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Counterion-Controlled Transition of a Cationic Gemini from Submicroscopic to Giant Vesicles

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Abstract: While much is known about the self-assembly of lipids on nanoscale, our understanding of their biologically relevant mesoscale organization remains incomplete. Here, we show for a cationic gemini lipid a sharp and reversible transition from small vesicles with an average diameter of approximately 40 nm to giant vesicles (GVs) with an average diameter of approximately 11 μm. This transition is dependent on proper [NaCl] and specific temperature. Below this transition and in the vicinity of the air/water interface, a series of mesoscale morphological transitions was observed, revealing complex structures resembling biological membranes. On the basis of microscopy experiments, a tentative [NaCl] versus temperature shape/size phase diagram was constructed. To explain this unprecedented transition, we propose a novel mechanism whereby a specific interaction of Cl^- counterion with the cationic gemini surfactant initiates the formation of a commensurate solute counterion lattice with low spontaneous curvature. In keeping with the high bending rigidity of NaCl crystal, this tightly associated ionic lattice enslaves membrane curvature and the mesoscale 3-D organization of this lipid.

Introduction

Complex organization of nature's amphiphilic molecules, lipids, into structures such as the plasma membrane and various internal organelle membranes is a ubiquitous feature of living cells. Living systems consist of complex polymers (proteins, nucleic acids, carbohydrates) and liquid crystalline amphiphiles, lipids. The various types of biological structures form, to a large extent, because of driving forces such as hydrophobicity and electrostatics acting together with specific, spatially matching intermolecular interactions, causing the spontaneous assembly of the constituents. A paradigm of this type of process is the formation of biological membranes, composed of a chemically diverse collection of lipids together with the embedded integral and surface-associated peripheral proteins. Characteristically to many-body systems, biomembranes organize into complex dynamic patterns on a wide range of time and length scales.[1] Because of their fundamental roles in the maintenance and control of the organization and function of biomembranes, the properties of lipids have been extensively investigated.[2] Key principles governing the formation and transition of the various types of nanoscale 3-D phases have been established[3] and relate to the effective shapes of the constituent molecules, involving the extent of hydration, charges, intermolecular hydrogen bonding, as well as the degree of thermally driven trans→gauche isomerization of the lipid acyl chains.[4] However, the determinants for the self-assembly and morphological transitions of biologically relevant mesoscale lipid structures such as nucleus, endoplasmic reticulum, mitochondria, and the eukaryotic cells themselves are poorly understood. Along these lines, we have recently shown how specific enzymatic reactions can cause dramatic changes on these length scales.[5] Likewise, certain anionic lipids can complex with a diverse range of cationic proteins to form fibrous structures on the 100−180 μm scale,[6] and bile salts mixed with sterols and phospholipids assemble into helical mesoscale structures.[7]

Gemini surfactants represent an interesting class of amphiphiles that has received vast academic and industrial interest during the past decade.[8] Their pleomorphic phase behavior

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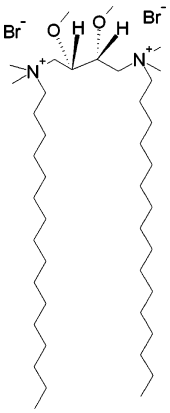

Figure 1. Chemical structure of gemini surfactant M-1.

resembles closely that of natural phospholipids, and their chemical structure allows for optimization of molecular shape to meet requirements of different technical applications such as nonviral gene transfer.[9] In the course of our studies on aqueous dispersions of the dicationic gemini surfactant (2*S*,3*R*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)-butane dibromide (M-1, for structure see Figure 1), we observed by visual inspection that millimolar M-1 solution turned instantaneously from optically clear to opalescent and bluish at 2 M NaCl and at specific temperature. Optical microscopy revealed remarkable mesoscale phase transition of this amphiphile from submicroscopic vesicles to giant liposomes, explaining the above observation. This interesting phenomenon was further characterized via dynamic light scattering, Langmuir monolayers, and differential scanning calorimetry.

Experimental Section

Experimental details are included in the Supporting Information.

