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Formation of a Hydrogen-Bonded Receptor Assembly in Niosomal Membranes

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One of the main goals of supramolecular chemistry is the development of dynamic systems that are stable in aqueous solutions. The directionality and specificity provided by hydrogen bonds make this noncovalent interaction an excellent tool for the formation of many self-assembled systems in solution.[1] However, most of the well-defined assemblies based exclusively on hydrogen bonding are not stable in water. Thus, when mimicking biological processes, these assemblies have a limited scope since water is omnipresent in biological systems. However, a closer look to the molecular recognition processes in biological systems reveals that many biorecognition processes occur at interfaces or at membrane surfaces between two aqueous compartments.

Thus, a more logical approach to mimicking biorecognition processes would be embedding of the synthetic self-assembled receptors in membranes. Until now, only synthetic ion channels[2] and membrane-anchored receptors[3] have been reported. To introduce more structural diversity and to allow selective binding of guest molecules, self-assembled receptors within bilayer membranes are especially attractive. To incorporate these self-assembled synthetic receptors into artificial membranes is challenging because this requires well-defined nanoscale self-assembled structures that can position the different functionalities in the correct orientation for guest complexation.

Here, we describe the inclusion of calix[4]arene dimelamine **1** and perylene-functionalized barbiturate (PerBAR) into nonionic surfactant-based vesicles (niosomes) and their self-assembly into hydrogen-bonded double rosettes $\mathbf{1}_3 \cdot (\text{PerBAR})_6$ (Figure 1).[4] This type of assembly functions as a receptor in organic solvents.[5]

The double-rosette assembly $\mathbf{1}_3 \cdot (\text{PerBAR})_6$ is spontaneously formed when 1 equiv of calix[4]arene dimelamine **1** is mixed with 2 equiv of PerBAR in chloroform or toluene via the formation of 36 hydrogen bonds.[4] ^1H NMR spectroscopy shows that assembly $\mathbf{1}_3 \cdot (\text{PerBAR})_6$ has a D_3-symmetry where the two melamine rings of each calix[4]arene **1** are in antiparallel orientation with respect to each other.

Due to the presence of chiral centers in the dimelamine **1** (*L*-Lys), only one diastereomer $((P)\text{-}\mathbf{1}_3 \cdot (\text{PerBAR})_6)$ is formed,[6] thus presenting a total induction of the supramolecular chirality.[7] This makes it possible to study double-rosette formation by circular dichroism (CD) spectroscopy.[8] Double-rosette assemblies exhibit a very strong induced CD signal ($|\Delta\epsilon_{\max}| \sim 100$ L mol^{-1} cm^{-1}), while the individual components are hardly CD active ($|\Delta\epsilon_{\max}| < 8$ L mol^{-1} cm^{-1}).

The double-rosette $\mathbf{1}_3 \cdot (\text{PerBAR})_6$ was incorporated into surfactant-based vesicles because they have a superior stability[9] and toughness compared to phospholipids. A mixture of polyoxyethylene alkyl ether surfactants $C_{12}EO_3$ and $C_{14}EO_3$ (Serdox NES3) was used to prepare the niosomes.[10] Niosomal solutions containing 2.2 μg/mL Serdox NES3 and 0.06 mM **1** and/or 0.12 mM PerBAR were prepared by sonication. Formation of niosomes in water
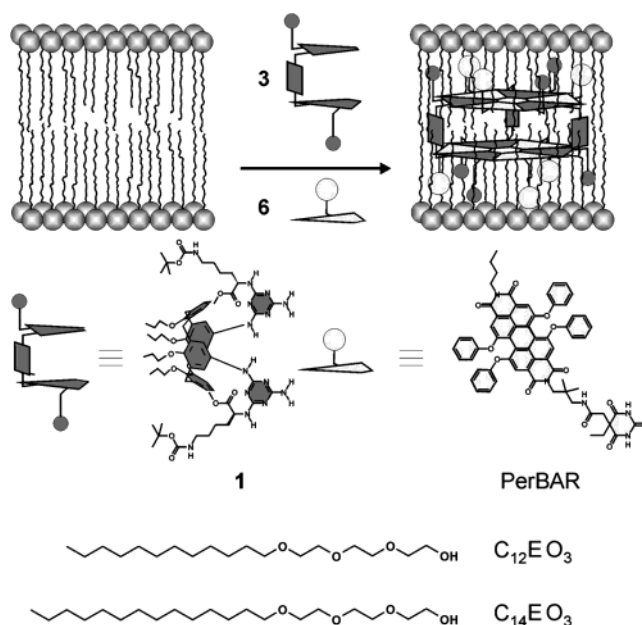

Figure 1. Schematic representation of the incorporation of **1** and PerBAR in a bilayer membrane and their self-assembly into double-rosette $\mathbf{1}_3 \cdot (\text{PerBAR})_6$. Molecular structures of the polyoxyethylene alkyl ether surfactants.
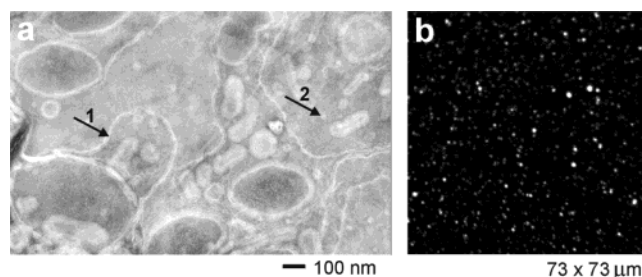

