Biomimetic Structures

DOI: 10.1002/anie.200805895

Polysaccharide-*block*-polypeptide Copolymer Vesicles: Towards Synthetic Viral Capsids**

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The directed self-assembly of amino acids, carbohydrates, and nucleic acids by the fine-tuning of intra- and intermolecular interactions produces the major components of living organisms, namely, bacteria, viruses, and cells. Various strategies based on supramolecular chemistry have been developed for the self-assembly of natural or synthetic molecules into soft and nanostructured materials with some essential features of natural materials.[1] One of the main structures in living organisms is the vesicle, which results from the self-assembly of amphiphilic biomolecules based on lipids, proteins, and carbohydrates. Natural vesicles vary in size: Large vesicles act as membranes to protect the intracellular components from the extracellular environment, whereas the role of small vesicles is the intracellular transport of biomolecules. Stable vesicular structures have been reproduced in the laboratory, as early as the 1960s[2] with liposomes assembled from naturally occurring phospholipids, and more recently with so-called polymersomes, which result from the self-assembly of block copolymers into vesicles.[3] Polymersomes have a short history but are expected to play a tremendous role in the development of biomedical applications, such as the delivery of therapeutics, and as microreactors that mimic the behavior of living cells. Polymersomes hold some advantages over liposomes, such as a lower critical aggregation concentration, a higher membrane viscosity, and elasticity. These characteristics enhance membrane stability and decrease the passive diffusion of encapsulated molecules; a greater chemical diversity for surface functionalization is also possible.[4] Most of the polymersomes described in the literature were assembled from synthetic amphiphiles with biocompatible or bioresorbable blocks, such as polylactide-*block*-poly(ethylene oxide),[5] polycaprolactone-*block*-poly(ethylene oxide),[6] and poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline).[7]

A further step towards the mimicking of natural vesicles would be the self-assembly of these systems from copolymers containing natural blocks, such as polysaccharides or polypeptides. The controlled ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCAs) has been thoroughly exploited to synthesize polypeptide-based block copolymers able to self-assemble into vesicles in an aqueous medium.[8] Interest in polysaccharide-based block copolymers arose in the late 1980s following the synthesis of poly(ethylene oxide)-*block*-oligosaccharide structures by end-to-end coupling techniques.[9] Since then, some examples of block copolymers in which a synthetic block is associated with a polysaccharide, such as dextran,[10] hyaluronan,[11] amylase,[12] and β-cyclodextrin,[13] have been synthesized by enzymatic polymerization, coupling techniques, or radical polymerization. Only a few of these copolymers underwent self-assembly in aqueous solutions to give micellar[10a–c,14] or vesicular[10f] aggregates. Finally, it was also reported recently that synthetic polymers with pendent monosaccharide moieties, such as glucose, may form submicronic vesicles in an aqueous environment.[15]

Herein, we present the synthesis of a very simple glycoprotein analogue, in which a polysaccharide block is linked linearly to a polypeptide block, and demonstrate that macromolecular structures of this type are able to self-assemble spontaneously into vesicles in water. Dextran, a water-soluble microbial polysaccharide composed of D-glucopyranose units linked by α-(1→6) glycosidic bonds with a low percentage of (1→3)-linked side chains, and poly(γ-benzyl L-glutamate) (PBLG) were used as model blocks. Both dextran and PBLG are recognized for their biocompatibility, which makes them suitable candidates for drug- or gene-delivery applications.[16] Previously, we described[17] a straightforward and versatile synthesis of polypeptide-based block copolymers by an approach based on a Huisgen 1,3-dipolar cycloaddition, or "click" chemistry, which combines mild experimental conditions, functional-group tolerance, and nearly quantitative yields.[18] We report herein a simple and versatile route to polysaccharide-*block*-polypeptide copolymers, as illustrated by the synthesis of dextran-*block*-PBLG (Scheme 1).