Results and Discussion

In water as well as in [NaCl] < 1.5 M, dispersions of 1 mM M-1 were optically clear, suggesting the formation of micelles or small unilamellar vesicles. However, at [NaCl] = 1.5 and 2 M and at approximately 60 °C, dispersions of M-1 became opalescent and slightly bluish and at 3 M [NaCl] became cloudy even at ambient temperature (approximately 21 °C). Cooling M-1 in [NaCl] = 1.5 and 2 M back to ambient temperature resulted in optically clear solutions, which upon further incubation for approximately 12 h revealed floating macroscopic aggregates that were easily disrupted by either vigorous mixing or heating.

The above prompted us to investigate the phase behavior of M-1 dispersions by microscopy. At [NaCl] > 1 M, a sequence of transitions became evident, exemplified by micrographs taken at [NaCl] = 2 M upon heating (Figure 2). At 24 °C, no visible structures were observed, in keeping with the optical clarity of the samples, suggesting surfactant to be organized into micelles or small vesicles. However, at approximately 29 °C, very large tubular structures appeared near the air/water interface of the solution (Figure 2A). With further increase in temperature to ~33 °C, these structures rapidly transformed (within seconds) into GVs that were concentrated at the air/water interface (Figure 2B). The average diameter of GVs varied between approximately 30 and 50 μm, while also larger vesicles were occasionally seen. These large GVs exhibited varying morphologies with some resembling living cells with internal organelles (Figure 2B, insets). At T > 36 °C, the size of the GVs started to decline and upon exceeding $T \approx 40$ °C morphologically homogeneous smaller GVs (diameters of ~10−15 μm) were observed throughout the sample cell (Figure 2C). No further changes were evident upon heating to 50 °C, thus suggesting the bluish appearance of M-1 solution at the hydration temperature (60 °C) to be caused by light scattering due to large population of smaller GVs. The above sequence of shape/size transitions was reversed upon cooling back to 24 °C. On the basis of microscopy data, a tentative [NaCl] versus temperature shape/size phase diagram for the structures formed by M-1 was constructed (Figure 3A). More specifically, we determined the dependency on [NaCl] of the transition temperatures T_g and T_s, defined as the temperatures at which large GVs in the vicinity of the air/water interface (T_g) and smaller GVs throughout the water phase (T_s) emerge. In brief, at [NaCl] = 0.5 M, no visible structures were observed within the temperature range from 24 to 50 °C, while at one molar salt occasional GVs were seen at T > 43 °C. However, at [NaCl] > 1 M, both T_g and T_s could be unambiguously defined and decreased linearly with increasing [NaCl] (Figure 3A).

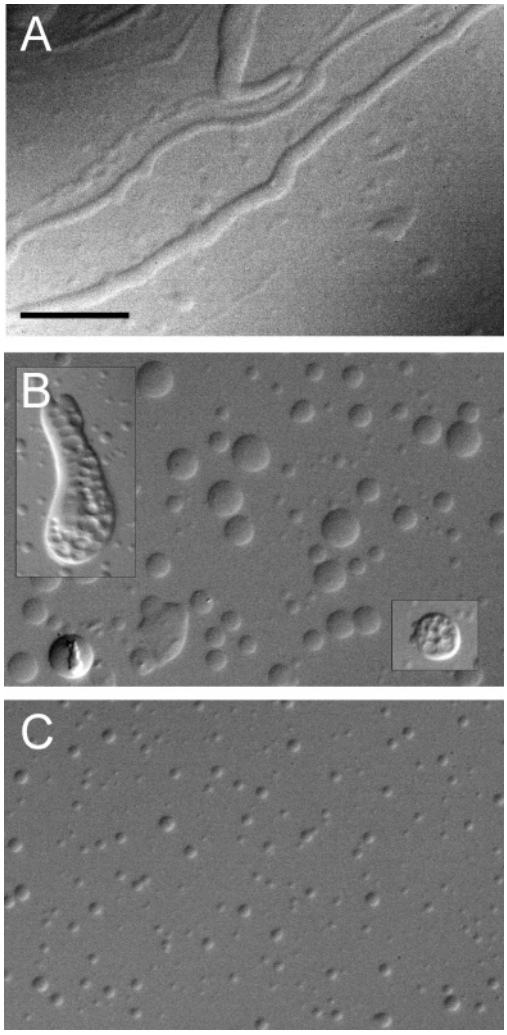

Figure 2. Microscopy images of 1 mM M-1 solution with 2 M NaCl. Temperatures were approximately 30 (A), 33 (B), and 43 °C (C). The scale bar represents 100 μm.

