The Heat of Transfer of Lipid and Surfactant from Vesicles into Micelles in Mixtures of Phospholipid and Surfactant

Ella Opatowski, Dov Lichtenberg, and Michael M. Kozlov
Department of Physiology and Pharmacology, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv 69978, Israel

ABSTRACT We study the heat associated with the transformation of vesicles into micelles in mixtures of bilayer-forming phospholipids and micelle-forming surfactants. We subdivide the total heat evolution ΔQ^{coex} within the range of coexistence of vesicles and micelles into three contributions related to the transition of dN_D^{m-b} molecules of surfactant and dN_L^{m-b} molecules of lipid from micelles to vesicles and to the extraction of dN_D^{m-w} molecules of surfactant from micelles to the aqueous solution, so that $\Delta Q^{coex} = \Delta H_D^{m-w} \cdot dN_D^{m-w} + \Delta H_D^{m-b} \cdot dN_D^{m-b} + \Delta H_L^{m-b} \cdot dN_L^{m-b}$ where ΔH_D^{m-w}, ΔH_L^{m-b}, and ΔH_D^{m-b} are the respective molar "transfer" enthalpies. We design a method for the evaluation of all three molar enthalpies, from isothermal calorimetric titrations conducted according to two different protocols of titration of lipid-surfactant mixtures. In the first protocol the mixture is titrated with an aqueous solution of pure lipid vesicles, and in the second the mixture is titrated with an aqueous solution of pure surfactant. Titration of the mixed systems by a buffer solution serves to verify the results obtained under these protocols. In addition to the values of molar enthalpies, our method yields the cmc value of the pure surfactant. We apply our method to investigating the heat evolution in mixtures of egg yolk phosphatidylcholine and the nonionic surfactant octylglucoside in a phosphate-buffered saline solution at 28°C. These studies gave the following values: $\Delta H_D^{m-w} = -1732$ cal/mol, $\Delta H_L^{m-b} = -592$ cal/mol, $\Delta H_D^{m-b} = 645$ cal/mol, and cmc = 23.5 mM. We discuss the possible physical insight of these values and the perspectives of applications of the proposed method.

GLOSSARY

R_e	(effective) ratio between surfactant and lipid in mixed aggregates
R_e^{sat}	the value of R_e at the onset of solubilization
R_e^{sol}	the value of R_e at the completion of solubilization
ΔR_e	the difference $R_e^{sol} - R_e^{sat}$
D_w	concentration of monomeric surfactant in aqueous solution
N_D^t	total number of surfactant molecules
N_D^a	number of surfactant molecules residing in aggregates
N_D^b	number of surfactant molecules in bilayers
N_D^m	number of surfactant molecules in micelles
N_D^w	number of monomeric surfactant molecules in aqueous solution
N_L^t	total number of lipid molecules
N_L^a	number of lipid molecules in aggregates
N_L^b	number of lipid molecules in bilayers
N_L^m	number of lipid molecules in micelles
N_L^w	number of lipid molecules in aqueous solution
dN_D^{m-w}	number of surfactant molecules that become extracted from micelles to water
dN_D^{m-b}	number of surfactant molecules transferred from micelles to bilayers
dN_L^{m-b}	number of lipid molecules transferred from micelles into bilayers
c_L	concentration of the lipid suspension in the titrant (protocol II)
L	concentration of the lipid suspension in the titration cell
c_D	concentration of the surfactant solution in the titrant (protocol III)
D	concentration of the surfactant solution in the titration cell
V	total volume
V_t	volume of one injection of the titrant
V_c	volume of the calorimetric cell
ΔQ	total heat evolution per titration step
ΔQ^{coex}	total heat evolution per titration step in the range of coexistence
ΔQ^{m-w}	heat of extraction of surfactant monomers from micelles into the aqueous solution per titration step
ΔQ^{trans}	heat of transformation of micelles to vesicles per titration step
ΔH_D^{m-w}	molar heat of extraction of surfactant molecules from micelles to water
ΔH_D^{m-b}	molar heat of transition of surfactant molecules from micelles to bilayers
ΔH_L^{m-b}	molar heat of transition of lipid molecules from micelles to bilayers
ΔH_{demic}	molar enthalpy of demicellization

Received for publication 20 December 1996 and in final form 10 June 1997.

Address reprint requests to Dr. Dov Lichtenberg, Department of Physiology and Pharmacology, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv 69978, Israel. Tel.: 972-3-6407305; Fax: 972-3-6409113; E-mail: physidov@ccsg.tau.ac.il.

0006-3495/97/09/1458/10 $2.00

INTRODUCTION

Solubilization and reconstitution of biological membranes are of fundamental importance in membrane biochemistry and biophysics. Much work has therefore been devoted to understanding the composition-induced transformation of vesicles into micelles in mixtures of membrane-forming

phospholipids and micelle-forming surfactants. These studies revealed that the self-assembly is determined by the ratio R_e of the two components in the mixed aggregates:

$$R_e = \frac{N_D^a}{N_L^a} \quad (1)$$

where N_D^a and N_L^a are the numbers of surfactant and lipid molecules residing in the aggregates.

When this ratio is lower than a critical value, R_e^{sat}, all of the phospholipid is contained in mixed bilayers (vesicles); when R_e is higher than another critical value, R_e^{sol}, the lipid is solubilized in the form of surfactant-lipid mixed micelles, and for R_e between R_e^{sat} and R_e^{sol}, mixed micelles of a composition R_e^{sol} and mixed vesicles of a composition R_e^{sat} coexist in the system.

In the preceding study, we changed the ratio R_e in a mixture made of phosphatidylcholine (PC) and the nonionic surfactant octyl glucoside (OG) by a stepwise dilution of the mixture with a buffer solution. As a result, the system was continuously driven from the micellar range via the range of micelle-vesicle coexistence into the vesicular range. From the measured heat of each step of dilution, we expected to evaluate the contributions of the heat of extraction of surfactant monomers from aggregates into the aqueous solution, ΔQ^{m-w} and the heat of transformation of micelles to vesicles, ΔQ^{trans}, within the range of micelle-vesicle coexistence. In fact, the results of those measurements indicated that the heat of extraction is much larger than the heat of transformation, $\Delta Q^{m-w} \gg \Delta Q^{trans}$. Hence the data were not sufficient to evaluate the values of ΔQ^{trans}.

The heat of transformation ΔQ^{trans} carries direct quantitative information on the comparative energetics of bilayers and micelles and is, therefore, of fundamental importance. The present study is devoted specifically to evaluating the heat of the micelle-to-vesicle transformation ΔQ^{trans} within the range of coexistence in mixtures of PC and OG.

