Communication

Nanoparticles on the Basis of Highly Functionalized Dextrans

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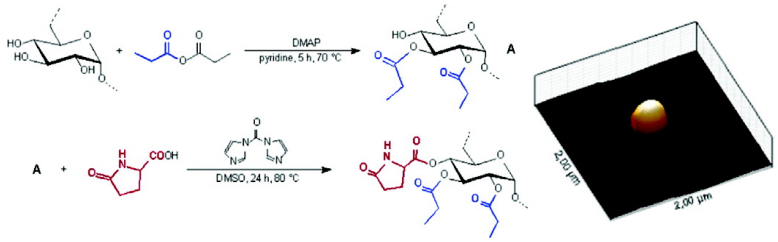
J. Am. Chem. Soc., **2005**, 127 (30), 10484-10485• DOI: 10.1021/ja052594h • Publication Date (Web): 07 July 2005



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Published on Web 07/07/2005

Nanoparticles on the Basis of Highly Functionalized Dextrans

Tim Liebert, Stephanie Hornig, Stephanie Hesse, and Thomas Heinze*

Center of Excellence for Polysaccharide Research, Friedrich Schiller University of Jena, Humboldtstrasse 10, D-07743 Jena, Germany

Received April 21, 2005; E-mail: Thomas.Heinze@uni-jena.de

Polymeric nanoparticles have gained considerable interest in the biomedical field, especially as carriers for drug targeting,[1−4] for contrast enhancement,[5] and as materials for nanocoating.[6] New nanoparticle-forming materials need to be nontoxic, biocompatible, and should have a high disease site selectivity. Thus, they are commonly prepared on the basis of biopolymers, for example, polysaccharides. The usual path is the partial derivatization of glucans, such as pullulan, with bulky hydrophobic substituents, including cholesterol.[7,8] The amphiphilic character of these polymers guaranties the self-assembling of regular nanospheres. However, a problem arises still from the presence of a large amount of unmodified hydroxyl groups tending to re-form the supermolecular interactions characteristic for polysaccharides yielding aggregation and, consequently, collapse of the nanoparticles. A more stable type of particles should be accessible by chemical modification of the majority of OH functions, avoiding the establishment of a hydrogen bond system. A nice approach in this regard is the conversion of acetylated pullulan with poly(ethylene glycol).[4]

The presented paper describes a new type of nanoparticles prepared on the basis of dextran via defined two-step esterification with biocompatible propionate and pyroglutamate moieties, leading to highly functionalized derivatives with an adjustable hydrophilic−hydrophobic balance and adjustable solubility (cf. Scheme 1).

The first step in the synthesis is the conversion of well-defined dextran from *Leuconostoc* spp. strain no. 10817 (M_w = 5430 g/mol) with propionic anhydride in the presence of pyridine for 5 h at 70 °C. The derivative obtained had a degree of substitution (DS_{Prop}) of 1.70, determined precisely from the spectral integrals of highly resolved polymer−^{1}H NMR spectra of peracetylated samples (dextran propionate acetate, DPA). The assignment of the chemical shifts of DPA has been done according to Gagnaire and Vignon[9] and via two-dimensional NMR (Figure 2). The complexity of the COSY signals for H2, H3, and H4 suggests a statistic pattern of propionylation.

The second esterification is achieved by homogeneous reaction of the dextran propionate with a pyroglutamic acid imidazolide, which is prepared in situ by conversion of pyroglutamic acid with *N*,*N*-carbonyldiimidazole (CDI).[10] The advantage of the application of CDI is the efficiency of the process, mild reaction conditions, and the selectivity of the reaction. In contrast to the commonly used activation of the carboxylic acid with DCC/DMAP, the reagents applied and the byproducts are nontoxic and completely removable. During conversion with CDI, only carbon dioxide and imidazole are formed. Thus, pure dextran propionate pyroglutamate (DPP) with DS values of DS_{Prop} = 1.70 and DS_{Pyrogl} = 0.26 (estimated by elemental analysis) is obtained simply by precipitation in ethanol and washing with ethanol. A ^{13}C NMR spectrum of the polysaccharide derivative is shown in Figure 3, confirming its structural purity. Relevant ^{13}C chemical shifts of the AGU of dextran and the two ester moieties are detected only.

