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Membranes for Artificial Cells

Vesicle Formation from Reactive Surfactants**

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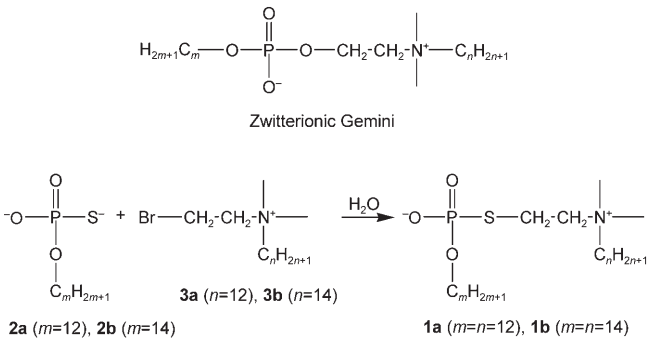
In recent years the synthesis of artificial cells in the laboratory has emerged as a realistic research goal.[1] Two complementary approaches in this endeavor can be distinguished. The bottom-up approach aims to construct a synthetic cell from the molecular level. Inspired by the RNA world scenario for the origin of life, many scientists envisage an RNA replicase that replicates inside a replicating lipid vesicle.[2] In the top-down approach, researchers try to reconstitute a minimal cell by reducing the complexity of biological organisms to a minimal set of DNA, RNA, and proteins that is, however, sufficient for replication and evolution.[3] In these synthetic-life studies the lipid components are either naturally occurring phospholipids[4] or fatty acid based systems.[5] It is a special property of fatty acid–soap systems to aggregate into micelles in a high-pH buffer while a simple drop in pH leads to a transformation into bilayer vesicles.[6] By utilizing this property, the formation of lipid vesicles from micelles and their controlled continuous growth have recently been achieved with fatty acids.[7] However, fatty acid vesicles are only stable within a narrow pH range and are stable only at low salt concentration.[8] While phospholipid membranes are stable under a variety of conditions, their formation and growth require a demanding biosynthetic apparatus involving several enzymes.[9] Phospholipids cannot be added as monomers or micelles to pre-existing vesicles to induce growth owing to their very low critical vesicle concentration (cvc) in the submicromolar range, and growth by vesicle–vesicle fusion is in many cases accompanied by extensive leakage.[10] It would therefore be very useful to develop a lipid system that retains the advantageous properties of phospholipids but can be built up from simple surfactants without using enzymes.

Johnsson et al. have recently introduced sugar-based gemini surfactants that show a pH-dependent aggregation behavior.[11] Sugawara and co-workers have presented a novel chemical system of self-reproducing giant vesicles. The precursors are an amphiphilic benzaldehyde and a lipophilic aniline derivative that react by imine formation to a long-chain vesicle-forming amphiphile bearing a quaternary ammonium head group.[12] We were, however, interested in biomimetic lipids that resemble phospholipids, which carry a zwitterionic head group and have two aliphatic chains. A new surfactant structure with these features has recently been reported by Menger and Peresypkin.[13] They have shown that so-called zwitterionic gemini surfactants (Scheme 1) aggregate into micelles, coacervates, vesicles, or gels depending on the chain lengths m and n. Zwitterionic geminis with $m,n > 10$ form predominantly vesicles. We reasoned that when the bridging oxygen atom is replaced by a sulfur atom (**1**), the aggregation properties should be retained. Furthermore, the sulfur bridge should allow the convergent synthesis of zwitterionic geminis in aqueous solution from suitably functionalized single-chain surfactants.



Scheme 1. Top: general structure of zwitterionic gemini surfactants. Bottom: formation of zwitterionic gemini **1** from functionalized precursors.

When the sulfur analogue, zwitterionic gemini **1a** ($m = n = 12$), was dispersed in water, it formed vesicles as expected (see the Supporting Information). We then mixed the single-chain surfactants **2a** ($m = 12$) and **3a** ($n = 12$) to prepare **1a** in situ. Phosphorothioate **2a** and quaternary ammonium salt **3a** were dissolved separately in aqueous buffer and then slowly mixed (2–4 h). After a few minutes the solution became turbid, indicating the formation of large aggregates. After complete mixing the suspension was further stirred for 1 h and examined by cryo-TEM. A heterogeneous mixture of large multilamellar vesicles and small unilamellar vesicles could be observed (Figure 1, left). Surfactants **2** and **3** were mixed at equal concentrations of 2–8 mM each, which is below the reported critical micellar concentration (cmc) for similar compounds. The cmc of the parent dodecyltrimethylammonium bromide (DTAB) was reported to be 15 mM, [14] and for the related *N*-(2-chloroethyl)-*N*,*N*-dimethyldodecylammonium chloride a cmc of 13.9 mM was given.[15] For *n*-dodecyl phosphate the cmc was determined to be between 10 and

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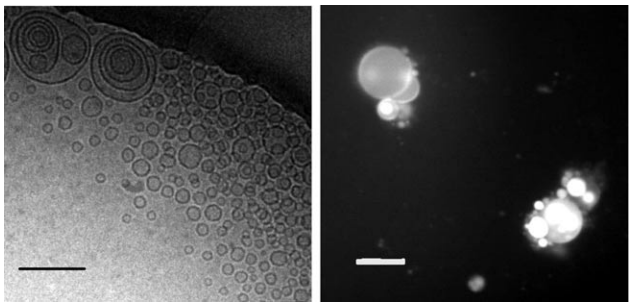

Figure 1. Left: cryo-TEM image of **1a** vesicles, formed in situ by the reaction of an equimolar mixture of **2a** and **3a**; scale bar: 200 nm. Right: fluorescent micrograph of FITC–dextran containing **1b** vesicles, formed by mixing **2b** and **3b** in the presence of FITC–dextran; scale bar: 5 μm.