We now consider the heat evolution within the range of coexistence to consist of three contributions, corresponding to 1) extraction of surfactant molecules from micelles to water, 2) transition of surfactant molecules from micelles to vesicles, and 3) transition of lipid molecules from micelles to vesicles.

For detailed analysis of the thermodynamic properties of the system, we designed a method based on the combined use of two different protocols of titration, namely titration of lipid-surfactant mixtures with solutions containing lipid vesicles on one hand, and with solutions of surfactant micelles on the other. Using this method, we determined the molar values of each of the three contributions to the total heat evolution.

MATERIALS AND METHODS

Preparation and characterization of vesicles and micelles

The chemicals used in this study were as described in the preceding communication. PC vesicles were made by ultrasonic irradiation of hydrated PC in buffer A (140 mM NaCl, 0.5 mM EDTA, 0.02% NaN_3 and 10 mM TRIS, pH 7.4), using a 350-V heat system sonicator. OG micelles and OG/PC mixed micelles were prepared as in the preceding study. Vesicles and micelles were characterized by photon correlation spectroscopy (PCS).

Calorimetric studies

All the calorimetric experiments were conducted at 28°C, using the OMEGA isothermal titration calorimeter as described in the preceding publication. We used three different protocols of titration of the mixture of the lipid and surfactant.

Protocol I involved titration of OG/PC mixtures with a buffer solution, as described in Fig. 1 *A* (*top*) and in the preceding report. In each titration step, a volume V_t of the buffer solution was added to a volume V_c of an OG/PC mixture. Hence, after each step of dilution the total numbers of molecules of surfactant, N_D^t, and the lipid, N_L^t, in the system did not change, whereas the total volume of the solution increased by V_t:

$$dV = V_t \qquad dN_D^t = 0 \qquad \text{and} \qquad dN_L^t = 0 \quad (2)$$

After equilibration of the mixture, a volume V_t of the diluted mixture was removed from the titration cell.

The states adopted by the mixture in the course of titration are presented in the upper panel of Fig. 1 *A* (Lichtenberg, 1993). The lines *a*, *b*, and *c* correspond to the dilution of various mixed micellar systems of different ratios of the total numbers of surfactant and lipid molecules, N_D^t/N_L^t.

Protocol II consisted of titration of an OG/PC mixture by a solution of small unilamellar vesicles (SUVs) of PC. The lipid concentration in the titrant was c_L. Each titration step of this protocol resulted in changes in the total volume V and the total number of lipid molecules N_L^t, whereas the total number of surfactant molecules N_D^t remained constant:

$$dV = V_t \qquad dN_D^t = 0 \qquad \text{and} \qquad dN_L^t = V_t \cdot c_L \quad (3)$$

The sequence of states of the lipid-surfactant mixtures in the course of this protocol are illustrated by the straight lines in Fig. 1 *A* (*middle panel*). The lines *a*, *b*, and *c* differ in the value of c_L.

Protocol III involved the titration of an OG/PC mixture by a solution of pure OG of a concentration c_D. Under this protocol, the total number of lipid molecules did not change as a result of each titration step, whereas the total volume and the total number of surfactant molecules changed:

$$dV = V_t \qquad dN_D^t = V_t \cdot c_D \qquad \text{and} \qquad dN_L^t = 0 \quad (4)$$

This protocol is illustrated in the bottom panel of Fig. 1 *A*. The straight lines *a*, *b*, and *c* correspond to different values of c_D.

THEORETICAL CONSIDERATIONS

In this section we consider theoretically the heat evolution ΔQ^{coex} per step of titration performed according to protocols I–III in the range of coexistence of mixed micelles and mixed vesicles. As mentioned in the Introduction, the total heat evolution due to each step of titration consists of three contributions:

$$\Delta Q^{coex} = \Delta H_D^{m-w} \cdot dN_D^{m-w} + \Delta H_D^{m-b} \cdot dN_D^{m-b} + \Delta H_L^{m-b} \cdot dN_L^{m-b} \quad (5)$$

In this equation dN_L^{m-b} and dN_D^{m-b} denote, respectively, the number of lipid and surfactant molecules transferred from micelles to vesicles; dN_D^{m-w} is the number of surfactant molecules extracted from micelles to water. All of these values relate to one titration step. The value ΔH_D^{m-w} is the molar heat of extraction of surfactant molecules from ag-