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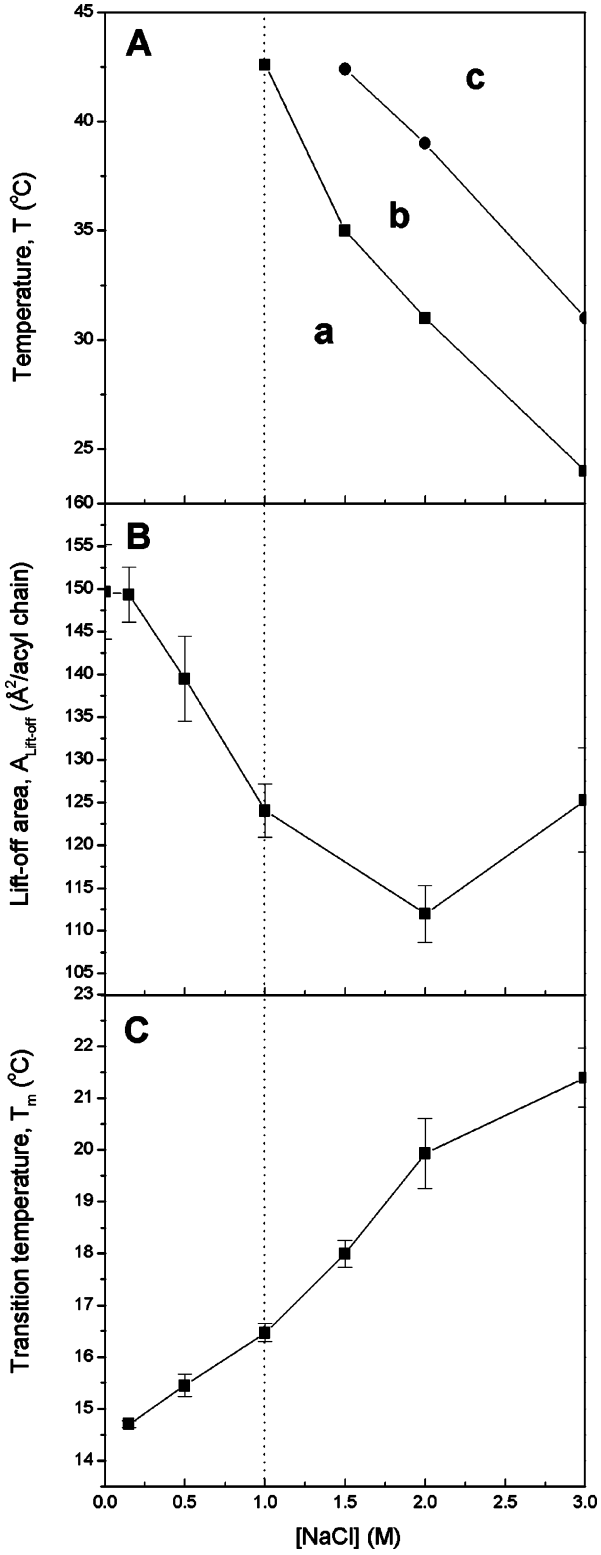

Figure 3. Tentative [NaCl] versus temperature vesicle shape/size phase diagram derived from microscopy experiments of 1 mM M-1 dispersion. Dashed vertical line represents [NaCl] after which GVs emerged (A). Submicroscopic structures (a), large giant vesicles (GVs), tubular, and cytomimetic structures in the vicinity of the air/water interface (b), and small GVs throughout the water phase (c). Lift-off areas ($A_{lift-off}$) determined from monolayer compression isotherms (see Supporting Information for details) recorded as a function of [NaCl] in the subphase (B). Main transition temperatures (T_m) obtained for 1 mM M-1 dispersions from DSC measurements as a function of [NaCl] (C). Error bars shown in panels B and C represent standard deviation calculated from at least three measurements.