Figure 2. (a) Transmission electron micrograph (TEM) of niosomes containing $\mathbf{1}_3 \cdot (\text{PerBAR})_6$. Niosomes are in a dominant coexistence with rodlike (1) and spherical (2) micelles. (b) Confocal micrograph of niosomes with $\mathbf{1}_3 \cdot (\text{PerBAR})_6$ (white dots). Niosome solutions are prepared in water containing 1% (v/v) glycerol. [Surfactant] = 2.2 μg/mL, [PerBAR] = 0.12 mM, [**1**] = 0.06 mM.

containing 1% (v/v) glycerol was confirmed by transmission electron microscopy (TEM).[11] The TEM graph (Figure 2a) indicates niosomes in dominant coexistence with rodlike and spherical micelles. The observed niosome sizes range from 100 to 600 nm. For all niosome solutions, dynamic light scattering gave z-average diameters between 430 and 480 nm. The incorporation of PerBAR into the membrane was confirmed by confocal microscopy. The observed fluorescent dots (Figure 2b), in strong contrast with the background in confocal micrographs, show that PerBAR is not
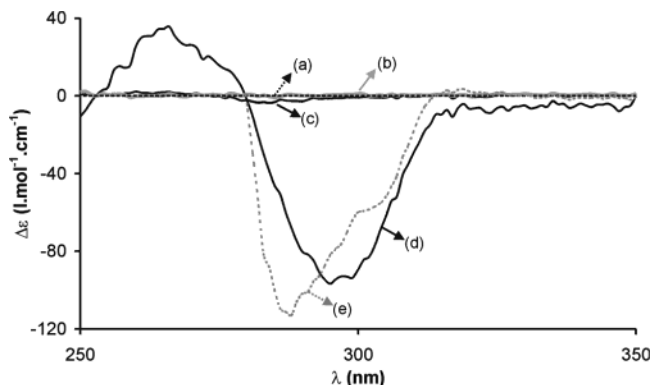
Figure 3. CD spectra of niosomes containing (a) no assembly components, (b) 0.12 mM PerBAR, (c) 0.06 mM **1**, and (d) 0.02 mM **1**$_3$·(PerBAR)$_6$ and (e) CD spectrum of 0.5 mM **1**$_3$·(PerBAR)$_6$ in toluene. Because the wavelength limit for toluene is 280 nm, the CD spectrum in toluene (e) was measured from 280 to 350 nm. Niosome solutions were prepared with 2.2 μg/mL surfactant in water containing 1% (v/v) glycerol.

present in the external aqueous media. The aqueous interior of the niosomes is identical to the external aqueous phase, excluding, therefore, the presence of PerBAR inside the vesicles.

Due to the strong CD intensity at $|\Delta\epsilon_{max}|$ (96 L mol^{-1} cm^{-1}) of the observed CD signal for niosomes containing both dimelamine **1** and PerBAR, we have very strong evidence that hydrogen-bonded double-rosettes **1**$_3$·(PerBAR)$_6$ are formed within the vesicular membranes (Figure 3). Differences observed between the induced supramolecular chirality of **1**$_3$·(PerBAR)$_6$ in niosomal membrane and toluene are probably due to differences in solvent–solute interactions.[12]

Solubilization of dimelamine **1** alone into vesicular membranes gave only a weak CD signal ($|\Delta\epsilon_{max}| \sim 3$ L mol^{-1} cm^{-1}), while niosomes with PerBAR or without assembly components are CD silent. The weak CD intensity observed for niosomes with dimelamine **1** indicates that it is incorporated in the bilayers. After filtering of samples where water (containing 1% (v/v) glycerol) was added to **1**, no CD activity was observed due to the insolubility of **1** in aqueous solution.

Solubilization of the assembly components into the niosomal membranes gives a high effective concentration of **1** and PerBAR that allows the formation of hydrogen-bonded assembly **1**$_3$·(PerBAR)$_6$.[13] Also the decrease of the local polarity within the membranes compared to water increases the stability of **1**$_3$·(PerBAR)$_6$. Furthermore, Serdox NES3 niosomes with a calculated membrane thickness of about 3 nm, allow the double-rosette assemblies with dimensions of 3 nm × 1.2 nm[4a] to fit very well in the bilayer membranes, either horizontally or vertically.[14]

To determine the stability of the assemblies **1**$_3$·(PerBAR)$_6$ in niosomes, CD spectra were recorded after storage for 1 week and 3 days at room temperature. Small decreases in the CD intensity (10 and 17% after 3 and 7 days, respectively) were observed at $|\Delta\epsilon_{max}|$,[15] indicating that assemblies **1**$_3$·(PerBAR)$_6$ in niosomes are quite stable under these conditions.

The results presented here show that niosomes prepared from nonionic surfactants C$_{12}$EO$_3$ and C$_{14}$EO$_3$ provide a stable system that allows self-assembly processes to occur in contact with aqueous environments. Incorporation of dimelamine **1** and PerBAR within niosomal membranes led to the formation of well-defined hydrogen-bonded assemblies **1**$_3$·(PerBAR)$_6$ within these membranes.

This system provides a useful scaffold to introduce synthetic receptors into artificial cell systems. Recently, dimelamine rings have been functionalized with peptides and carbohydrates, which are able to complex biomolecules.[5a,16] These self-assembled receptors could possibly, after their incorporation into niosomes, respond to different external triggering processes.[17] Efforts in this direction are going on in our laboratory as a first step toward artificial cell mimics.

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(14) When the same experiments where performed with the chiral *L*-(α)-phosphatidylcholine lipids instead of Serdox NES3 surfactants, incorporation of **1** and PerBAR was observed. However, assembly formation could not be detected. The presence of charges in *L*-(α)-phosphatidylcholine vesicles may prevent the formation of the hydrogen-bonded double rosettes.
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