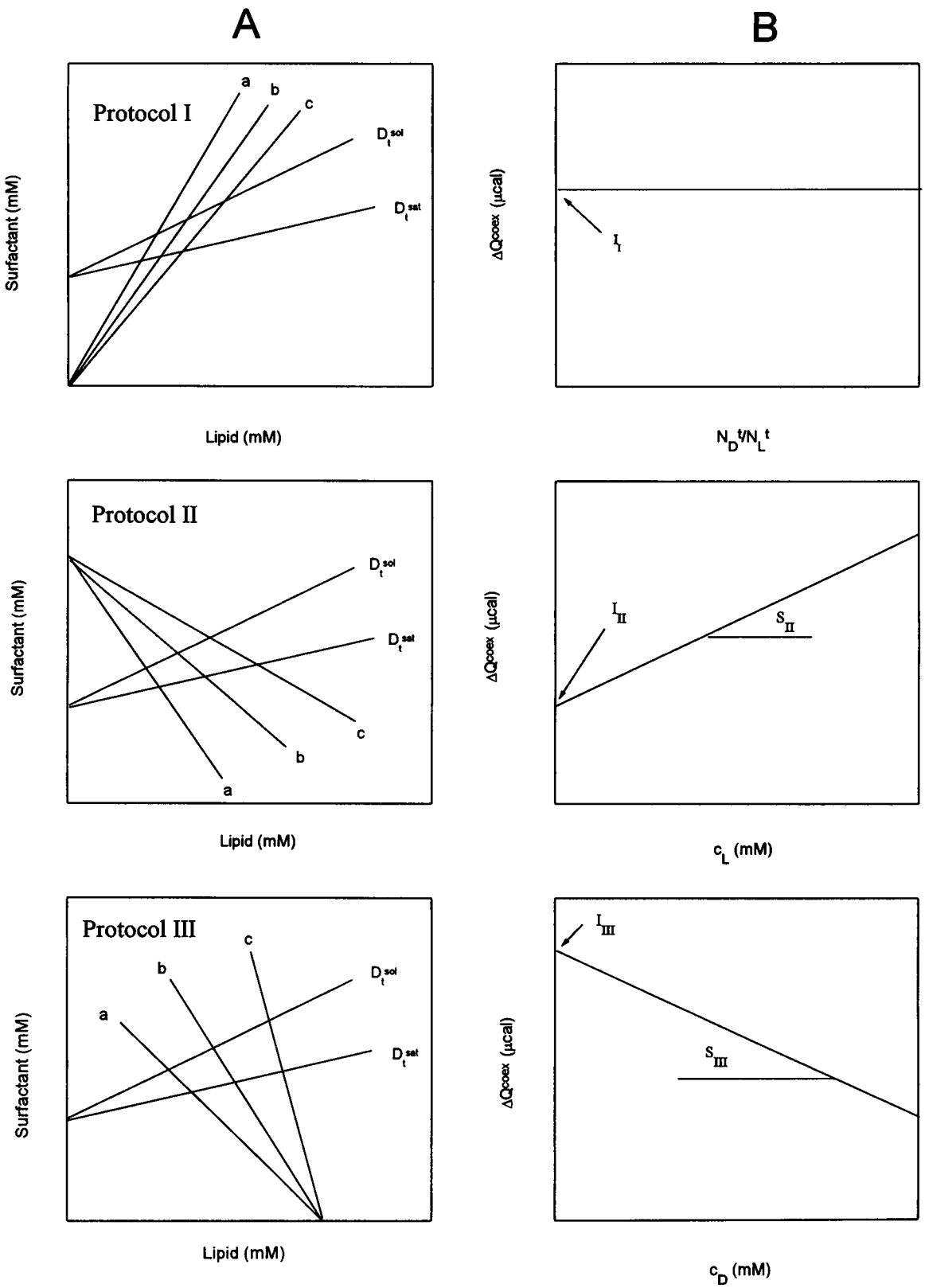

FIGURE 1 (A) Schematic description of the three different protocols used in this study: protocol I (*top*), protocol II (*middle*), and protocol III (*bottom*). The different lines (*a*, *b*, and *c*) in the top panel (Protocol I) depict different ratios of the total numbers of surfactant and lipid molecules N_D^t/N_L^t. In the middle panel (Protocol II) the different lines refer to different values of c_L. In the bottom panel (Protocol III) the different lines refer to different values of c_D. (B) Theoretical dependencies of the heat evolution (ΔQ^{coex} per step) in the range of coexistence. In the top panel (Protocol I), ΔQ^{coex} is given as a function of the ratio of the total number of surfactant and lipid molecules. In the middle panel (Protocol II), ΔQ^{coex} is described as a function of the lipid concentration in the titrant. In the bottom panel (Protocol III), ΔQ^{coex} is described as a function of the surfactant concentration in the titrant.

gregates to water, and ΔH_D^{m-b} and ΔH_L^{m-b} are, respectively, the molar heats of transition of surfactant and lipid molecules from micelles into vesicles.

Our aim is to relate the heat evolution per step of titration within the range of coexistence, ΔQ^{coex}, to the experimentally controlled values, namely the volume of one injection, V_t; the lipid concentration in the titrant, c_L, in protocol II; and the surfactant concentration in the titrant, c_D, in protocol III. The experimental results will then be analyzed in terms of these dependencies.

General relationships

In the range of vesicle-micelle coexistence, the molecules of lipid and surfactant are distributed between mixed micelles, mixed vesicles, and aqueous solutions of monomers. We will denote the numbers of lipid and surfactant molecules in the vesicles by N_L^b and N_D^b, in the micelles by N_L^m and N_D^m, and in the form of monomers by N_L^w and N_D^w, respectively.

According to previous results (Almog et al., 1990; Lichtenberg, 1996), we will assume that the compositions of the mixed vesicles and micelles within the whole range of coexistence are constant (independent of the total concentrations of the components) and equal to

$$R_e^{sol} = \frac{N_D^m}{N_L^m} \tag{6}$$

$$R_e^{sat} = \frac{N_D^b}{N_L^b} \tag{7}$$

respectively.

The thermodynamic consequence of Eqs. 6 and 7 is that within the whole range of coexistence the aqueous concentration of surfactant monomers, D_w, is constant.

Accordingly, the number of surfactant monomers in water, N_D^w, is given by

$$N_D^w = V \cdot D_w \tag{8}$$

where V is the total volume of the aqueous solution.

Because the critical micellar concentration (cmc) of the lipid is very low (Small, 1986), we will neglect the presence of lipid monomers in water, assuming that

$$N_L^w = 0 \tag{9}$$

The total number of surfactant molecules in the mixture is

$$N_D^t = N_D^m + N_D^b + N_D^w \tag{10}$$

whereas according to Eq. 9, the total number of lipid molecules is given by

$$N_L^t = N_L^m + N_L^b \tag{11}$$

Using Eqs. 6–11, we can express the numbers of lipid molecules in micelles and vesicles through the total numbers of lipid and surfactant molecules in the system:

$$N_L^m = \frac{N_D^t - R_e^{sat} \cdot N_L^t - V \cdot D_w}{\Delta R_e} \tag{12}$$

$$N_L^b = \frac{R_e^{sol} \cdot N_L^t - N_D^t + V \cdot D_w}{\Delta R_e} \tag{13}$$

where $\Delta R_e = R_e^{sol} - R_e^{sat}$.

Using Eqs. 12 and 13, together with Eqs. 6 and 7, we can express the numbers of surfactant molecules in micelles and vesicles:

$$N_D^m = R_e^{sol} \cdot N_L^m \tag{14}$$

$$N_D^b = R_e^{sat} \cdot N_L^b \tag{15}$$

As shown in Materials and Methods, in all of our experimental procedures we control the changes in the total amount of the components dN_L^t and dN_D^t, as well as the change in the total volume dV per step of titration. Using Eqs. 12–15, we can determine the corresponding changes in the numbers of molecules of lipid and surfactant in the micelles and vesicles, and compute the numbers of molecules exchanged between the two types of aggregates. This will lead us to determination of all three contributions to the heat evolution (Eq. 5).

