Micelle-to-Vesicle Transition: A Time-Resolved Structural Study

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Amphiphilic molecules spontaneously self-assemble in solution into a variety of microstructures. While their equilibrium properties are well understood, little is known about the kinetics of structural transitions and the existence and properties of metastable states. We demonstrate for the micelle-to-vesicle transition that the evolution of aggregate structures can be followed using time-resolved light and neutron scattering. The polymerlike micelles are found to first transform into nonequilibrium disks, which finally evolve into vesicles following an exponential time dependence. [S0031-9007(99)08805-5]

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Surfactants in solution exhibit a complex aggregation behavior resulting from a delicate balance of opposing forces. The size and shape of the aggregates not only depend on the chemical composition, but also dramatically change in response to variations in solution parameters, such as temperature, concentration, pH, or ionic strength. While the knowledge on their behavior under equilibrium conditions has made significant progress [1], only limited information is available on structural transitions concerning kinetics as well as existence, properties, and evolution of nonequilibrium or metastable states.

Transformations between aggregates of different monolayer or bilayer membrane topologies represent a particularly important topic [2], as they provide the basis for processes ranging from technological to biological systems. Typical examples include vesicle formation, membrane reconstitution, digestion, or drug delivery. A classical example of such a structural transition is the micelle-to-vesicle transition. Although extensively studied [3–8], several aspects of its temporal and structural evolution remain highly controversial. The exact sequence of structures and the existence of intermediates, such as connected networks or perforated bilayers, is still not conclusively determined [4,5]. Moreover, not even the question whether vesicles represent true equilibrium structures is unambiguously solved.

Scattering methods have proven to be particularly powerful techniques in order to investigate surfactant systems providing detailed structural information on a broad range of length scales down to almost molecular resolution. Recent instrumental developments [9,10] now enable us to follow the temporal evolution of aggregate structures using time-resolved static (SLS) and dynamic (DLS) light and small-angle neutron scattering (SANS). These experiments thus not only advance our understanding of an important surfactant phase transition, but also highlight the new opportunities in time-resolved structural studies on self-assembling systems.

Aqueous mixtures of lecithin and bile salt are prime examples of mixed surfactant solutions that exhibit a spontaneous micelle-to-vesicle transition. They are not only important and frequently used model systems for the investigation of self-assembly in mixed surfactant solutions [6,7], but are also of considerable importance in biology, physiology, and pharmaceutical applications. Depending on the bile salt-to-lecithin-ratio and the total lipid concentration, a variety of different structures form, and a detailed small-angle neutron scattering study of the equilibrium structures resulted in the following picture (Fig. 1): Upon dilution of a micellar stock solution the shape of the relatively monodisperse micelles changes from small spherical to elongated, flexible and locally cylindrical, polymerlike structures. This is caused by the different monomer solubilities, i.e., critical micellization

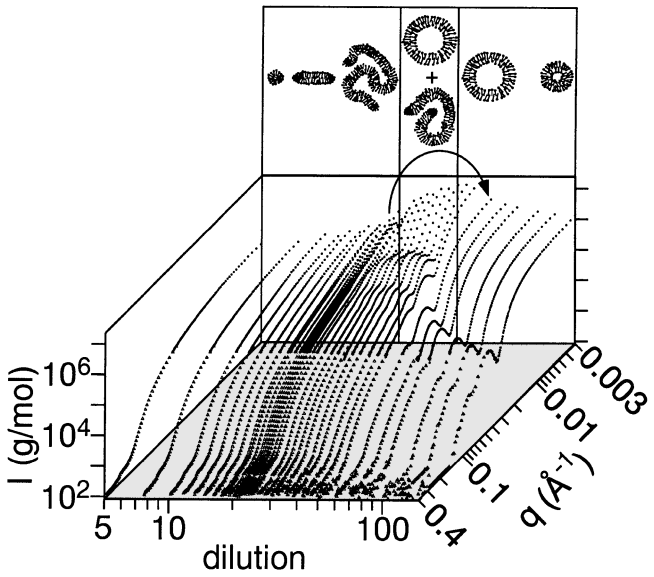

FIG. 1. Small-angle neutron scattering results for a dilution series of equilibrated aqueous mixtures of egg yolk lecithin and bile salt (taurochenodeoxycholic acid sodium salt). Normalized scattering intensity $I(q, t)$ as a function of scattering vector q. The observed aggregates and phase boundaries are represented schematically. The arrow indicates the dilution-induced micelle-to-vesicle transition shown in Figs. 2 and 3.