First, an alkyne group was introduced at the reducing end of dextran ($M_n = 6600\ g\,mol^{-1}$, polydispersity index: 1.35) by reductive amination with propargylamine in acetate buffer (pH 5.0) in the presence of sodium cyanoborohydride, which reduces double bonds in Schiff bases selectively.[19] Even though the terminal alkyne functionality was not detected by ^1H NMR spectroscopy of the purified end-functionalized dextran in [D$_6$]DMSO, the full disappearance of peaks for reducing-end-group anomeric hydrogen atoms (peak for the α anomeric H atom centered at $\delta = 6.7$ ppm, and peak for the β anomeric H atom centered at $\delta = 6.3$ ppm) was a strong

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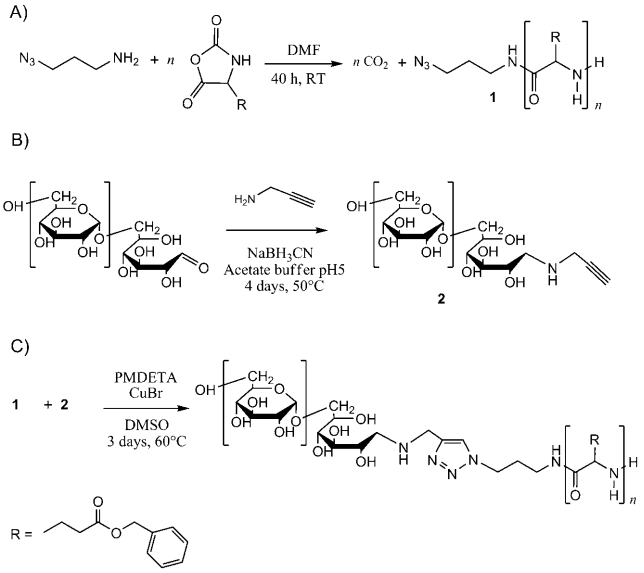
[**] We thank D. Taton and W. Agut for helpful discussions and technical assistance.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200805895.



Scheme 1. Synthesis of the dextran-*block*-poly(γ-benzyl L-glutamate) copolymer. A) Synthesis of PBLG end-functionalized with an azido group through the polymerization of BLG NCA initiated by 1-azido-3-aminopropane. B) Synthesis of dextran end-functionalized with an alkyne group by the reductive amination of dextran with propargylamine. C) Block coupling by click chemistry. DMF = *N*,*N*-dimethylformamide, DMSO = dimethyl sulfoxide.

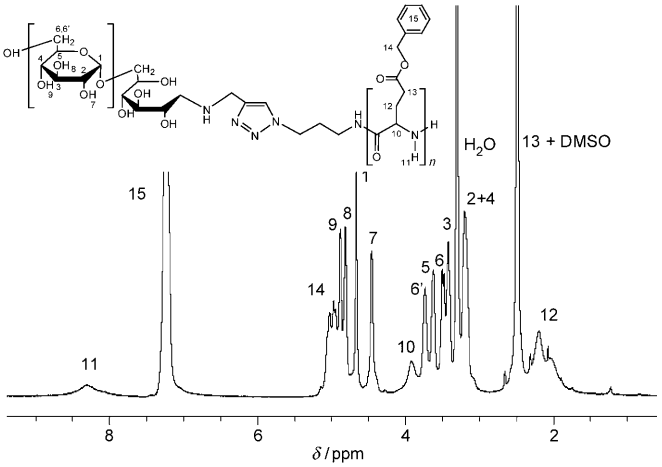


Figure 1. ^{1}H NMR spectrum of dextran-*block*-poly(γ-benzyl L-glutamate) in [D$_6$]DMSO.

indication of a quantitative reaction[20] (see the Supporting Information). In a second step, PBLG that was end-functionalized with an azide group and had a degree of polymerization DP = 59 was obtained through the ring-opening polymerization of γ-benzyl L-glutamate *N*-carboxylic anhydride (BLG NCA) initiated by 1-azido-3-aminopropane, according to a previously described procedure.[17] The polysaccharide and polypeptide blocks were coupled in DMSO, a good solvent for both blocks, at room temperature in the presence of a copper(I) catalyst (CuBr) and the ligand pentamethyldiethylenetriamine (PMDETA). Two equivalents of end-functionalized dextran were used to favor full conversion of the azido-functionalized PBLG. The reaction medium was dialyzed against water, a poor solvent for PBLG, by using a membrane with a molecular-weight cutoff of 50 kDa to ensure the full removal of excess dextran. ^{1}H NMR spectroscopy confirmed the synthesis of the polysaccharide-*block*-polypeptide copolymer structure (Figure 1). The disappearance of the azide peak at 2100 cm^{-1} in the IR spectrum also indicated that all azido-functionalized PBLG had reacted with alkyne-functionalized dextran blocks (see the Supporting Information).