PROTOCOL I

Under this protocol, titration results in alteration of the total volume V, whereas the total numbers of lipid and surfactant molecules remain constant. Differentiating Eqs. 12–15 with respect to V and using Eq. 2 for the change in the volume, we obtain

$$dN_L^m = -\frac{D_w \cdot V_t}{\Delta R_e} \tag{16}$$

$$dN_L^b = \frac{D_w \cdot V_t}{\Delta R_e} \tag{17}$$

$$dN_D^m = -\frac{R_e^{sol} \cdot D_w \cdot V_t}{\Delta R_e} \tag{18}$$

$$dN_D^b = \frac{R_e^{sat} \cdot D_w \cdot V_t}{\Delta R_e} \tag{19}$$

The change in the number of surfactant monomers in aqueous solution is

$$dN_D^w = D_w \cdot V_t \tag{20}$$

Dilution by buffer results in partial transformation of mixed micelles into mixed vesicles. During this process, a part of the surfactant molecules that leave the micelles enter vesicles, while another part remains in the aqueous solution in the form of monomers. As a result, the number of surfactant molecules transferred from micelles to vesicles is given by $dN_D^{m-b} = dN_D^b$. Similarly, for the number of transferred lipid

molecules, we obtain $dN_L^{m-b} = dN_L^b$. The injected volume does not contain any surfactant. Therefore, the number of surfactant monomers that become extracted from micelles into aqueous solution is simply equal to $dN_D^{m-w} = dN_D^w$. Using these relationships and inserting Eqs. 17, 19, and 20 into Eq. 5, we obtain for the heat evolution

$$\Delta Q^{coex} = D_w \cdot V_t \cdot \left(\Delta H_D^{m-w} + \frac{\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b}}{\Delta R_e} \right) \quad (21)$$

According to Eq. 21, the heat resulting from one titration step is expected to be independent of the total amounts of the components in the mixture, N_D^t, N_L^t, i.e., it is expected to be constant throughout the whole range of coexistence, as illustrated by Fig. 1 *B* (*upper panel*).

PROTOCOL II

In this case, the total number of surfactant molecules, N_D^t, remains constant, whereas the total number of lipid molecules, N_L^t, and the total volume V change according to Eq. 3. Differentiating Eqs. 12–15 with respect to V and N_L^t and using Eq. 3, we obtain the following changes in the numbers of molecules in the aggregates:

$$dN_L^m = -\frac{(R_e^{sat} \cdot c_L + D_w) \cdot V_t}{\Delta R_e} \quad (22)$$

$$dN_L^b = \frac{(R_e^{sol} \cdot c_L + D_w) \cdot V_t}{\Delta R_e} \quad (23)$$

$$dN_D^m = -\frac{R_e^{sol} \cdot (R_e^{sat} \cdot c_L + D_w) \cdot V_t}{\Delta R_e} \quad (24)$$

$$dN_D^b = \frac{R_e^{sat} \cdot (R_e^{sol} \cdot c_L + D_w) \cdot V_t}{\Delta R_e} \quad (25)$$

The change of the number of surfactant molecules in water, dN_D^w, is given by Eq. 20.

As a result of injecting a volume V_t of a solution of pure lipid vesicles, a portion of preexisting mixed micelles undergoes transition into mixed vesicles. Because the lipid solubility in water is negligible, all of the lipid molecules that leave the micelles enter vesicles. Therefore, the number of lipid molecules transferred from micelles into vesicles is $dN_L^{m-b} = -dN_L^m$. The surfactant molecules that leave the micelles are redistributed between the vesicles and the aqueous solution. Therefore, the number of surfactant molecules undergoing transition from micelles into vesicles is equal, as in the previous case, to $dN_D^{m-b} = dN_D^b$. Because the titrant does not contain surfactant, the number of extracted monomers is equal, as in the previous case, to $dN_D^{m-w} = dN_D^w$.

According to Eqs. 5, 22, 25, and 20, the heat evolution of one titration step is given by

$$\Delta Q^{coex} = D_w \cdot V_t \cdot \left(\Delta H_D^{m-w} + \frac{\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b}}{\Delta R_e} \right) + V_t \cdot c_L \cdot R_e^{sat} \cdot \left(\frac{\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b}}{\Delta R_e} \right) \quad (26)$$

Hence the heat evolution (Eq. 26) is a linear function of the lipid concentration in the titrant c_L, as illustrated in the middle panel of Fig. 1 *B*.

The slope of this dependence is

$$S_{II} = V_t \cdot R_e^{sat} \cdot \frac{\Delta H_L^{m-b} + R_e^{sol} \cdot \Delta H_D^{m-b}}{\Delta R_e} \quad (27)$$

and the intercept is

$$I_{II} = D_w \cdot V_t \cdot \left(\Delta H_D^{m-w} + \frac{\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b}}{\Delta R_e} \right) \quad (28)$$

Note that this intercept is equal to the heat evolution expected in protocol I (Eq. 21).

PROTOCOL III

In this protocol, each titration step leads to changes in the volume, V, and in the total number of surfactant molecules, N_D^t, whereas the total number of lipid molecules does not change, as given by Eq. 4. Differentiating Eqs. 12–15 with respect to V and N_D^t under these conditions leads to

$$dN_L^m = \frac{V_t \cdot (c_D - D_w)}{\Delta R_e} \quad (29)$$

$$dN_L^b = -\frac{V_t \cdot (c_D - D_w)}{\Delta R_e} \quad (30)$$

$$dN_D^m = R_e^{sol} \cdot \frac{V_t \cdot (c_D - D_w)}{\Delta R_e} \quad (31)$$

$$dN_D^b = -R_e^{sat} \cdot \frac{V_t \cdot (c_D - D_w)}{\Delta R_e} \quad (32)$$

The change in the number of surfactant monomers in water is given by Eq. 20.

In this case, the titrant of a volume V_t contains pure surfactant micelles and surfactant monomers, the concentration of which is equal to the cmc, which is higher than the final aqueous concentration of monomers D_w. Accordingly, each titration results not only in transformation of mixed vesicles into mixed micelles, but also in the simultaneous transition of surfactant monomers from the aqueous solution into mixed micelles, so that the monomer concentration relaxes to the equilibrium value D_w. Note that both of these processes are the opposite of those occurring in protocols I and II. The numbers of lipid and surfactant molecules that are transferred from micelles into bilayers is given by $dN_L^{m-b} = dN_L^b$ and $dN_D^{m-b} = dN_D^b$, respectively. To calculate

the number of surfactant monomers that become extracted from mixed aggregates into the aqueous solution after each injection, we have to take into account the monomers pre-existing in the titrant. The resultant number of extracted monomers is $dN_D^{m-w} = dN_D^w - cmc \cdot dV$. Accounting for Eq. 20, this number is given by

$$dN_D^{m-w} = (D_w - cmc) \cdot V_t \tag{33}$$

Note that this value is also negative because monomers are incorporated into the mixed aggregates rather than being extracted from them.

