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Glycopolymer vesicles with an asymmetric membrane

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Direct dissolution of glycosylated polybutadiene–poly(ethylene oxide) block copolymers can lead to the spontaneous formation of vesicles or membranes, which on the outside are coated with glucose and on the inside with poly(ethylene oxide).

Polymer vesicles, also referred to as "polymersomes,"[1] are considered as model biomembranes for applications in life science and biomedicine.[2] Block copolymer vesicles with broadly adjustable properties including stability, fluidity, and dynamics can have a better performance than the phospholipid membranes of biological cells.[1–5] Especially interesting are peptide- and sugar-based biohybrid polymers and vesicles, which may inherit all the advantageous features of synthetic polymers (elasticity, solubility, *etc.*) and biological polymers (functionality, biocompatibility, *etc.*).[6,7] Glycopolymers are currently "booming" because of their easy availability[8] and potential use in cell sensing, therapeutics, and synthetic biology.[9]

Herein we describe the spontaneous formation of glycopolymer vesicles with an asymmetric membrane,[10–12] the outside of which is covered by glucose (Glc) and the inside by poly(ethylene oxide) (PEO) (see the illustrations in Fig. 1), in dilute aqueous solution. The vesicular structure of the aggregates was identified by light and X-ray scattering and electron microscopy. 2D-NOESY-NMR and surface-enhanced Raman spectroscopy (SERS) were applied to demonstrate the asymmetric structure of the membrane.

The glycopolymers **1** and **2** (see structure in Fig. 1A) used for this study were synthesized by photoaddition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose onto 1,2-polybutadiene-*block*-poly(ethylene oxide) (PB_x–PEO_y; x = 65, y = 212 → **1** and x = 68, y = 34 → **2**), as described earlier.[13,14] The protected glycopolymers contained on average 33 (**1**) or 30 (**2**) glucose units per chain, and 6–9% of the double bonds remained unreacted (elemental analysis and ^{1}H NMR). The apparent polydispersity indexes of the samples were 1.1 and 1.2, respectively (size-exclusion chromatography, SEC). The deacetylation of the glucose units was achieved in a quantitative yield (^{1}H NMR and FT-IR).†

Owing to the large weight fraction of hydrophilic units (Glc + PEO), $w_{hydrophilic}$ = 0.76 (**1**) and 0.58 (**2**), both glycopolymers could be directly dispersed in pure water. According to static and dynamic light scattering (SLS/DLS) (each series of measurements were done with four samples containing 0.025–0.1 wt% polymer at scattering angles between 12° and 150°), the dispersions contained very large aggregates with $R_{g,0}$ = (550 ± 20) nm, $R_{h,0}$ = (520 ± 20) nm (**1**) and $R_{g,0}$ = (270 ± 40) nm, $R_{h,0}$ = (280 ± 30) nm (**2**) (R_g: radius of gyration, R_h: hydrodynamic radius). The dimensions of the aggregates and the values of the characteristic ratio $R_{g,0}/R_{h,0} \sim 1$ suggest that the aggregates of **1** and **2** were vesicles.[15] Combined SLS and DLS analysis[16] reveals a high softness of the particles, indicative for vesicles with a quite thin shell.† The existence of unilamellar vesicles could be confirmed by transmission electron microcopy (TEM, Fig. 1B) and by small-angle X-ray scattering (SAXS).†

The spontaneous aggregation of **1** and **2** is seemingly driven by the hydrophobic effect. The hydrophobic core consists of hydrocarbon chains (formerly PB) and is shielded from water by glucose and PEO segments. The hydrophobic membrane measures about 5 nm across, as evidenced by TEM analysis of the vesicles stained with OsO_4 (selective for residual double