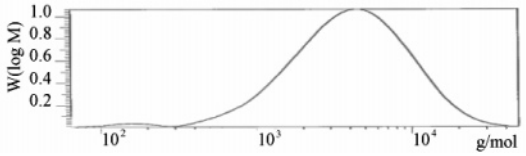


Figure 1. Molar mass distribution of dextran from *Leuconostoc* spp. strain No. 10817 (M_w = 5430 g/mol) obtained by GPC analysis.

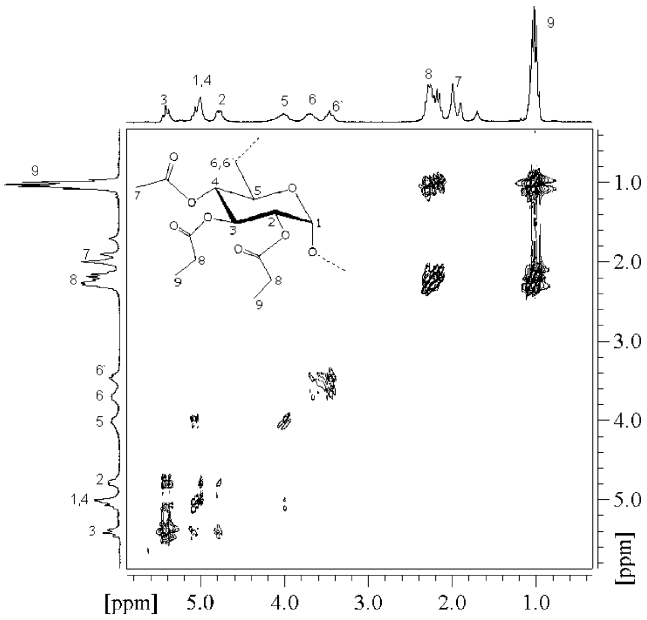
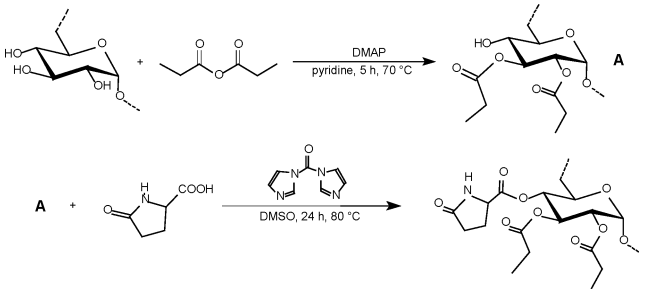


Figure 2. ^{1}H,^{1}H COSY NMR spectrum of dextran propionate acetate (DPA; in DMSO-d_6).

Scheme 1. Synthesis Path Applied for the Preparation of Highly Functionalized Amphiphilic Dextran Propionate Pyroglutamate



Noteworthy is that an inverse synthesis path does not give amphiphilic polymers because of the fact that the NH functions of the pyroglutamic moiety responsible for the hydrophilic character of the molecule are converted with the propionic anhydride to give a hydrophobic amide-type *N*-propionate.[11]

Preparation of the nanoparticles was carried out by a dialysis process;[3] 40 mg of DPP was dissolved in 10 mL of purified DMAc

10.1021/ja052594h CCC: $30.25

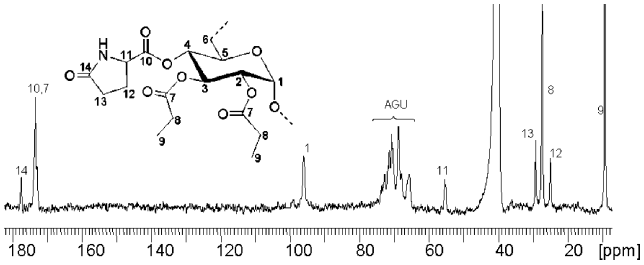


Figure 3. ^{13}C NMR spectrum of dextran propionate pyroglutamate (DPP; in DMSO-d_6).