Inserting Eqs. 30, 32, and 33 into Eq. 5 gives us an expression for the heat evolution:

$$\Delta Q^{coex} = (D_w - cmc) \cdot V_t \cdot \Delta H_D^{m-w} + D_w \cdot V_t \cdot \left(\frac{\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b}}{\Delta R_e} \right) - c_D \cdot V_t \cdot \left(\frac{\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b}}{\Delta R_e} \right) \tag{34}$$

Hence the heat evolution per titration under this protocol is a linear function of the surfactant concentration in the titrant, c_D. This is illustrated in the bottom panel of Fig. 1 *B*. The slope of this dependence is

$$S_{III} = -V_t \cdot \left(\frac{\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b}}{\Delta R_e} \right) \tag{35}$$

and the intercept is

$$I_{III} = I_{II} - cmc \cdot V_t \cdot \Delta H_D^{m-w} \tag{36}$$

RESULTS

Fig. 2 *A* presents examples of isothermal calorimetric titrations conducted under the three different protocols described above. As is obvious from these examples, the heat evolution in each of the titration experiments exhibited distinctly different ranges corresponding to the three states of the OG/PC mixture: micellar range, vesicular range, and a range of coexisting vesicles and micelles.

Under protocol I (Fig. 2 *A*, *top*), the exotherms observed in the middle of the series of titration steps (steps 11–14) were larger than in either the preceding or following steps. As confirmed independently by PCS (not shown), titration steps 11–14 corresponded to coexistence between the two types of aggregates, so that each titration step resulted in the transformation of micelles into vesicles. The preceding steps (numbers 1–10) were performed in the micellar range, and the following steps (numbers 15–30) were performed in the vesicular range. In both latter regions the composition of the mixed aggregates changed upon dilution, but no phase transformation occurred.

Under protocol II (Fig. 2 *A*, *middle*), the initial steps of titration (numbers 1–4) resulted in complete solubilization of the added PC vesicles and formation of mixed micelles. The subsequent two steps (5 and 6) resulted in the formation of mixtures of coexisting vesicles and micelles and, consequently, transition of the lipid and surfactant molecules from micelles to vesicles. In the following steps of titration (7–10), the dispersion contained only mixed vesicles.

Under protocol III (Fig. 2 *B*, *bottom*), the initial state of the mixture (steps 1–10) was vesicular. Subsequent titration steps (11–13) resulted in formation of the state of coexistence of micelles and vesicles and the related transfer of lipid and surfactant molecules from vesicles to micelles. Steps 14–25 occurred in the micellar range.

Under all of the protocols in each of the series of titration steps, the heat evolution per titration step, within the range of coexistence, ΔQ^{coex}, is expected to be constant (see Theoretical Considerations, above). In fact, the experimentally determined ΔQ^{coex} varies slightly (Fig. 2 *A*). Because the variation does not show a clear dependence on the injection number (i.e., on the concentrations of surfactant and lipid within this range), we cannot attribute it to deviation from our assumption of a constant D_w. Instead, the variation may be attributed, at least partially, to incomplete equilibration. We therefore think that the extremal value of ΔQ^{coex} is the closest estimate of the equilibrium value of ΔQ^{coex}. Accordingly, we use the extremal heat evolution observed in the calorimetric curve as the representative value of ΔQ^{coex} characterizing the region of coexistence.

The upper panel of Fig. 2 *B* depicts the values of the heat evolution ΔQ^{coex} obtained under protocol I at different ratios of the total numbers of the surfactant and lipid molecules, N_D^t/N_L^t. As expected from our theoretical predictions (Eq. 21), ΔQ^{coex} is indeed almost independent of N_D^t/N_L^t.

The middle panel of Fig. 2 *B* depicts the dependence of the heat evolution, ΔQ^{coex}, measured under protocol II on the concentration of lipid in the titrant, c_L. As predicted theoretically (Eq. 26), ΔQ^{coex} depends linearly on c_L. The slope of the line describing this dependence is equal to $S_{II} = 37.5 \pm 2.5$ μcal/mM, and the intercept has the value $I_{II} = -563 \pm 113$ μcal.

The lower panel of Fig. 2 *B* presents the heat evolution ΔQ^{coex} resulting from titration under protocol III as a function of surfactant concentration in the titrant, c_D. In agreement with the theoretical predictions (Eq. 34), this dependence is linear. The slope of the straight line in this figure is $S_{III} = -3.03 \pm 0.3$ μcal/mM, and the intercept equals $I_{III} = 182 \pm 55$ μcal.

Using Eqs. 27 and 35 for S_{II} and S_{III}, together with the corresponding experimental values obtained for these slopes, we can calculate the molar heats of transition of surfactant and lipid molecules from micelles to vesicles, ΔH_D^{m-b} and ΔH_L^{m-b}. Using the values of R_e^{sat} and R_e^{sol} obtained in the preceding study (1.6 and 3.1, respectively), we obtain