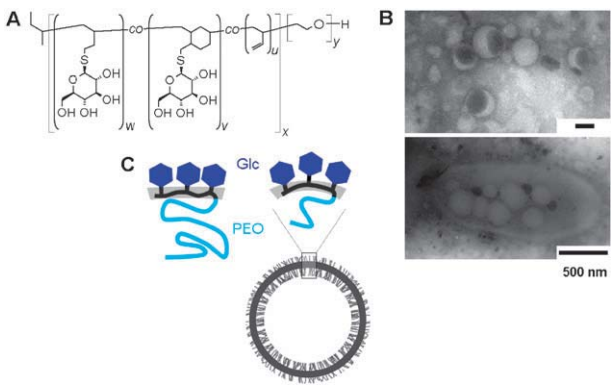

Fig. 1 (A) Chemical structure of the glycopolymers **1** (w + v = 0.51, u = 0.06, x = 65, y = 212) and **2** (w + v = 0.44, u = 0.09, x = 68, y = 34). (B) Transmission electron micrographs of collapsed vesicles of **1**. Samples (0.2 wt% **1** in water) were prepared as monolayers on Pioloform coated copper grids and stained for 60 minutes in OsO_4 vapour; images were taken at room temperature. (C) Illustration of the spontaneous curvature of **1** (left) and **2** (right) and the possible assembly of chains into an asymmetric monolayered membrane.

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bonds and sulfur atoms) (Fig. 1B) and SAXS.† Whether the membrane has a monolayered or a bilayered structure[1] cannot be judged on the basis of experimental data. However, a packing of chains consisting of one hydrophobic and two hydrophilic chains (Glc and PEO) seems only possible in a monolayer, as illustrated in Fig. 1C, like for ABC triblock copolymers.[12]

The aggregation itself may not be surprising, but it could not be expected that **1** and **2** would form vesicles. Block copolymers like PB–PEO with $w_{\text{hydrophilic}} > 0.5$ are known to form (compact) micelles in dilute aqueous solution and not (hollow) vesicles.[17] Whereas linear chains are oriented perpendicular to the interface,[1] the "comb-shaped" glucose-grafted PB segments would be oriented parallel to the interface (Fig. 1C),[13,14] thus promoting the formation of layers.

Another peculiarity is that the vesicles formed by the more hydrophilic **1** were about two times larger than those of **2** (Glc: $(w + v)\ x \sim$ const., PEO: $y = 212 \rightarrow 34$). Conventionally, the relation between the packing parameter of the amphiphile and curvature ($\propto 1/R$) of the aggregate would have suggested the opposite trend.[4] The scaling behavior observed for **1** and **2** can only be rationalized assuming that the vesicle membrane exhibits an asymmetric structure, as shown in Fig. 1C. The glucose units should be located on the outside of the membrane and PEO on the inside, based on geometric and solubility arguments. The surface area per chain is essentially determined by the spatial requirements of the glucose-grafted segment and should be the same for both glycopolymers. The necessity of stabilizing the same inner surface area with shorter PEO chains (**2**) as with longer PEO chains (**1**) leads to a stronger bending of the membrane and formation of smaller vesicles. Curvature should have decreased if PEO was on the outside or PEO–glucose chains were distributed on either side of the membrane.

So far it is just a hypothesis that the glycosomes **1** and **2** have an asymmetric membrane with a Glc outside and a PEO inside. For confirmation, a ~1 wt% solution of **1** in D_2O was exemplarily studied by 2D-NOESY-NMR[18] and SERS.[19]‡

The 2D-^{1}H, ^{1}H-NOESY-NMR spectrum of **1** (Fig. 2A) shows cross peaks between the signals of methine protons of the glucose units ($\delta \sim 4.45$, 3.85–3.65, 3.55–3.25 ppm),[20] but none between Glc and PEO ($\delta = 3.64$ ppm). This absence of a nuclear Overhauser effect (NOE) seems to indicate that there are no 3D spatial correlations[21] and no attractive hydrogen bonding interactions between the two hydrophilic segments.[22]§

Raman spectroscopy and SERS were applied to decide which of the phase-separated hydrophilic segments is located on the outside/inside of the membrane. In a simple case, the SERS spectrum is like a conventional Raman spectrum but with enhanced signal intensity. However, the selection rules are dominated by the metal surface–molecule interactions and in particular by the orientation of the molecule on the surface. SERS may eventually show additional modes but modes may also disappear.