The solution behavior of the amphiphilic dextran-*block*-PBLG copolymer was investigated in water. PBLG is known to adopt a rigid α-helical conformation in the bulk state by means of intramolecular hydrogen bonding. This conformation favors strong side-by-side interactions between peptide-bond dipoles, which lie nearly parallel to the helix axis.[21] To favor the disaggregation of the block copolymer and its self-assembly in water, we used the so-called nanoprecipitation method,[22] a process similar to the well-known ethanol-injection method used to form small unilamellar liposomes.[23] In this case, an excess volume of milliQ water was added slowly to a solution of the copolymer in DMSO to induce a stepwise displacement of water-miscible DMSO. After the removal of DMSO by dialysis against water, the dispersion of the polysaccharide-*block*-polypeptide was characterized by scattering and microscopy techniques. Dynamic light scattering (DLS) measurements of the aqueous copolymer dispersion showed a single relaxation mode, which is typical of a monomodal size distribution (Figure 2). A linear variation of Γ with q^2, whereby the line passes through the origin, is the hallmark of a translational diffusive process typical of spherical objects (see the Supporting Information).

DLS analysis provided a hydrodynamic radius (R_H) of 45 nm with a low polydispersity ($\sigma = 0.20$). To further characterize the micellar morphology, we carried out small-angle neutron scattering (SANS) experiments. A plot of the scattering intensity $I(q)$ versus the scattering vector q displayed a q^{-2} to q^{-4} dependency, in agreement with a

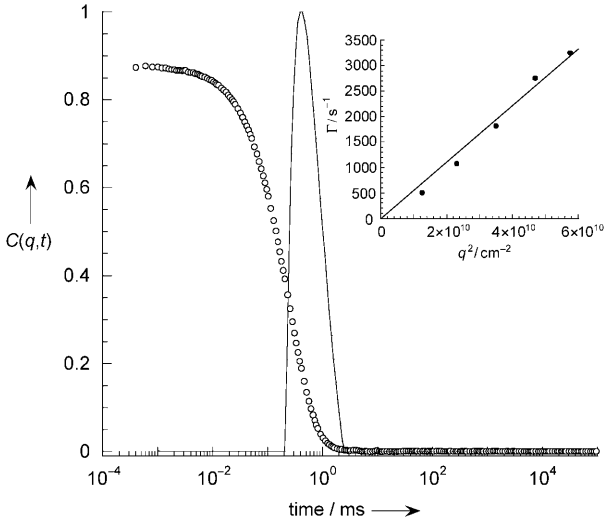


Figure 2. Dynamic light scattering autocorrelation function of the dispersion of the dextran-*block*-PBLG copolymer in water and its relaxation-time distribution at a scattering angle of 90°. Inset: Dependence of the decay rate (Γ) on q^2, the square of the scattering vector.

hollow structure (Figure 3). The scattering curve was indeed well-fitted, with a vesicle form factor[24] from which the vesicle radius ($R = 45$ nm) and membrane thickness ($\delta = 21$ nm) could be derived. The Kratky–Porod representation

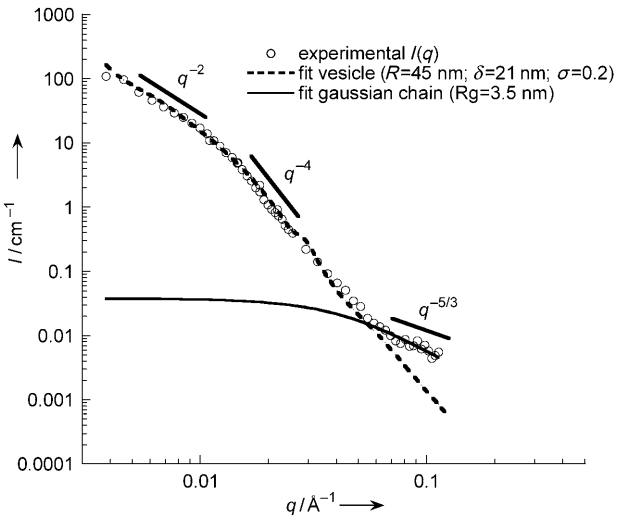


Figure 3. Small-angle neutron scattering of the dextran-*block*-PBLG copolymer in water. Experimental data were fitted with a vesicle form factor with a radius of 45 nm, a membrane thickness of 21 nm, and a polydispersity of 0.2. The Gaussian chain form factor used to fit the data in ranges of high q values showed that dextran chains adopt a random-coil conformation on either side of the membrane vesicles. Rg = radius of gyration.