$$\Delta H_D^{m-b} = 645 \pm 110 \text{ cal/mol} \tag{37}$$

$$\Delta H_L^{m-b} = -592 \pm 178 \text{ cal/mol} \tag{38}$$

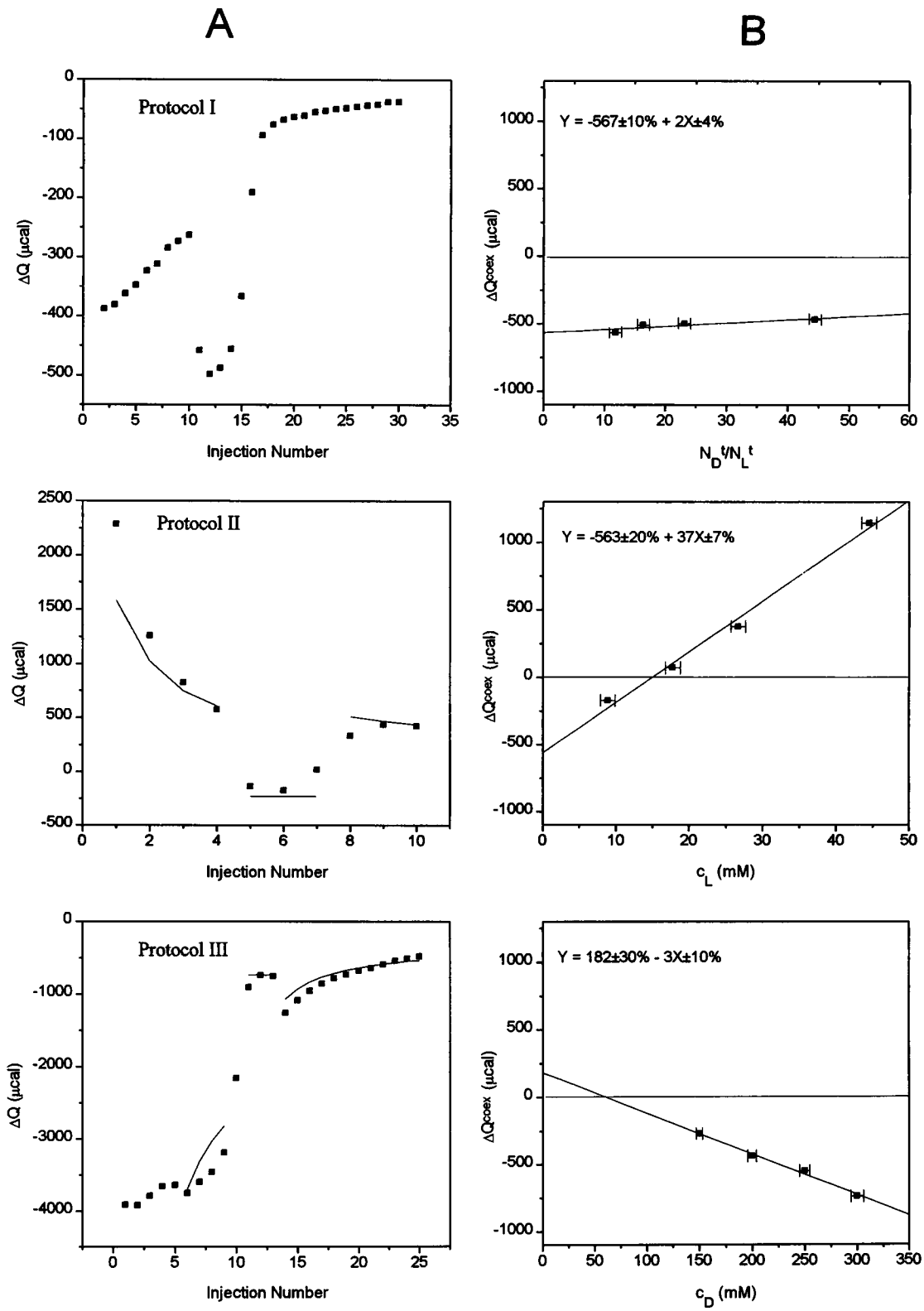

FIGURE 2 (A) Examples of ITC titration curves. Each panel depicts the results of a calorimetric titration experiment. The top panel describes the stepwise titration of OG/PC mixed micelles of a composition of 22 mM OG and 0.95 mM PC with a solution of buffer A. The middle panel describes the stepwise titration of a solution of OG with initial concentration of 20 mM with a dispersion of small unilamellar vesicles at a concentration of 8.9 mM. The bottom panel describes a stepwise titration of a dispersion of lipid vesicle at an initial concentration of 4 mM with a pure OG solution with a concentration of 300 mM. The volume of each injection step was 25 μl for the top and the middle panels, and 10 μl for the bottom panel. The solid lines in the middle and bottom panels are theoretical curves, simulated as described in the Appendix. (B) Experimental dependencies of the heat evolution in the range of coexistence, as described theoretically in Fig. 1 *B* (see text for details).

Inserting the obtained values of the intercepts I_{II} and I_{III} into Eqs. 28 and 36, we can determine the heat of extraction ΔH_D^{m-w} and the cmc of the pure OG. For that we need a value for the aqueous concentration of monomers, D_w. This value, assumed to be constant in the present study, showed slight variations in the measurements of the preceding study (between D_w^{sat} and D_w^{sol}). As we do not understand these variations, which may be due to experimental errors or contributions of other factors that have yet to be investigated, we have chosen to use for the computations in this study an average value, $D_w = (D_w^{sat} + D_w^{sol})/2 = 15.75$ mM. These computations yielded the following values:

$$\Delta H_D^{m-w} = -1732 \pm 294 \text{ cal/mol} \quad (39)$$

$$\text{cmc} = 23.5 \pm 4.5 \text{ mM} \quad (40)$$

DISCUSSION

This study presents a new approach to analyzing the heat evolution of all of the processes involved in the structural transitions in lipid/surfactant mixtures. Using this approach, we were able to calculate the heat of transition of surfactant and lipid molecules from micelles to vesicles as well as the heat of extraction of surfactant molecules from aggregates to water. In addition, this treatment yielded the value of the cmc of pure OG.

The validity of our analysis is supported by the close agreement between the latter value (cmc = 23.5 mM) and the cmc determined by many other investigators, using various independent approaches (Paula et al., 1995; Miguel et al., 1989; Ollivon et al., 1988; Parternostre et al., 1995).

The close agreement between the value of ΔH_D^{m-w} derived from our analysis ($\Delta H_D^{m-w} = -1732$ cal/mol) and the value obtained for the molar enthalpy of demicellization of pure OG micelles ($\Delta H_{demic} = -1691$ cal/mol) lends further support to our analysis.

The values of the heats of transition of surfactant and lipid molecules from micelles to vesicles (ΔH_D^{m-b} and ΔH_L^{m-b}, respectively) have opposite signs: ΔH_L^{m-b} is negative (exothermic), whereas ΔH_D^{m-b} is positive (endothermic). This means that transferring a surfactant molecule from a micelle into a vesicle costs energy, whereas transferring a lipid molecule from a micelle to a vesicle releases energy. This could be explained by the tendency of surfactant molecules to aggregate along curved surfaces and that of lipid molecules to aggregate along flat surfaces. This tendency, expressed in terms of spontaneous curvature, reflects the molecular geometries of the two amphiphiles and contains contributions from the energy of headgroup hydration and solvation of the hydrophobic moieties in the various aggregates. Given the precision of our calorimetric data, separate evaluation of the latter contributions requires further experimentation and theoretical considerations.

Similar tendencies were found for the heat associated with the phase transformation in mixtures of 1-palmitoyl-2-oleoyl-phosphatidylcholine and the nonionic surfactant $C_{12}EO_8$ (Heerklotz et al., 1996). The solubility of the latter surfactant in aqueous media is very low (cmc = 70–90 μM) (Deguchi and Meguro, 1972; Heerklotz et al., 1996). Hence extraction of surfactant monomers from mixed aggregates to the medium made a negligible contribution to the overall heat evolution. This, of course, simplifies the determination of ΔH_L^{m-b} and ΔH_D^{m-b}, so that it was sufficient to perform the measurements at only one concentration of titrant.

