Excess Enthalpies of Mixing in Phospholipid-Additive Membranes

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Isothermal titration calorimetry (ITC) allows the measurement of composition-dependent mixing heats of amphiphiles. A number of experimental protocols are now established to measure molecular transfer heats between, for example, micellar and lamellar aggregates. This study deals with the principle understanding of the physical effects contributing to the ITC data. The physical state of the mixture is described in terms of the molar excess enthalpy as a function of its composition $h_E(X)$. A relation is derived between this system property and the observable heat per mole of titrant (q_{obs}) as $q_{obs} = (X_{syr} - X)(\partial h_E/\partial X) + h_E(X) - h_E(X_{syr})$ with X and X_{syr} being the mole fractions of one chosen component within the mixed aggregates in the sample cell and in the injection syringe, respectively. According to this differential equation, one may derive information about the second and further derivatives (i.e., the curvature) of the excess enthalpy function. This can serve to construct the $h_E(X)$ plot based on the ITC data. We emphasize that for aggregates mixing nonideally (which must be considered rather the rule than the exception) one has to carefully distinguish between observed mixing heats and enthalpic state of the mixture. The formalism is presented at the example of mixtures of the phospholipid POPC and detergents of the type $C_{12}EO_n$ with $n = 3-6$. For instance, the system $C_{12}EO_3$/POPC was found to show an extremely asymmetric mixing enthalpy function with an attractive part (i.e., $h_E < 0$) for low and a repulsive one for higher detergent contents in the mixed membranes. Such excess enthalpy functions could be modeled by a polynomial equation and discussed in terms of cooperative interactions between the molecules.

Introduction

Recently, it was shown that the incorporation heats of amphiphilic molecules into lipid membranes can be very sensitively and conveniently studied by isothermal titration calorimetry. Various protocols allow characterizing the "binding" of amphiphile monomers to membranes[1−6] as well as the transfer of detergents from micelles to lipid membranes.[3,4,8−11] As a result, a consistent system of transfer heats of the molecules between the various pseudo-phases was established. As long as one assumes the characteristic molar enthalpy in each of the states (monomers, micelles, and bilayers) to be constant, the transfer heats directly reflect the enthalpy differences of the transferred molecule between the initial and the final state, respectively.

We emphasize that this does not remain true when the molar enthalpies of the different molecules in a mixed membrane or micelle depend on the aggregate composition. For many surfactants, the partition coefficients to lipid membranes were found to decrease with increasing surfactant content[12−15] (i.e., the chemical potential gain upon incorporation decreases). Because the composition dependent packing effects of hydrocarbon chains[1,2] as well as that of hydrated headgroups[6,9] in lamellae give rise to both enthalpic and entropic effects which essentially cancel out each other, the molar enthalpies are suggested to depend even more sensitively on aggregate composition.

Unfortunately, little is known about these nonideality effects up to now. Johann et al.[16,17] modeled heat capacity curves (i.e., DSC data of phospholipid mixtures) considering asymmetric nonideal mixing properties in terms of a polynomial model. ITC experiments dealing with the incorporation of surfactant monomers into membranes ("partitioning protocols") have not been suitable to resolve a composition dependence of the enthalpy.[2−4] Most of the few data available for the micelle to bilayer transfer presently lack a consistent quantitative modeling and interpretation.[8,9] Only for the rather strongly cone-shaped detergents $C_{12}EO_7$ and $C_{12}EO_8$ a simple pair interaction approach allowed a good fit of composition dependent enthalpies,[9] giving rise to substantial endothermic nonideality parameters.

In this study, we present a general approach to evaluate nonideal mixing heats obtained by ITC in terms of an excess enthalpy function. Whereas experimental transfer data include the heat required for the complete process of reequilibration after an injection (dynamic information), the excess enthalpy function characterizes the enthalpic state of a molecule at a given composition regardless of the history of the sample.