$\ln(q^2 I(q))$ versus q confirmed the δ value (see the Supporting Information). The $q^{-5/3}$ dependency of $I(q)$ in the high scattering range demonstrates that dextran chains on both sides of the membrane adopt a random-coil conformation under good solvent conditions. We can therefore conclude that vesicles are stabilized sterically by the hydrophilic dextran corona. Analysis of the sprayed sample by transmission electron microscopy (TEM) clearly confirmed the vesicular morphology (Figure 4). The membrane thickness estimated from the TEM images ($\delta = 20$ nm) corroborates the value determined previously by SANS.

In light of these results, one can conclude that the dextran-*block*-PBLG copolymer self-assembles in water into small polymersomes with a low polydispersity. The origin of this vesicular structure must be related to both the strong interactions between the rigid and hydrophobic α-helical PBLG blocks and the hydrophilicity of the dextran blocks: The former interactions favor the formation of a flat membrane, and the hydrophilicity of dextran imparts solubility and fluidity to the membrane.[8a] Interestingly, the hydrophilic mass ratio of the copolymer ($f = 34\,\%$) is close to the expected value for polymersomes on the basis of the empirical prediction of Discher and co-workers ($f = 35 \pm 10\,\%$).[25] Finally, a basic model of the membrane vesicles was derived (Figure 4). If one assumes that the PBLG blocks adopt an α-helical conformation and are stacked in a strictly antiparallel orientation, as in the bulk state, one can estimate the PBLG thickness in the membrane to be 9 nm.[26] From

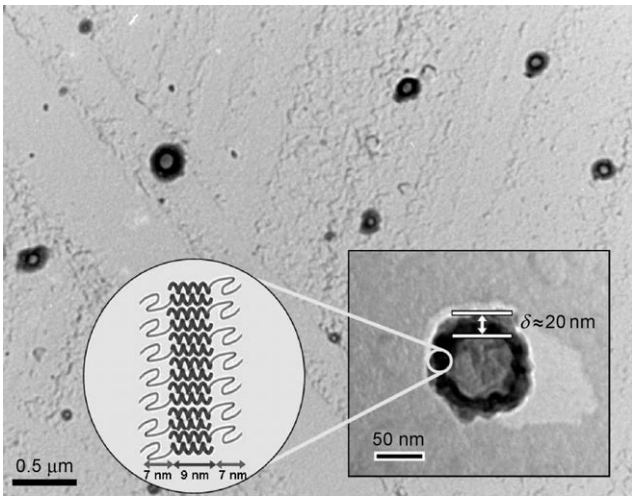


Figure 4. TEM image of a dried dispersion of the dextran-*block*-PBLG copolymer. The inset shows evidence of a vesicle-like structure with a membrane thickness of approximately 20 nm, which is close to the value found for the proposed model.

light-scattering measurements, the size of the dextran blocks was estimated to be 7 nm on either side of the PBLG layer. Thus, the overall membrane thickness would be 23 nm, in close agreement with the experimentally determined value ($\delta \approx 21$ nm).

In conclusion, we believe that the proposed synthetic strategy, owing to its ease and versatility, could probably be applied to a large range of polypeptide and polysaccharide molecules of biological interest, such as polysialic acid or hyaluronic acid. Hence, the development of polysaccharide-*block*-polypeptide copolymers may play a significant role in future applications of polymers in biology. For example, their closely related glycoprotein structure makes them suitable model compounds for glycomics research.[27] Furthermore, the ability of these copolymers to self-assemble into small vesicles could be used to construct a new generation of drug- and gene-delivery systems with a high affinity for the surface glycoproteins of living cells. It has also been noted previously that the structure and properties of polymersomes are similar to those of viral capsids.[28] The creation in this study of a capsular structure composed of molecules of a polysaccharide-*block*-polypeptide is a further step towards the mimicking of virus morphology.

Received: December 3, 2008
Published online: February 26, 2009

Keywords: click chemistry · polymersomes · polypeptides · polysaccharides · self-assembly

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