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concentrations (cmc), resulting in a dilution-induced decrease of the bile salt-to-lecithin ratio in the aggregates, which appears to lower the average spontaneous curvature of the mixed micelles. The highly curved end caps are therefore avoided, and the micelles are forced to grow. At even higher dilutions the formation of locally flat, lamellar structures is favored, and a spontaneous transition from micelles to vesicles is induced. After passing through a coexistence region of polymerlike micelles and vesicles, one enters a single phase of relatively monodisperse vesicles, whose size decreases with increasing dilution. This dependence of the vesicle size upon bilayer composition remains one of the most puzzling questions since most theoretical models predict an opposite behavior [11].

Based on the well established equilibrium phase behavior, we started an investigation of the kinetics and the possible existence and structure of intermediates during the micelle-to-vesicle transition using time-resolved SLS, DLS, and SANS. This attempt to resolve some of the open questions has become possible due to a new generation of instruments [9,10] ideally suited for the time scale of this transition [3,4].

The transition was induced by rapidly diluting an equilibrated micellar stock solution to a concentration where vesicles exist at equilibrium (Fig. 1, arrow). The light scattering (LS) experiments were performed on a dedicated fiber-optics-based multiangle instrument, which simultaneously collects the scattered light at nine angles and allows for parallel dynamic and static measurements [9]. SLS yields the time dependence of the normalized scattered intensity $I(q,t)$ as a function of the magnitude of the scattering vector q [Fig. 2(a)]. In the limit $q \to 0$, $I(q,t)$ corresponds to the apparent weight average molar mass of the aggregates. The time dependence of the apparent average hydrodynamic radius $R_h(q,t)$ obtained from DLS is shown in Fig. 2(b).

The data indicate that dilution induces a structural reorganization of the micelles initially present in the equilibrated stock solution (Fig. 2, open squares) which is very rapid on the time scale of the experiment. While the mass of the particles formed directly after the dilution step is almost the same, R_h decreased significantly from its initial value $R_h = 225$ Å (open squares) to $R_h \approx 150$ Å (first filled circles) obtained in the first kinetic measurement made 25 s after dilution. The combination of DLS and SLS thus indicates a rapid formation of nonequilibrium intermediary structures with a more compact shape than the originally present polymerlike micelles. These intermediates are then slowly transformed into the final vesicular structures with much larger size and mass, which is reflected by the increase of $I(q,t)$ and $R_h(q,t)$ with time. Equilibrium values are reached after approximately 4 h.

It is known [12] that the rate constant for surfactant exchange ("backward reaction" of a single surfactant molecule leaving the micelle, k_{out}) is of the order

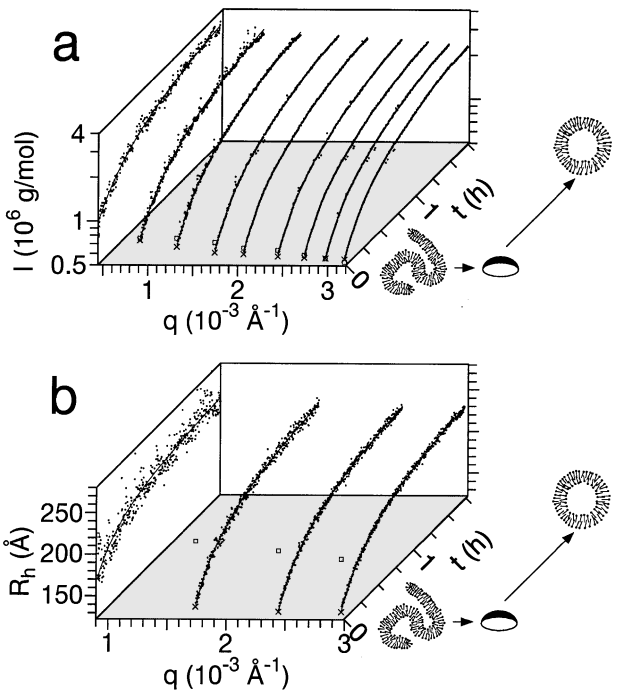

FIG. 2. Dilution-induced micelle-to-vesicle transition as observed by time-resolved static and dynamic light scattering. Temporal evolution of the normalized scattering intensity $I(q,t)$ (a) and hydrodynamic radius $R_h(q,t)$ (b) as a function of scattering vector q, respectively. The data obtained from the initial, equilibrated mixed micellar solution is represented by open squares. The fit to the kinetic model is shown as the solid lines and the values for the intermediate structures as obtained by this fit are represented by crosses. The observed aggregates are represented schematically.

of $k_{\text{out}} \sim 10^9\ (\text{s mol/L})^{-1} \times \text{cmc (mol/L)}$, which yields $k_{\text{out}} \sim 10^6\ \text{s}^{-1}$ for the bile salt and $k_{\text{out}} \leq 0.1\ \text{s}^{-1}$ for the lecithin. If the intermediate formation is due to a release of bile salt and a subsequent structural rearrangement without any modification of the total number of aggregates, k_{out} immediately sets the time scale for this process, which is much faster than the measurement time, in agreement with our findings. However, the formation of the vesicles requires substantial aggregate growth, i.e., a significant decrease in the total number of particles, which from the micellar kinetics is known to be much slower than the simple release of surfactant molecules and has often led to the observation of ultraslow relaxation processes.