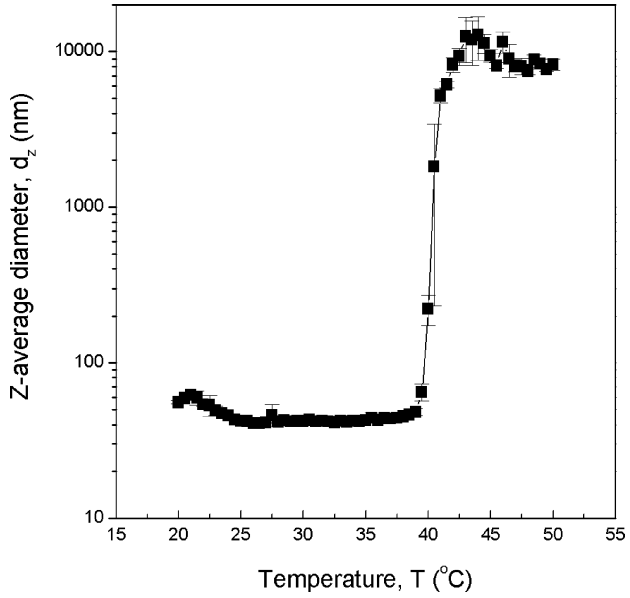

Figure 4. *Z*-average diameters (d_z) of aggregates formed in 1 mM M-1 in 2 M NaCl determined by dynamic light scattering as a function of temperature. Error bars shown represent standard deviation calculated from three measurements.

To evaluate the importance of the air/water interface for these transitions, we conducted microscopy experiments also in a sealed glass compartment instead of an open chamber otherwise used. The transition to large GVs and more complex aggregates, that is, the transition between regions a and b (Figure 3A), was absent in the sealed compartment, revealing this transition required an air/water interface with the surfactant monolayer. Importantly, the transition to numerous GVs was observed at $T_s \approx 39$ °C also in a sealed sample compartment.

These findings were substantiated by measuring *Z*-average diameters (d_z) of the aggregates formed by dynamic light scattering (DLS). Accordingly, a temperature scan for 1 mM M-1 in 2 M NaCl demonstrates a sharp transition at $T_s \approx 40$ °C, whereby small submicroscopic vesicles with $d_z \approx 40$ nm rapidly transformed into giant vesicles with $d_z \approx 11$ μm (Figure 4). Because the formation of complex membrane structures in the transition between regions a and b (Figure 3A) takes place immediately below the air/water interface, it was not evident in the DLS data showing scattering from the bulk of the dispersion.

The small-scale 3-D phase behavior of lipid assemblies is generally described by the molecular geometry of the effective shapes of lipids.[4] This relationship is rationalized in terms of a packing parameter p[3]:

$$p = \frac{v}{al}$$

where v is the volume of hydrocarbon chain(s), a is the area occupied by the headgroup, and l is the maximum length of hydrocarbon chains. In brief, conventional spherical micelles are formed when $p < {}^1/_3$, nonspherical (e.g., rodlike or discoidal) micelles form when ${}^1/_3 < p < {}^1/_2$, and bilayers form when ${}^1/_2 < p < 1$. If $p > 1$, inverted structures with negative spontaneous curvature, such as inverted hexagonal phase, form. The transition described here involves a change from submicroscopic vesicles with a high positive curvature to nearly planar bilayers of GVs,

requiring a significant increment in p. This is likely to be due to efficient screening of the dicationic headgroup of M-1 by Cl^- ions reducing the Coulombic repulsion between the amphiphiles and dehydrating headgroup, thus causing condensation and reduced a. Evidence for reduced a and, accordingly, also increased p were obtained from both monolayer and DSC experiments. The lift-off areas ($A_{lift-off}$) determined from compression isotherms of M-1 monolayers as a function of [NaCl] (Figure 3B) decreased progressively from approximately 150 $Å^2$/acyl chain upon exceeding 150 mM NaCl, with a minimum at [NaCl] = 2 M and without further condensation at higher [NaCl]. In keeping with the above, at [NaCl] = 150 mM, dispersion of M-1 exhibited a single endotherm peaking at T_m = 14.7 °C, whereas increasing [NaCl] elevated T_m, reaching 21.4 °C at 3 M salt (Figure 3C) and suggesting condensation of the membrane. The impact of electrostatics and counterions on phase behaviors of charged lipids is well documented. For instance, the specific nature and concentration of counterions affect the sphere→rod transition of anionic micelles[10] as well as cationic gemini surfactants,[11] increased protonation of the headgroup at low pH causes vesicle→micelle transition,[12] and increasing surface charge density due to a cationic amphiphile in a mixture with zwitterionic phosphatidylcholine induces lamellar bilayer→interdigitated bilayer transition.[13] Mesoscale morphological transitions have been previously reported for charged amphiphiles such as anionic phospholipids[14,15] and gemini surfactants.[16,17] However, the transition described here is unique as it involves a spontaneous and rapid coalescence of submicroscopic lipid aggregates into mesoscale structures similar in size and morphology to biological lipid assemblies.

