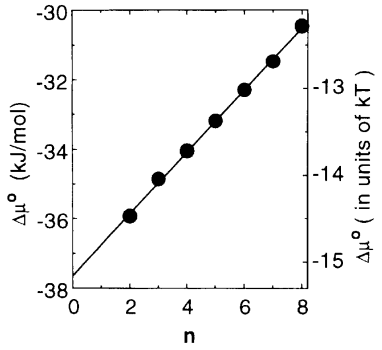

Fig. 8. The standard chemical potential difference $\Delta\mu^{\circ}$ upon transfer of the detergents from water to the membrane versus EO-chain length, n. $\Delta\mu^{\circ}$ are calculated using $P_{DD}^{b/w}$ data from Table 1 and Eqs. (9), (10) and (11). The line shows the result of the linear regression with $\Delta\mu^{\circ} = -37.6 + 0.9n$ kJ/mol.

4.5. Contribution to $\Delta\mu^{\circ}$ per EO-unit

The standard chemical potential differences $\Delta\mu^{\circ}$ of the detergents $C_{12}EO_n$ between membranes and water calculated by Eq. (11) are shown as a function of n in Fig. 8. It can be very well described by the linear dependence:

$$\Delta\mu^{\circ}(C_{12}EO_n) = n \cdot \Delta\mu^{\circ}(\text{-EO-}) + \Delta\mu^{\circ}(C_{12}EO_0) \quad (28)$$

with $\Delta\mu^{\circ}(\text{-EO-}) = +0.9$ kJ/mol and an intercept of -37.6 kJ/mol.

Leo et al. [25] showed that in general the chemical potential difference of a molecule can be approximated by a sum of appropriate increments of $\Delta\mu^{\circ}$ for arbitrarily chosen subunits which are found to depend on the given solvent pair. Therefore, the linearity observed is a strong indication that every EO-unit added from $n = 3$ to 8 is incorporated into a similar non aqueous environment within the membrane.

$\Delta\mu^{\circ}$(-EO-) can be estimated alternatively assuming additivity of the contributions of the ether oxygen and of the methylene groups:

$$\Delta\mu^{\circ}(\text{-EO-}) = 2 \cdot \Delta\mu^{\circ}(\text{-}CH_2\text{-}) + \Delta\mu^{\circ}(\text{-O-}) \quad (29)$$

Using values published for the standard partition system octanol/water: $\Delta\mu^{\circ}_{oct/w}(\text{-}CH_2\text{-}) = -2.8$ kJ/mol and $\Delta\mu^{\circ}_{oct/w}(\text{-O-}) = +5.6$ kJ/mol [25] one finds $\Delta\mu^{\circ}_{oct/w}(\text{-EO-}) \approx 0$. A linear correlation is given between the $\Delta\mu^{\circ}$ for water/octanol and $\Delta\mu^{\circ}$ for other solvent pairs [25,26]. Consequently, $\Delta\mu^{\circ}_{b/w}(\text{-EO-}) \approx 0$ should be expected for the bilayer/water system. The $\Delta\mu^{\circ}_{b/w}(\text{-EO-}) = +0.9$ kJ/mol obtained experimentally indicates in fact a considerable compensation of the hydrophilicity of the ether oxygen by the two methylene groups.

The intercept of $\Delta\mu^{\circ}(n)$ corresponds to $C_{12}EO_0$, i.e., dodecanol, if the observed linearity holds also for $n \to 0$. Jain et al. [27] measured membrane/water partition coefficients for alcohols from butanol to nonanol, using egg yolk lecithin membranes. Their extrapolated molal partition coefficient of dodecanol can be converted yielding $\Delta\mu^{\circ}(C_{12}EO_0) \approx 36$ kJ/mol.

If one suggests two hydrogen bonds to be the origin of $\Delta\mu^{\circ}$(-O-) [28], one could conclude that the discrepancy between $\Delta\mu^{\circ}$ of dodecanol and $\Delta\mu^{\circ}$ of $C_{12}EO_n$ for $n \to 0$ is due to a lower hydration of the first or second EO-unit. This would be consistent with the hydration study of Volkov [28] who found one EO-unit per detergent molecule not to be hydrated by means of extrapolation $n \to 0$.

But, because of the errors that may occur in deducing both values, the significance of the observed deviation $\Delta\mu^{\circ}$(dodecanol) $< \Delta\mu^{\circ}(C_{12}EO_n,\ n \to 0)$ is questionable and must be the object of further investigations.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 294).

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