These qualitative findings suggests that this transition could be described by the following scheme:

$$\text{micelles} \xrightarrow{\text{very fast}} \text{intermediates} \xrightarrow{\text{slow}} \text{vesicles}.$$

Based on this hypothesis we can develop a simple kinetic model to quantitatively describe the temporal evolution of $I(q,t)$ and $R_h(q,t)$. Since the transition from the micelles to the intermediate structures appears to be instantaneous on the time scale of the light scattering experiment (first measurement after 25 s, duration

of individual measurements 10 s), we assume that the contribution of the micelles to $I(q,t)$ and $R_h(q,t)$ can be neglected during the time course of the experiment. Furthermore, the above-mentioned increase in mass indicates that the transformation from the intermediates to the equilibrium vesicles might involve a further short-lived intermediate, which, however, cannot directly be observed by the present scattering experiments due to the weighting by the scattering intensity. The transition is thus described by a conversion from intermediates, characterized by $I_i(q)$ and $R_{h,i}(q)$, at time $t = 0$ s to vesicles, characterized by $I_v(q)$ and $R_{h,v}(q)$, at time $t \to \infty$. This implies that intermediates of all shapes and sizes, if present, transform according to the same time dependence. As the simplest time dependence we assume an exponential decay of the concentration of intermediates with time constant τ. The measured intensity $I(q,t)$ then consists of the time-dependent contributions of the intermediates $[I_i(q)e^{-t/\tau}]$ and vesicles $[I_v(q)\,(1 - e^{-t/\tau})]$, and the DLS experiment, which measures the collective diffusion constant $D(q,t) \sim R_h(q,t)^{-1}$, yields the intensity weighted average of the inverse hydrodynamic radius $R_h(q,t)^{-1}$,

$$I(q,t) = I_i(q)e^{-t/\tau} + I_v(q)\,(1 - e^{-t/\tau}),$$

$$R_h(q,t)^{-1} = [I_i(q)e^{-t/\tau}R_{h,i}(q)^{-1} + I_v(q)\,(1 - e^{-t/\tau})R_{h,v}(q)^{-1}]/I(q,t).$$

The experimental data are thus at all times characterized by a single, q-independent time constant τ and two sets of time-independent scattering functions and hydrodynamic radii for the intermediates $[I_i(q), R_{h,i}(q)]$ and vesicles $[I_v(q), R_{h,v}(q)]$ only. While the latter can be obtained from the equilibrated vesicular sample, $I_i(q)$ and $R_{h,i}(q)$ have to be determined by a fit to the measured $I(q,t)$ and $R_h(q,t)$ using the above equations. A fit according to this model (Fig. 2, solid lines) shows very good agreement with the data. It yields $\tau = 73$ min, and the resulting values for $I_i(q)$ and $R_{h,i}(q)$ (Fig. 2, crosses) indicate the existence of polydisperse aggregates with an average overall size of about 300 Å.

These experiments provide valuable information on the time constant τ and the overall sizes of the aggregates at all times. From a combination of $I_i(q)$ and $R_{h,i}(q)$ we can deduce that the intermediate structure has to be quite compact and possesses a certain degree of polydispersity. However, the indirect nature and the limited resolution of the SLS experiment, which is approximately bracketed by $300 \leq 1/q \leq 3000$ Å, is not sufficient to obtain detailed insight into the structure of these intermediates, and various structural models would be in agreement with the data.

The spatial resolution can dramatically be improved by SANS, which covers a q range that is ideal for such studies [7]. SANS experiments were performed on the instrument D22 (ILL, Grenoble), which offers unique possibilities for time-resolved studies due to its very high neutron flux and the large q range covered in a single instrumental setting [10]. Figure 3 shows the temporal evolution of $I(q,t)$ during the dilution-induced micelle-to-vesicle transition, which only approximately corresponds to the transition shown in Fig. 2 (for the SANS experiment D_2O was used instead of H_2O [7]). A measurement time of 60 s already results in astonishingly good statistics, although the total surfactant concentration is less than 1 mg/ml to avoid interparticle interaction effects. SANS reveals the same qualitative behavior as LS. At low values of q, which corresponds to similar length scales as SLS, $I(q)$ increases reflecting the formation of vesicles from smaller intermediate structures. The intermediate and high q part of the data exhibit much more structural details than accessible by SLS. In particular, we observe the minima and maxima of the vesicle form factor, which provide information on the size and polydispersity of the vesicles, and from the high q part of $I(q)$ we can determine details of the bilayer structure such as its thickness or scattering length density distribution across the bilayer. The positions of the minima and maxima of $I(q)$ remain constant throughout the experiment but become more pronounced with time. This indicates that the vesicles already form with a narrow size distribution around their final equilibrium size, while their number fraction increases during the course of the micelle-to-vesicle transition.