The approach is illustrated for mixtures of the nonionic detergents $C_{12}EO_n$ with $n = 3-7$ and the phospholipid POPC. For these detergents, sufficiently high experimental concentrations can be chosen (in the millimolar range) so that the fraction of monomers in water is negligible. Then, all the measured heats can be interpreted in terms of nonideality effects in the bilayers and, for $n = 5-8$, the transformation of the detergents from the micellar to the lamellar state. The results show that the transfer heats of the detergent or the lipid to the mixed membranes may differ from the excess enthalpies even in sign,

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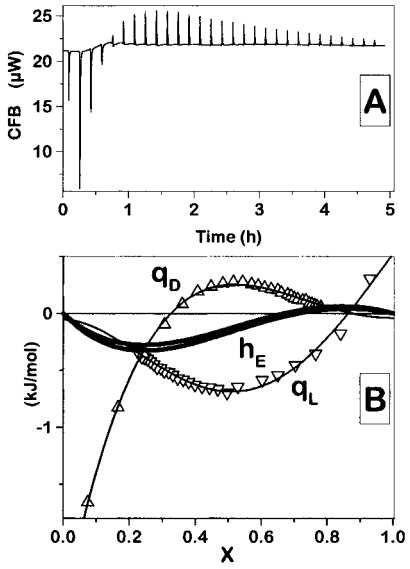

Figure 1. Experimental raw data collected by an ITC experiment injecting 10 μL aliquots of a 39 mM $C_{12}EO_3$ vesicle dispersion to a 2.45 mM POPC suspension (*A*) and the corresponding observed heats q_{obs} vs the $C_{12}EO_3$ mole fraction X (*B*, △). Additionally, the data of the injection of 15 mM POPC to 1 mM $C_{12}EO_3$ are displayed as ▽. The fit lines correspond to eqs 11 and 12, respectively. The thick solid lines represent the excess enthalpy functions $h_E(X)$ according to eq 10 and the fit parameters.

illustrating the major importance of this differentiation for a proper interpretation of the experimental results.

Experimental Section

The lipid 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) was purchased from Avanti Polar Lipids, Birmingham, AL, and the oligo (ethylen oxide) dodecyl ethers were from Nikko Chemicals, Japan. The substances were used without further purification.

The dry phospholipid POPC was suspended in water by vortexing and subsequent extrusion through Nuclepore polycarbonate membranes of 100 nm pore size. This procedure was checked to ensure it yielded essentially unilamellar vesicles of 100 nm diameter and to ensure caused no significant loss of material. The detergent was dispersed in water and vortexed rapidly. In dilute aqueous dispersion, $C_{12}EO_3$ and $C_{12}EO_4$ form bilayer vesicles and the detergents $C_{12}EO_n$ with $n = 5-8$ aggregate as micelles at room temperature.[18,19]

The experiments were done at a MicroCal MCS isothermal titration calorimeter (ITC).[20] Typically, about 20 injections of $3-10$ μL each are performed using computer-controlled titration syringe (syringes with total volume of about 60, 130, and 300 μL available) to the sample cell of 1.3 mL volume. The cell is filled completely prior to the experiment so that concentration data are subject to a slight correction for replacement effects. The heat of mixing after each injection is detected by means of a power peak of the cell feedback heater (CFB, cf. Figure 1A) to balance temperature differences between the sample and reference cell, both residing within an adiabatic jacket. During all the experiments, the cell is stirred with 100 or 400 rounds/min. The lower rate increases the time for reequilibration slightly but essentially prevents heat effects arising from viscosity changes upon injection.

The detergent fraction in the aqueous dispersion and its variations are negligible at the millimolar concentrations of detergent or lipid chosen for this study. Generally, this approximation is better for higher lipid and/or detergent concentrations, higher partition coefficients (i.e., $C_{12}EO_n$ with lower n), less exothermic heats of demicellization (i.e., lower n), and less endothermic heats of transfer to mixed bilayers (i.e., lower n). In particular, the effective detergent mole fraction in the mixed membranes is by -0.001 or less lower than the total X used. The subsequent maximum absolute error of the molar titration heats shown (-0.5 kJ/mol) arises for the first injection of $C_{12}EO_7$ (cf. Figure 3), where 2% of the injectant is transferred to the water causing -0.26 kJ/(mol injected) instead of the $+0.24$ kJ/(mol injected) expected for the detergent incorporation into membranes, which corresponds to a relative error of 4%. The maximum absolute error for $C_{12}EO_3$ and $C_{12}EO_4$ occurs for the first injection of the latter and amounts to -0.1 kJ/(mol injected) (cf. Figure 2).