42 mM depending on the pH value and counterions.[16] A likely pathway starts therefore from monomers, which, upon mixing, first form mixed micelles and then transform into vesicles with progressing reaction.

Vesicles can also be obtained just by mixing cationic and anionic surfactants without any chemical reaction occurring between the two amphiphiles. This type of vesicle is known as a catanionic vesicle.[17] They can be prepared from a variety of surfactants but require an excess of one surfactant since equimolar mixtures tend to precipitate.[18] To confirm that the vesicles in Figure 1 were indeed composed mainly of the zwitterionic gemini **1a**, first a negative control with the corresponding nonfunctionalized surfactants was carried out. When the nonfunctionalized surfactant DTAB was mixed with **2a**, no visible turbidity and no particles larger than 10 nm could be observed. In the related catanionic system DTAB–disodium dodecanephosphonate only mixed micelles were observed but no vesicles.[19] When the mixtures of **2** and **3** were worked up by extraction and recrystallization, the zwitterionic geminis **1** could be repeatedly isolated in quantitative yields (>90%). This high yield is most likely due to the alignment of the reactive groups at the interface between the aggregate and the bulk aqueous phase.

To check for functional stability of the vesicles, fluorescent dye molecules were encapsulated in the interior volume. Fluorescently labelled dextran (FITC–dextran, $M_w = 20000$) was dissolved in a small volume of buffer, and equimolar amounts of **2b** ($m = 14$) and **3b** ($n = 14$) were continuously added over 2 h. Non-encapsulated dextran was then removed by gel filtration. When the lipid-containing fractions were examined by fluorescence microscopy, stable vesicles containing FITC–dextran could be observed (Figure 1, right). Similar experiments with the dodecyl compounds were unsuccessful owing to strong leakage. This observation is in accordance with earlier reports in which it was found that 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) vesicles cannot encapsulate water-soluble molecules, whereas vesicles made from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) can do so.[20,21] Also in terms of physical characteristics the zwitterionic geminis behave remarkably similarly to the corresponding phosphatidylcholines. For hydrated bilayers of **1a** the main transition melting temperature (T_m) is below 0 °C compared to −3 °C for DLPC; for **1b** the T_m value is 28 °C compared to 24 °C for DMPC. The cvc for **1a** is (180 ± 30) nM, compared to 25 nM for DLPC[22] and (65 ± 20) nM for **1b**. The lower value for the corresponding phospholipid may be attributed to the fact that the zwitterionic head group is separated from the chain assembly by the glycerol backbone. Also in catanionic systems the critical aggregation concentration (cac) is below the cac of the individual components by 2–3 orders of magnitude,[23] whereas in the zwitterionic gemini system reported here the covalent bond generates a 10^5-fold reduction in the cac.

Finally we wanted to evaluate whether the reaction between **2** and **3** can also sustain the growth of vesicles that are already present in the medium. For that we prepared a vesicle suspension of **1a**, extruded through 100-nm pores to a size of (77 ± 3) nm. To that we added one equivalent of each **2a** ($m = 12$) and **3a** ($n = 12$) over a two-hour period. The size of the vesicles was periodically monitored by using dynamic light scattering. We found that the size of the particles increased steadily in diameter, up to a final value of (111 ± 2) nm (Figure 2). This result corresponds to a doubling of the

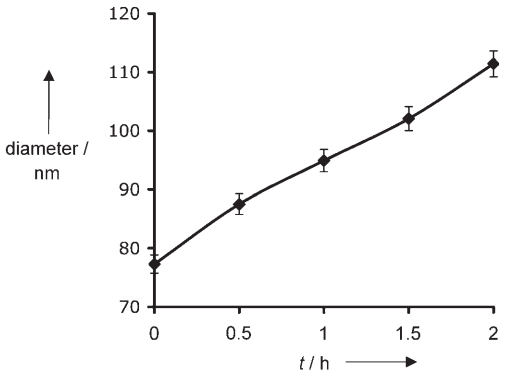

Figure 2. Growth of **1a** vesicles in response to feeding with one equivalent of both **2a** and **3a** over a two-hour period, monitored by dynamic light scattering.

surface area ($111^2/77^2 \approx 2$) and is therefore consistent with the quantitative incorporation of the added surfactants **2** and **3** into the preformed vesicles and subsequent reaction to give **1** within the bilayer.

Vesicles from zwitterionic gemini **1** can be prepared in media from pH 5 to 10 and up to 1M NaCl. These favorable stability and growth characteristics make us optimistic that the development of artificial cells in the laboratory will greatly benefit from this new approach to vesicle formation from reactive surfactants. Another interesting aspect is their similarity to natural phospholipids, except that they lack, however, the latter's flexibility for the attachment and exchange of fatty acids and head groups. One may therefore speculate that this flexibility constitutes one of the main driving forces behind the natural selection of phospholipids over alternative structures such as the zwitterionic geminis.

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