In the general case of mixed lipid/surfactant systems, where extraction of surfactant is considerable and cannot be neglected, our approach should be used to dissect the contributions of the various processes and by that process to determine the values of the molar enthalpies of the contributing processes. These enthalpies are likely to depend not only on the composition and structure of the mixed aggregates, but also on the properties (i.e., on the molecular structure) of the lipid and surfactant as well as on the temperature and the composition of the aqueous medium (e.g., pH, ionic strength, presence, and concentrations of water-soluble solutes). The general nature of our method creates the possibility of studying all of these effects. As an example, we think that the value of ΔH for any given component in the system reflects its tendency to be assembled in monolayers of different curvature. This would mean that the value of ΔH for phase transformation can serve as an experimentally attainable measure of the spontaneous curvature of amphiphiles.

In our preceding study (Opatowski et al., 1997) we have analyzed the results of calorimetric titrations conducted according to protocol I under the assumption that $\Delta Q^{coex} = \Delta Q^{m-w}$, i.e., that ΔQ^{coex} is much larger than the heat of transformation of micelles to vesicles (ΔQ^{trans}). This assumption was made to explain the finding that at 40°C all of the heats were extremely small throughout the whole range of concentrations. The validity of this assumption for titrations conducted at other temperatures has yet to be confirmed. Nonetheless, under this assumption, we obtained a reasonable agreement between the calorimetric results obtained at 28°C and the independently measured phase boundaries (R_e^{sat} and R_e^{sol}).

Using the results of the present study, we can compare ΔQ^{m-w} and ΔQ^{trans} for 28°C. According to Eq. 21,

$$\Delta Q^{coex} = \Delta Q^{m-w} + \Delta Q^{trans}$$

where $\Delta Q^{m-w} = D_w \cdot V_t \cdot \Delta H_D^{m-w}$ and $\Delta Q^{trans} = D_w \cdot V_t \cdot ((\Delta H_L^{m-b} + R_e^{sat} \cdot \Delta H_D^{m-b})/\Delta R_e)$. Substituting R_e^{sat}, ΔR_e, ΔH_D^{m-w}, ΔH_L^{m-b}, and ΔH_D^{m-b} with the values obtained in the present study, we get $\Delta Q^{m-w} = -D_w \cdot V_t \cdot 1732$ and $\Delta Q^{trans} = D_w \cdot V_t \cdot 303$. This means that ΔQ^{trans} is in fact equal to ~20% of ΔQ^{m-w}. A 20% deviation, of course, is not "negligible." Nonetheless, given the sensitivity of the values of ΔH of the various processes to temperature and the unresolved question of possible slight variations in the concentration of monomers (D_w) in the range of coexistence, we think that the general conclusion of the preceding study regarding the predominance of the contribution of the heat of extraction is valid.

The close agreement between the value of ΔH_D^{m-w} in the range of coexistence as obtained in this study and the value used in the previous one for the whole range of concentrations supports the other assumption made in the preceding study (Opatowski et al., 1997), namely, that the heat evolution due to extraction of surfactants from mixed aggregates into water ΔH_D^{m-w} is only slightly dependent, if at all, on the composition and structure of aggregates from which the surfactant is extracted. Much more precise determinations are required to study the possible differences that may exist between the heat of extraction from micelles, mixed micelles, and vesicles of different compositions.

APPENDIX

In the main part of the paper we analyzed the calorimetric results obtained in the range of coexistence of mixed micelles and vesicles. In this appendix, we analyze the calorimetric data obtained in the ranges of pure micellar and vesicular phases. We show how results of the present and preceding studies can be used to simulate the heat evolution measured in the ranges of pure phases under Protocols II and III. From the comparison between these simulations and the experimental data, we will estimate the accuracy of our analysis.

The present calculations will be based on the results of the preceding paper, which indicates that the equilibrium composition of mixed aggregates, R_e^a, is a universal function of the aqueous concentration of surfactant monomers, D_w. In addition, we assume that:

1. The molar heat of transition of surfactant molecules from the aggregates to water does not depend on either the type of aggregate or their composition.
2. $\Delta H_D^{b-w} = \Delta H_D^{m-w} = -1732$ cal/mol, the molar heat of transition of lipid between vesicles and micelles, does not depend on the composition of the two types of aggregates and is equal to $\Delta H_L^{m-b} = -592$ cal/mol, as obtained in the coexistence range.
3. The heat of transition of surfactant and lipid molecules from pure aggregates to mixed aggregates of the same type is negligible.

The major effect of titration in the ranges of pure phases is transition of surfactant monomers between the aqueous solution and the aggregates. Therefore, the starting point of the analysis is calculation of the number of molecules of surfactant exchanged between water and the aggregates as a result of one injection. In the following titration steps, the exchange of surfactant molecules between water and aggregates will coincide with the change in the number of surfactant molecules within the aggregates dN_D^a.

The equation we use relates the number of surfactant molecules in aqueous solution, N_D^w, and in the aggregates, N_D^a, to the total number of surfactant and lipid molecules, N_D and N_L, respectively. Taking into account that $N_D^w = V \cdot D_w$, and $N_D^a = R_e^a(D_w) \cdot N_L$, where V is the total volume and D_w is the aqueous concentration of surfactant monomers, we obtain

$$V \cdot D_w + R_e^a(D_w) \cdot N_L = N_D \tag{A1}$$

One injection of volume V_t contains, in the general case, surfactant and lipid of concentrations c_D and c_L, respectively. The resulting changes in the total numbers of molecules of the two components are such that $dN_D = c_D \cdot V_t$, $dN_L = c_L \cdot V_t$, whereas the increase in the total volume is $dV = V_t$. Differentiating Eq. A1 and accounting for the relationships given above, we obtain the following dependence of the change of the aqueous concentration of the surfactant monomers, dD_w, on the composition of the mixed aggregates and the titrant:

$$dD_w = \frac{(V_t/V_c) \cdot (c_D - D_w - R_e^a \cdot c_L)}{1 + L \cdot (dR_e^a/dD_w)} \tag{A2}$$

where V_c is the volume of the experimental cell, and $L = N_L/V_c$ is the total concentration of the lipid.