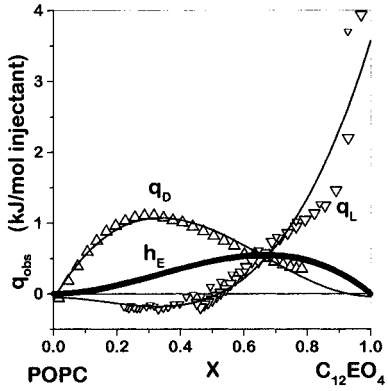

Figure 2. Observed heats for the titration of 101 mM $C_{12}EO_4$ to 2.5 mM POPC (△) and for 15 mM POPC injected to 2.5 mM (larger ▽) and 1 mM (smaller ▽) $C_{12}EO_4$, respectively. The fit lines are according to eqs 11 and 12; the thick solid line represents the corresponding excess enthalpy function according to eq 10.

Theory

Excess Enthalpy and Observed Heats. We consider an aqueous lipid/detergent mixture in the composition range where lamellar aggregates are formed exclusively. For simplicity, let us assume that the partition coefficient of the detergent and the experimental concentrations are high enough to make detergent monomers in water negligible. That means that the effective mole fraction of detergent in the mixed membrane (often denoted X_e) can be approximated by the total X:

$$X_e \approx X = \frac{N_D}{N_D + N_L} \tag{1}$$

with N_D and N_L denoting the mole numbers of detergent and lipid, respectively.

The enthalpy of the mixed lamella $H(X)$ can be written as the weighted sum of the partial molar enthalpies of the lipid and detergent h_L and h_D:

$$H(X) = N_D h_D(X) + N_L h_L(X) \tag{2}$$

with the partial molar enthalpies considered to be a function of the bilayer composition X.

The mixing excess enthalpy $h_E(X)$ is defined as the difference between the mean molar enthalpy of the mixture and the ideal average of the molar enthalpies of the pure components $h_D(1)$ and $h_L(0)$:

$$h_E(X) = X[h_D(X) - h_D(1)] + (1 - X)[h_L(X) - h_L(0)] \tag{3}$$

yielding with eqs 1 and 2,

$$H(X) = N_D h_D(1) + N_L h_L(0) + (N_D + N_L) h_E(X) \quad (4)$$

The heat Q consumed or released upon reequilibration of the system after mixing injectant and cell content is just the enthalpy difference between the previous and the new equilibrium state. It is conveniently expressed in terms of the observable heat per mole of injectant q_{obs}:

$$q_{obs} = \frac{Q}{\Delta N_D + \Delta N_L} \quad (5)$$

The relation between this experimentally observable quantity and the system state specified by means of the excess enthalpy is derived in the Appendix, yielding:

$$q_{obs} = (X_{syr} - X)\frac{\partial h_E(X)}{\partial X} + h_E(E) - h_E(X_{syr}) \quad (6)$$

For single component injectants, the titrant composition X_{syr} amounts to 0 (lipid injection) or 1 (detergent injection) and the last term vanishes. The first term of eq 6 describes the variation of the sample enthalpy upon variation of its composition X caused by the injection. Note that X is increased by detergent injections ($(X_{syr} - X) > 0$ for $X_{syr} = 1$) but decreased by lipid injections ($(X_{syr} - X) < 0$ for $X_{syr} = 0$) to a mixture ($0 < X < 1$) and that the variation of X caused by a series of equal injections decreases with X approaching X_{syr}. The equation applies also to injection of mixtures ($0 < X_{syr} < 1$) which can be important if the pure detergent cannot be homogeneously dispersed in water. The change of the sample composition causes a heat dependent on the local slope of the excess enthalpy function $\partial h_E/\partial X$. The difference $h_E(X) - h_E(X_{syr})$ represents the enthalpy change of the injected material. Writing this difference in terms of an infinite sum according to the Taylor law,

$$-[h_E(X_{syr}) - h_E(X)] = -\sum_{k=1}^{\infty} \frac{\partial^k h_E(X)}{\partial X^k} \frac{(X_{syr} - X)^k}{k!} \quad (7)$$

eq 6 can be rewritten as

$$q_{obs} = \sum_{k=2}^{\infty} \frac{\partial^k h_E(X)}{\partial X^k} \frac{(X_{syr} - X)^k}{k!} \quad (8)$$

eq 8 leads to the important conclusion that the experimental q_{obs} data which are observed in the ITC experiment contain information only about the second and higher derivatives of $h_E(X)$. That means that only the local curvature but not the absolute value or linear slope of the excess enthalpy function are experimentally accessible from a chosen value $q_{obs}(X)$.