While high ionic strength is a prerequisite for the formation of GVs and causes augmented lipid packing (and thus also increases p), the change in packing parameter is highly unlikely to provide an adequate explanation for the observed transitions at T_s. More specifically, taking into account the relatively small change in curvature between structures in regions b and c (Figure 3A), the corresponding change in p would be minute on the length scales involved. In addition, the critical importance of temperature for the transitions at T_g and T_s is difficult to reconcile in terms of effective molecular shapes. Interestingly, gemini isomers differing from the mesoform M-1 only in stereochemical conformation of the spacer and thus having slightly different spacing between cationic charges of the headgroup interact vigorously with NaCl (but not with NaBr, for instance), forming crystalline aggregates (Säily et al., to be published), suggesting specific interaction between these surfactants and Cl^-. Accordingly, it is tempting to propose that the dicationic headgroup of M-1 with a fixed distance between its two positive charges (Figure 1) acts as nucleating surface for Cl^- counterions to form a planar, commensurate pseudocrystalline lattice corresponding to the 111-facet for Cl^- in a NaCl crystal and associated with the vesicle surface. Adjacent to this layer of Cl^- is a lattice of Na^+, followed by another Cl^- layer, and so on. In accordance with the crystal structure of NaCl, the arrangement of the above ion-counterion lattices is planar and should therefore promote the formation of giant vesicles instead of smaller ones with high positive or negative membrane curvature. In other words, the rigid pseudocrystalline counterion lattice, because of favoring planarity, slaves the curvature of the associated cationic lipid surface. To this end, the effective concentration of the ions in the vicinity of the charged amphiphile surface is likely to be very high, as recently pointed out by Romsted and co-workers.[11] The ion-counterion lattice would form only after a threshold [NaCl] reached at lower temperatures (Figure 3A) and is likely to be sensitive to the phase state of the nucleating membrane. More specifically, to efficiently initiate the formation of NaCl crystal lattice, the amphiphiles in the bilayer would have to adapt their mean molecular area to the lattice spacing; that is, alkyl chains should be in the fluid state. However, if the membrane is very elastic, as it is near T_m in the gel−liquid coexistence regime,[18] it may not be sufficiently stable so as to constitute a template for the growth of the pseudocrystalline NaCl lattice adjacent to the lipid surface. Consistent with the above, T_s is much higher than T_m. Yet, despite complying with our observations, the above mechanism remains speculative at this stage, and several factors, such as the role of the orientation of the hydroxyl moieties in the spacer and changes in the hydration of the headgroup[19] of M-1, remain to be studied.

To our knowledge, this is the first study reporting spontaneous temperature and [NaCl]-dependent formation of GVs in an amphiphile suspension. A surfactant capable of spontaneous vesiculation that is sensitive to both temperature and ionic strength opens the road for practical applications requiring precisely determined self-assembly, for example, enhanced liposomal drug encapsulation and controlled release systems, including gene delivery by liposome−DNA complexes.[20] Yet, the above mechanism for the control of membrane curvature by pseudocrystalline counterion lattices could have biological relevance. A dramatic example for a mesoscale transition is provided by vesiculation of the nuclear membrane preceding cell division and its subsequent reappearance after the formation of two daughter cells.[21] The underlying mechanisms of the above remain elusive. While biomembranes certainly are much more complex than the amphiphile dispersion used in this study, mechanisms similar to the above may well be involved. To this end, mitochondrial membranes exhibit a rich polymorphism[22] and have a high content of cardiolipin, a natural dianioinic gemini phospholipid known to form complex mesoscale assemblies in the presence of $CaCl_2$.[23] Spontaneous formation of cytomimetic vesicles supports these types of structures being

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an important intermediate in the origin of life[24] and demonstrates that morphological transitions similar to those observed in biological systems could take place in the absence of any protein machinery, such as in a protocell consisting only of self-replicating RNA and a surrounding lipid membrane.[25]

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Supporting Information Available: Experimental section with NMR spectra of M-1 and complete ref 9. This material is available free of charge via the Internet at http://pubs.acs.org.

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