The change in the number of surfactant molecules in the aggregates can be expressed by

$$dN_D^a = N_L \cdot dR_e^a + R_e^a \cdot dN_L \tag{A3}$$

Taking into account that $dR_e^a = (dR_e^a/dD_w) \cdot dD_w$ and $dN_L = c_L \cdot V_t$ and using Eq. A2, it follows that

$$dN_D^a = \frac{V_t \cdot [(c_D - D_w) \cdot (L \cdot (dR_e^a/dD_w)) + c_L \cdot R_e^a]}{1 + L \cdot (dR_e^a/dD_w)} \tag{A4}$$

Equation A3 relates to both the micellar and vesicular ranges. Applying it below to a particular range, we will replace the index a with m or b for micelles and vesicles, respectively. For the sake of these calculations it is convenient to approximate the universal function $R_e^a(D_w)$ by fitting functions separately in the two ranges. According to the results of the preceding study, $R_e^a(D_w)$ in the micellar range will be taken as

$$R_e^m(D_w) = 39.97 - 5.20 \cdot D_w + 0.20 \cdot D_w^2 \tag{A5}$$

and in the vesicular range as

$$R_e^b(D_w) = 1.73 - 0.2 \cdot D_w + 0.01 \cdot D_w^2 \tag{A6}$$

Using these expressions, we can consider the heat evolutions expected under Protocols II and III in the different ranges.

Protocol II

When the pure lipid vesicles are injected into the mixture, the concentration of lipid changes with the injection number n according to

$$L = c_L \cdot \left[1 - \left(\frac{V_c}{V_c + V_t}\right)^n\right] \tag{A7}$$

Micellar range

There are two contributions to the heat evolution: the first is related to transition of lipid molecules from the injected vesicles to mixed micelles, and the second results from transition of surfactant molecules between the aqueous solution and the mixed micelles. The resulting heat is therefore given by

$$\Delta Q = -\Delta H_L^{m-b} \cdot c_L \cdot V_t - \Delta H_D^{m-w} \cdot dN_D^m \tag{A8}$$

where dN_D^m is given by Eq. A4, where we insert Eqs. A5 and A7 and $c_D = 0$.

The values of ΔH_L^{m-b} and ΔH_D^{m-w} are taken as obtained in the main part of the paper. The result of the calculation of ΔQ is shown in Fig. 2 *A* (micellar range).

Vesicular range

Neglecting the possible heat of transition of lipid molecules from pure lipid vesicles to the mixed vesicles, the expected heat evolution is

$$\Delta Q = -\Delta H_D^{m-w} \cdot dN_D^b \tag{A9}$$

where dN_D^b is given by Eq. A4, accounting for Eqs. A6 and A7 and $c_D = 0$. The heat evolution given by Eq. A9 is presented in Fig. 2 *A* (vesicular range).

Protocol III

A solution of detergent of a concentration c_D higher than the cmc is injected into the mixture. The lipid concentration in the titration cell changes with the injection number according to

$$L = L_0 \cdot \left(\frac{V_c}{V_c + V_t}\right)^n \qquad \text{(A10)}$$

Micellar range

The first contribution to the heat evolution relates to monomerization of the micelles preexisting in the titrant; the second contribution results from the exchange of the surfactant molecules between the aqueous solution and the mixed micelles. The resulting heat evolution is given by

$$\Delta Q = \Delta H_D^{m-w} \cdot (c_D - \text{cmc}) \cdot V_t - \Delta H_D^{m-w} \cdot dN_D^m \qquad \text{(A11)}$$

where the second term is given by Eq. A4, accounting for Eqs. A5 and A10 and $c_L = 0$. The result of these calculations is presented in Fig. 2 *A* (micellar range).

Vesicular range

As in the preceding case, the heat evolution is presented by

$$\Delta Q = \Delta H_D^{m-w} \cdot (c_D - \text{cmc}) \cdot V_t - \Delta H_D^{m-w} \cdot dN_D^b \qquad \text{(A12)}$$

where the second term is given by Eq. A4, accounting for Eqs. A6 and A10 and $c_L = 0$. The results of these calculations are presented in Fig. 2 *A* (vesicular range).

REFERENCES

Almog, S., B. J. Litman, W. Winley, J. Cohen, E. J. Wachtel, Y. Barenholz, A. Ben-Shaul, and D. Lichtenberg. 1990. States of aggregation and phase transformation in mixtures of phosphatidylcholine and octyl glucoside. *Biochemistry.* 29:4582–4592.

Deguchi, K., and K. Meguro. 1972. The determination of critical micelle concentration of non-ionic surfactants by charge-transfer solubilization of 7,7,8,8-tetracyanoquinidimethan. *J. Colloid Interface Sci.* 38: 596–600.

Heerklotz, H., G. Lantzsch, H. Binder, G. Klose, and A. Blume. 1996. Thermodynamic characterization of dilute aqueous lipid/detergent mixtures of POPC and $C_{12}EO_8$ by means of isothermal calometry. *J. Phys. Chem.* 100:6764–6774.

Lichtenberg, D. 1993. Micelles and liposomes. *In* Biomembranes—Physical Aspects, Vol. 1. M. Shinitzky, editor. VCH, Weinheim. 63–96.

Lichtenberg, D. 1996. Liposomes as a model for solubilization and reconstitution of membranes. *In* Handbook of Nonmedical Applications of Liposomes: Models for Biological Phenomena, Vol. 2. Y. Barenholz and D. D. Lasic, editors. CRC Press, Boca Raton, FL. 199–218.

Miguel, M. G., O. Eidelman, M. Ollivon, and A. Walter. 1989. Temperature dependence of the vesicle-micelle transition of egg phosphatidylcholine and octyl glucoside. *Biochemistry.* 28:8921–8928.

Ollivon, M., O. Eidelman, R. Blumenthal, and A. Walter. 1988. Micelle-vesicle transition of egg-phosphatidylcholine and octyl glucoside. *Biochemistry.* 27:1695–1703.

Olofsson, G. 1985. Microtitration calorimetric study of the micellization of three poly(oxyethylene) glucol dodecyl ethers. *J. Phys. Chem.* 89: 1473–1477.

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.

Paternostre, M., O. Meyer, C. Grabielle-Madelmont, S. Lensieur, M. Ghanham, and M. Ollivon. 1995. Partition coefficient of a surfactant between aggregates and solution: application to the micelle-vesicle transition of egg phosphatidylcholine and octyl β-$_D$-glucopyranoside. *Biophys. J.* 69:2476–2488.

Paula, S., W. Suess, J. Tuchtenhagen, and A. Blume. 1995. Thermodynamics of micelle formation as a function of temperature: a high sensitivity titration calorimetry study. *J. Phys. Chem.* 99:11742–11751.

Small, D. M. 1986. The Physical Chemistry of Lipids: Handbook of Lipid Research, Vol. 4. Plenum Press, New York.