Experimental Determination of the Excess Enthalpy Function. Let us denote the heat observed upon detergent and lipid titration $q_D = q_{obs}(X_{syr} = 1)$ and $q_L = q_{obs}(X_{syr} = 0)$, respectively. To gain the physical property h_E based on the experimental data q_D and/or q_L, one has to solve the differential eq 6 for h_E.

On one hand, the excess enthalpy value at a given composition X can be calculated on the basis of $q_D(X)$ and $q_L(X)$ according to the relation:

$$h_E(X) = X q_D(X) + (1 - X) q_L(X) \quad (9)$$

which can be easily proven using eq 6. We should note that the mixing heats q_D and q_L are not independent. For given values of $q_D(X)$, one can calculate $q_L(X)$ and vice versa according to the formulas:

$$q_L(X) = -\frac{X}{1 - X} q_D(X) + \int_0^x \frac{q_D(X')}{(1 - X')^2} dX' \quad (9a)$$

$$q_D(X) = -\frac{1 - X}{X} q_L(X) + \int_0^x \frac{q_L(X')}{X'^2} dX' \quad (9b)$$

Equations 9a,b can be derived by differentiation of eq 9 and using eq 6.

On the other hand, eq 6 can be solved using a model function for $h_E(X)$. This solution should obey two conditions: It must vanish for $X \rightarrow 0$ and for $X \rightarrow 1$ in accord with the definition of the excess enthalpy (cf. eq 3) and it must be analytically differentiable to apply eq 6. Assuming an polynomial expression,[16,17,21]

$$h_E^b(X) = X(1 - X)[\rho_0 + \rho_1 X + \rho_2 X^2 + \rho_3 X^3 + ...] \quad (10)$$

the observed heat (eq 5) becomes, according to eq 6 for the detergent injection experiment ($X_{syr} = 1$),

$$q_D = (1 - X)^2[\rho_0 + 2\rho_1 X + 3\rho_2 X^2 + 4\rho_3 X^3 + ...] \quad (11)$$

and for the lipid injection experiment ($X_{syr} = 0$),

$$q_L = X^2[(\rho_0 - \rho_1) + 2X(\rho_1 - \rho_2) + 3X^2(\rho_2 - \rho_3) + 4X^3(\rho_3 - ...)...] \quad (12)$$

The functions (11) or (12) allow fitting the experimental data by adjusting the parameters ρ_0, ρ_1, ρ_2, etc., determining $h_E(X)$ according to eq 10. This formal approach is empirical and does not require the parameters to have a distinct physical meaning.

We state that eq 9 requires both the q_D and the q_L at the composition X to calculate $h_E(X)$. Using eq 11 or 12 one can calculate h_E based on the complete data set of only one experiment, q_D or q_L.

Statistic Motivation in Terms of Cooperative Interactions. If one stops the polynomial in the bracket in eqs 10−12 after ρ_0, one obtains the well-known formula for pair interaction statistics in randomly mixed systems (cf. regular solution model[22,23]) and the subsequent ITC fitting eqs[3,9] where the factor ρ_0 represents a measure of the nonideal pair interaction enthalpy. This special case constitutes the first approximation in a series also in the physical sense. In general, the interaction between two molecules can be considered to depend on other molecules in the environment as well. Then, interactions of higher cooperativity than pair interactions must be taken into account to model the enthalpy of the mixture.[24]