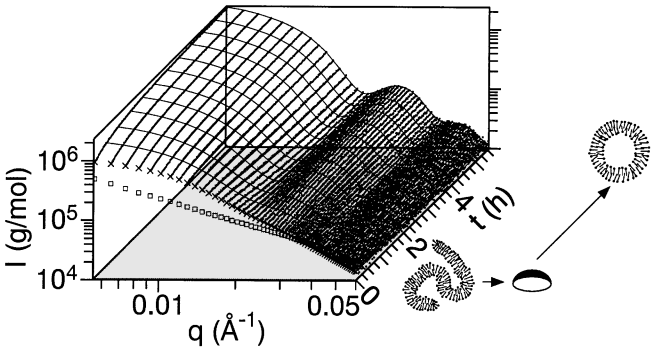

FIG. 3. Dilution-induced micelle-to-vesicle transition as observed by time-resolved small-angle neutron scattering. Temporal evolution of the normalized scattering intensity $I(q,t)$ as a function of scattering vector q. In addition, every 30 min $I(q,t)$ is highlighted as solid lines. Note that every second measurement is shown only, and the data plotted only represent the initial time dependence as the transition was followed for a much more extended period of time in order to ensure that the equilibrium $I_v(q)$ required for the fits was reached. The data obtained from the initial, equilibrated mixed micellar solution are represented by open squares. The fit to the kinetic model is shown as the solid lines, and the resulting intensities $I_i(q)$ for the intermediate structures using a model of polydisperse disks are represented as crosses. The observed aggregates are represented schematically.

A fit to $I(q,t)$ with the same model used previously to analyze the light scattering experiments results in good agreement with the data and therefore confirms the conclusions drawn from LS (data not shown). An

inverse Fourier transformation [13] of the resulting fitted scattering function $I_i(q)$ of the intermediates indicates a sheetlike (bilayer) structure with a thickness of about 50 Å and a maximum size of approximately 350 Å and is, in particular, incompatible with small vesicles or a coexistence of vesicles and locally cylindrical micelles. Based on this additional information the scattering function of a polydisperse solution of bilayer disks [14] with thickness 50 Å is used instead of fitting $I_i(q)$ for all values of q. This significantly reduces the number of free fitting parameters to the mean radius $\langle R \rangle$ and polydispersity σ of the disks only. We obtain excellent agreement for $\tau = 143$ min and polydisperse disks with $\langle R \rangle = 170$ Å and $\sigma = 0.2$ as the intermediate structures (Fig. 3, solid lines), which is also consistent with the molar mass calculated from $I(q \rightarrow 0, t)$ [15].

As demonstrated above, the combination of light and neutron scattering experiments together with recent instrumental developments have provided us for the first time with a set of time-resolved structural data on the temporal evolution of self-assembling aggregates during a micelle-to-vesicle transition. Based on these data we were able to detect the existence of nonequilibrium, intermediary aggregates and to derive a quantitative model for their structure down to almost molecular resolution, as well as to characterize the time dependence of their transformation to vesicles. It seems conceivable that the locally cylindrical micelles very quickly change their morphology to disks as a response to the modified spontaneous curvature. The disks then transform into closed unilamellar vesicles in a much slower process. We believe that this represents a significant step towards a full understanding of this important structural transition that occurs in a variety of different surfactant systems. It may solve the puzzling question on how the initially present wormlike micelles transform into closed bilayer structures. There are several theoretical models that describe the energetics of a disk-to-vesicle transition [16], which were originally developed in order to explain the micelle-to-vesicle transition based on the previously postulated and recently rejected model of a disk-like structure for the mixed micelles. Our new findings may now provide the missing link between the theoretical models and the aggregate structures existing in these systems.

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[15] The difference in the time constants measured with LS and SANS is related to the fact that the starting and end points of the transitions studied with light and neutron scattering, respectively, are not identical due to the different isotopic compositions of the solvent [7]. However, while τ depends on the exact solution composition, the overall pattern of a transition from micelles to vesicles via the formation of transient disks is common for all experiments performed so far. Although light scattering does not allow one to directly deduce a detailed structural model for the intermediates, the light scattering data are in quantitative agreement with intermediate disks.
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