The Raman spectrum of the vesicles of **1** in D_2O (Fig. 2B, top) showed all the expected bands for Glc and PEO (see ESI†).[23] In SERS, the addition of gold nanoparticles (average diameter: 20 nm) to the vesicle solution of **1** caused a suppression of the signals of glucose whereas the signals of PEO remained essentially unchanged (Fig. 2B, bottom). Evidently,

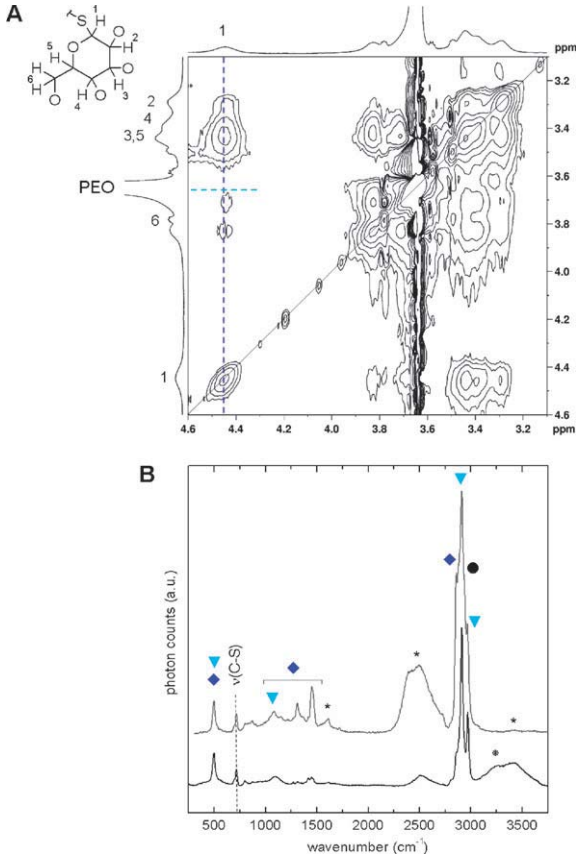

Fig. 2 (A) 2D-^{1}H, ^{1}H-NOESY-NMR spectrum (500.17 MHz, mixing time: 150 ms, negative levels)[18] and (B) Raman (top) and surface-enhanced Raman (bottom) spectra of glycopolymer vesicles **1** in D_2O at room temperature. Spectra in (B) were normalized to the intensity of the thioether vibration at 717 cm^{-1}. Symbols indicate the signals of Glc (◆), PEO (▼), and the hydrocarbon chain (●). Signals marked with stars are due to the solvent (★) and gold nanoparticles (*).

the gold nanoparticles only come into contact with the glucose, which therefore must be located on the outside of the membrane, but not with PEO. Due to the favorable Au–S interaction, the gold nanoparticles are trapped at the outer periphery of the vesicles and prevented from passing the membrane and entering the inner compartment.¶

In summary, glycosylated PB–PEO block copolymers can spontaneously assemble into vesicles in dilute aqueous solution (SLS/DLS, SAXS, and TEM), the membranes of which exhibit an asymmetric structure with a coating of sugar on the outside and PEO on the inside (2D-NOESY-NMR and SERS). Such vesicles could potentially be used for biomedical applications for which the cellular interactions for instance with bacteria are the key to success. Future work is devoted to the development of "smart" glycopolymer vesicles or bioreactors with pH- and/or thermo-responsive inner compartments.

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Notes and references

‡ Visualization of the asymmetric structure of a membrane would require application of cryogenic 3D electron microscopy or tomography. However, cryo-fixation of the samples failed—this might be attributed to the ability of glycopolymers (**1**) to change the structure of ice (data not shown).[24]

§ Due to the problem of NOE zero-crossing, there is a possiblity that no NOE signal is detected although PEO and glucose units are forming a mixed shell. In this case, multiple hydrogen bonding interactions between PEO and Glc should have noticeably affected the dynamics of the chains. However, the PEO resonances are sharp (see NMR spectra in ESI) reflecting high segmental motion.

¶ Unfortunately, the location of the gold nanoparticles in 3D space cannot be resolved by conventional TEM because the image would show just the 2D projection of a collapsed vesicle.

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