Let us consider clusters of three molecules every one of which is in contact with both the others in a two-dimensional hexagonal array. Four types of clusters are possible in the mixture of lipid (L) and detergent (D) molecules: LLL, LLD, DDL, and DDD. Note that the pure lipid and detergent clusters, LLL and DDD, exhibit no excess enthalpy. Consequently, the excess enthalpy of a randomly arranged two-component mixture is given by the sum of the enthalpies of the LLD and DDL clusters only (denoted ρ_{LLD} and ρ_{DDL}), weighted by their relative incidences, p_{LLD} and p_{DDL}:

$$h_E^b(X) = p_{LLD}\rho_{LLD} + p_{DDL}\rho_{DDL} \quad (13)$$

The superscript "b" added to h_E serves to specify the fact that the mixed and both the pure phases have the same (e.g., bilayer) structure. This is important for the consistency of the equations with those for micellar detergents introduced below. Assuming random mixing, one obtains with $p_{LLD} = (1 - X)^2X$ and $p_{DDL} = X^2(1-X)$:

$$h_E^b(X) = X(1 - X)[\rho_{LLD}(1 - X) + \rho_{DDL}X] \quad (14)$$

We emphasize that eq 14 is equivalent with eq 10 for

$$\rho_{LLD} = \rho_0 \quad (15)$$

$$\rho_{DDL} = \rho_0 + \rho_1 \quad (16)$$

and ρ_2, ρ_3, ... = 0. An analogous approach for clusters of four molecules is found to correspond to eq 10 considering the terms up to ρ_2, with

$$\rho_{LLLD} = \rho_0 \quad (17)$$

$$\rho_{LLDD} = \rho_1 + 2\rho_0 \quad (18)$$

$$\rho_{LDDD} = \rho_0 + \rho_1 + \rho_2 \quad (19)$$

Summarizing, we conclude that the number of terms of the polynomial in eqs 11 and 12 required to fit the data can be interpreted as a measure for the cooperativity of the molecular interactions (i.e., of the minimum size of clusters which must be taken into account to model the molecular interactions in the mixture). In the frame of this approach, the fit parameters of the empirical function eq 10 possess a physical meaning regarding cluster enthalpies. However, we emphasize again that the empirical application of eq 10 to plot $h_E(X)$ is not subject to the validity of any assumption made in this chapter.

Results and Discussion

Derivation of the Excess Enthalpy Functions. The Figures 1−4 illustrate the experimental data obtained for the detergents $C_{12}EO_n$ with $n = 3-6$ in mixtures with POPC. Generally, the data collected upon detergent injections (i.e., the "upscans" in the X-scale) are displayed as up triangles and the lipid injection data (i.e., "downscans") by down triangles.

The blank experiment injecting lipid dispersions to water yielded titrant dilution heat of about −0.054 kJ/mol. The detergent dilution heat of about −0.04 kJ/mol was measured by injections of the concentrated detergent dispersions to detergent dispersions above the cmc (in the absence of lipid). These values were considered as constant base line shifts in the fit procedures.

The detergents $C_{12}EO_3$ and $C_{12}EO_4$ (cf. Figures 1 and 2) which form lamellae in aqueous dispersion at room temperature show rather low mixing heats over the complete composition range. The experimental data collected by lipid and detergent titration experiments could be fitted consistently with one set of parameters ρ_0 and ρ_1 using eqs 11 and 12, respectively.

The pure detergents $C_{12}EO_n$ with $n = 5-8$ form micelles in aqueous dispersion. Consequently, a composition-driven phase transition from mixed lamellae to micelles occurs at a distinct composition X, indicated by marked breakpoints of the observed heats (cf. Figures 3 and 4). A quantitative treatment of the transfer heats between the different phases was introduced in ref 3. However, we can employ the excess enthalpy formalism also to these systems. Generally, h_E is defined to describe the difference between the mean enthalpy of the molecules in the real compared to an ideal mixture. The latter is given in terms of the weighted average of the enthalpies of the pure phases of the components, $h_D(1)$ and $h_L(0)$, giving rise to eq 3. In the present case, $h_L(0)$ and $h_D(1)$ stand for pure lipid bilayers and pure detergent micelles, respectively. Indeed, one can find such a function $h_E(X)$ which accounts fairly well for the experimental data q_D, as well as q_L, according to eq 6 (cf. solid lines in Figure 3).

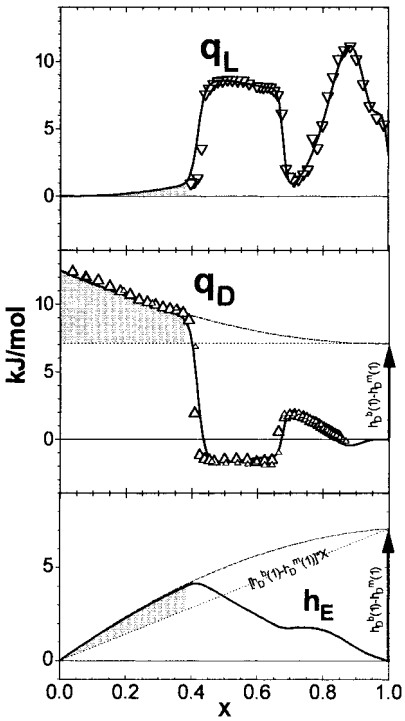

Figure 3. Observed heats q_D (part A)and q_L (cf. part B) and the corresponding excess enthalpy h_E (cf. part C) for the system $C_{12}EO_7$/POPC as a function of the $C_{12}EO_7$ mole fraction X. Experimental parameters: (*A*) Titration of 4.9 mM $C_{12}EO_7$ with 28 × 10 μL injections of POPC 15 mM (▽), (*B*) Titration of 5 mM POPC with 20 × 3 μL + (syringe changed) 28 × 10 μL $C_{12}EO_7$ 100 mM (larger △) and (new experiment) with 30 × 10 μL + (syringe refilled) 11 × 25 μL $C_{12}EO_7$ 100 mM (smaller △). The thick solid lines represent one simultaneous fit of all data sets shown and the corresponding excess enthalpy function h_E (fitting eqs obey eq 6 and are not presented explicitly). The gray filled area in part C marks h_E^b (cf. eq 20). The other lines are explained in the text.

Of course, this "overall" or "apparent" excess enthalpy cannot be modeled in terms of eq 10 because it contains contributions from the interactions of the molecules within the bilayers and/or micelles as well as from the phase transformation of the molecules. An approach to split these contributions to h_E within the lamellar range is described by eq 20:

$$h_E(X) = h_E^b(X) + X[h_D^b(1) - h_D(1)] \quad (20)$$

and illustrated by Figure 3. Accordingly, an intermediate enthalpy state $h_D^b(1)$ is introduced which can be imagined as the molar enthalpy of imaginary pure detergent bilayers. The transfer to this state would cause the heat $h_D^b(1) - h_D(1)$ as indicated by the arrows in Figure 3B, C. The fact that each detergent molecule in the mixed lamella "contains this enthalpy" contributes to the mean enthalpy of all molecules in the lamella weighted by the detergent content X. With equations 6 and 20 we find for the heat upon detergent injection q_D into a sample in the exclusively lamellar range:

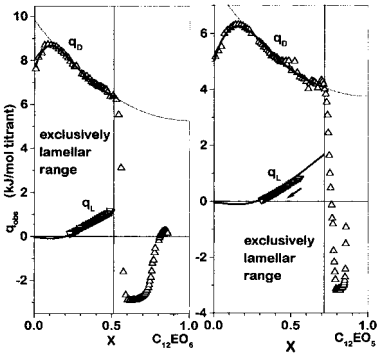

Figure 4. Observed heats on titration of 100 mM $C_{12}EO_6$ (left, △) and 100 mM $C_{12}EO_5$ (right, △) to 5 mM POPC, respectively, and upon titration of 25 mM POPC to mixtures 2.5 mM POPC + 2.5 mM $C_{12}EO_6$ (left, ▽) and 2.9 mM POPC + 4 mM $C_{12}EO_5$ (right, ▽). The behavior in the exclusively lamellar range was fitted (solid lines) according to eqs 11, 12, and 21. The corresponding excess enthalpy functions are displayed in Figure 4. The extrapolation to estimate $[h_D^b(1) - h_D^m(1)]$ (thin dash/dot lines) was done for $X > 0.2$ with $\rho_o = 3.9$ and 4.6 kJ/mol for $n = 5$ and 6, respectively, and $\rho_1, \rho_2, \ldots = 0$.

$$q_D = (1 - X)\frac{\partial h_E^b(X)}{\partial X} + h_E^b(X) + [h_D^b(1) - h_D(1)] \quad (21)$$

Note that q_L is not affected by a phase transformation in the lamellar range because $h_L(0) = h_L^b(0)$.

Hence, the dotted lines in Figure 3 can be imagined to separate the contributions to q_D and h_E arising from the lipid/detergent interactions in the membrane (marked in gray) and from the injectant phase transformation.

The quantity h_E^b is directly comparable to the excess enthalpy functions for lamellar detergents (the difference $h_E - h_E^b$ vanishes for $h_D^b(1) = h_D(1)$) and has all properties of an excess enthalpy function with the only restriction that the reference state for the pure detergent phase is not the equilibrium state.

Note that the extrapolation procedure we applied to estimate $h_D^b(1) - h_D(1)$ (cf. dash/dot lines in Figures 3 and 4) assumes the absence of rather highly cooperative, detergent dominated clusters which would not be detectable inside the experimentally accessible range.

The experimental data could be modeled fairly well according to the cooperative interaction model eq 10 (cf. 15−19) with one to five parameters ρ_i and a constant for all detergents investigated. The fact that, for all the detergents, both q_L and q_D measured independently were found to correspond to the same excess enthalpy function can be considered a proof of the approach.

Interpretation of the Excess Enthalpy Functions. The excess enthalpies h_E^b of mixed lamellae made up of POPC and detergents $C_{12}EO_n$ ($n = 3-8$) are observed to be more endothermic as the detergent headgroup becomes larger (cf. Figure 5). This rather unspecific effect can be, at least qualitatively, explained in terms of the curvature strain introduced by the detergent into the membrane as argued earlier.[9]

An essential new information extracted from the excess enthalpy formalism is the asymmetric shape of the excess enthalpy functions with respect to $X = 0.5$. Whereas the symmetric regular solution function considering pair interactions obeys an exclusively convex curvature, the $h_E^b(X)$ curves for $n = 3-6$ exhibit a considerably ($n = 3$ or 4) or slightly ($n = 5$ or 6) increasing slope at low X. This property can be imagined as a measure for cooperative interactions involving more than two molecules.The data are compatible with the idea that a

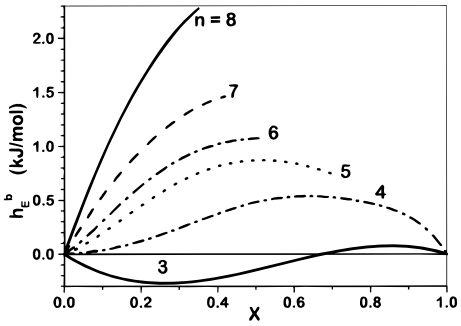

Figure 5. Intrinsic bilayer excess enthalpy functions h_E^b vs detergent mole fraction in the membrane X for the homologous series of the detergents $C_{12}EO_n$ with $n = 3-8$. The curve for $C_{12}EO_4$ differs by a slightly steeper right flank from the one displayed in Figure 2. It was derived fitting the data at high X more precisely by more adjustable parameters. The $C_{12}EO_8$ parameters have been derived in ref 3.

randomly occurring arrangement of two $C_{12}EO_3$ molecules neighboring to one POPC results in an enthalpy gain of −2.3 kJ/mol compared to the pure components. The respective advantage for two $C_{12}EO_4$ plus one POPC amounts to −0.05 kJ/mol only. In contrast, clusters containing two detergents and one lipid molecule are enthalpically unfavorable by +1.1 and +3.8 kJ/mol, respectively. The behavior for the larger headgroup detergents $C_{12}EO_5$ and $C_{12}EO_6$ can be modeled only if one introduces higher order terms to the fit functions. That means, the specific, enthalpically favorable interaction is not only weaker but exhibits also a higher cooperativity. In other words, more lipid molecules are required to interact with one detergent to form the favorable arrangement. Note that the values for h_E^b observed (cf. Figure 4) are considerably lower than the thermal energy (2.5 kJ/mol) and thus support the assumption of a random arrangement of the molecules, which was assumed in the statistical motivation. About the molecular origin of this effect we can only speculate at the moment. Does the favorable cluster constitute a unit with vanishing spontaneous curvature? Does the detergent "fill a space" the phospholipid headgroups leave free because of dipole or hydration forces? Do the different hydrocarbon chains of the lipid and the detergent play a role for this specific interaction? These and other hypotheses are to be proven or abandoned by further studies and alternative methods.

Conclusions

The main aim of this study was to specify the information content of mixing heats gained by means of ITC. A formalism was established to relate the experimental heats to the enthalpic state of the molecules in the mixture. We emphasize that one has to carefully distinguish between both quantities as soon as nonideal mixing effects have to be considered.

In particular, mixtures of POPC and the detergents $C_{12}EO_3$ and $C_{12}EO_4$ forming lamellae in aqueous dispersion at room temperature exhibit a markedly asymmetric excess enthalpy function. This behavior can be modeled taking into account cooperative interactions between three, randomly neighbored molecules. Obviously, arrangements of one detergent molecule with two or more phospholipids are enthalpically favored. This effect gradually disappears with increasing headgroup size of the detergents. Accordingly, the cluster interactions become weaker and the minimum cluster sizes seem to grow.

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Appendix: Derivation of Equation 5

For the determination of the heat Q arising from the reequilibration after mixing, we have to concern a semiclosed system exchanging no material but only heat with outside. Such system must include the cell content and the syringe and the access tube of the cell, to which some cell content is displaced due to the injection. Then, Q is given by the change of the enthalpy content of this system:

$$Q = \Delta H(\text{cell}) + \Delta H(\text{replaced}) + \Delta H(\text{syringe}) \quad (22)$$

The variation of H inside the cell, i.e., upon a slight change N_D and/or N_L can be approximated by the differential:

$$\Delta H(\text{cell}) = \frac{\partial H}{\partial N_D}\cdot\Delta N_D + \frac{\partial H}{\partial N_L}\Delta N_L \quad (23)$$

The enthalpy content of the replaced material can be neglected as long as the injection volume is low and the injectant concentration is high, both compared to the cell content:

$$\Delta H(\text{replaced}) \approx 0 \quad (24)$$

Then, the mole number variations in the syringe upon an injection are just $-\Delta N_D$ and $-\Delta N_L$. Because the injectant leaves the syringe unchanged, we can apply eq 4 for its enthalpy content:

$$\Delta H(\text{syringe}) = -\Delta N_D[h_D(1) + h_E(X_{syr})] - \Delta N_L[h_L(1) + h_E(X_{syr})] \quad (25)$$

Now the absolute heat released (Q) can be written inserting eqs 23−25 into eq 22. A more convenient form to express the data is the observed heat per mole of injectant, q_{obs}, as defined by eq 5 (cf. Theory section). Note that the injected mole numbers ΔN_D and ΔN_L are related to each other in terms of the detergent mole fraction in the injection syringe, $X_{syr} = \Delta N_D/(\Delta N_D + \Delta N_L)$ (cf. eq 1). Hence, we find

$$q_{obs} = \left[\frac{\partial H}{\partial N_D} - h_D(1) - h_E(X_{syr})\right]X_{syr} + \left[\frac{\partial H}{\partial N_L} - h_L(1) - h_E(X_{syr})\right](1 - X_{syr}) \quad (26)$$

By differentiation of eq 4, we obtain

$$\frac{\partial H}{\partial N_D} = h_D(1) - h_E(X) + (N_D + N_L)\frac{\partial X}{\partial N_D}\frac{\partial h_E}{\partial X} \quad (27)$$

$$\frac{\partial H}{\partial N_L} = h_L(0) + h_E(X) + (N_D + N_L)\frac{\partial X}{\partial N_L}\frac{\partial h_E}{\partial X} \quad (28)$$

From eq 1 it follows that

$$(N_D + N_L)\frac{\partial X}{\partial N_D} = 1 - X \quad (29)$$

and

$$(N_D + N_L)\frac{\partial X}{\partial N_L} = -X \quad (30)$$

Considering eqs 27−30, eq 26 simplifies to eq 6.

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