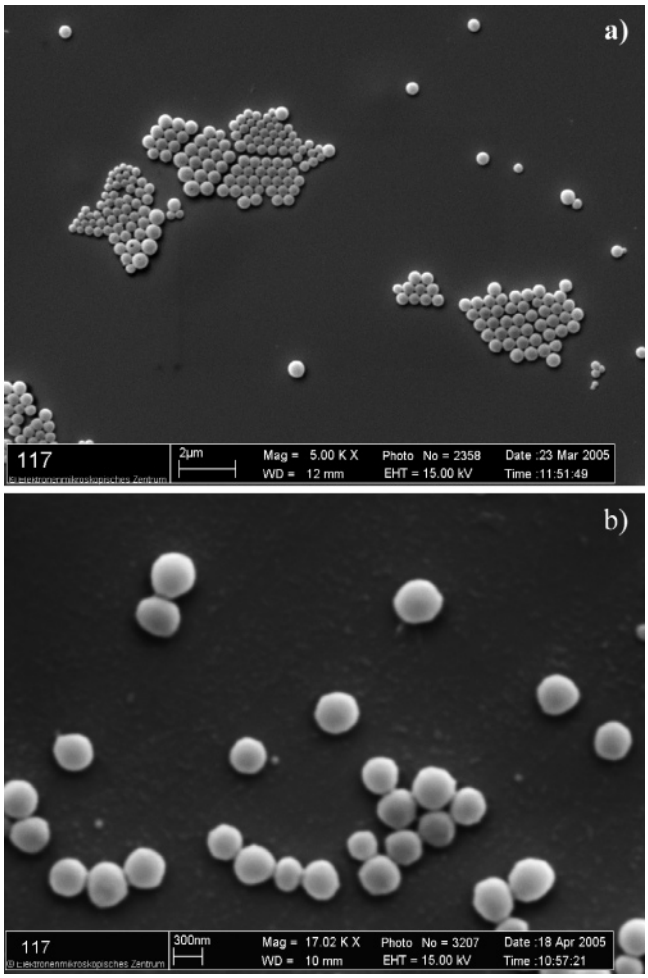


Figure 4. SEM images of dextran propionate pyroglutamate (DPP) nanoparticles on a graphite-covered mica surface (a) directly taken after the dialysis and (b) after 3 weeks storage in water.

and was dialyzed against distilled water for 4 days. The suspension obtained was investigated by scanning electron microscopy (SEM) and atomic force microscopy (AFM). For this purpose, a droplet of approximately 0.2 mL was placed on a mica surface covered with graphite. The system was lyophilized for 6 h.

After covering with gold, the structures were studied with an SEM equipment LEO-1450 VP (LEO, Oberkochen, Germany). The nanospheres observed had dimensions in the range of 200−600 nm. The major fraction consists of particles of 370 nm in diameter

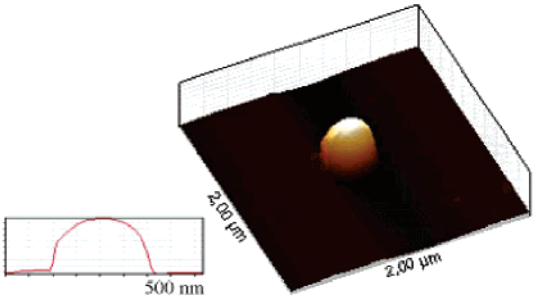


Figure 5. AFM image (noncontact mode) of a dextran propionate pyroglutamate (DPP) nanoparticle on a graphite-covered mica surface.

(cf. Figure 4a). The SEM image in Figure 4b verifies that nanospheres in the suspensions did not undergo any morphological changes within 3 weeks. The turbidity determined during this period was in the range of 11 000 NTU.

AFM investigations were carried out on non-gold-plated nanoparticles with a DualScope C-21 (DME, Denmark) and silicon nitride tips (60.0 N/m, 0.20 nN, 310 kHz). DPP nanoparticles were found to be in the range of 200−500 nm, which confirms the SEM measurements. The synthesis of DPP opens up a new path for the preparation of polymeric nanoparticles with an average size of about 300 nm. The polysaccharide derivative prepared is characterized by a high degree of functionalization of the OH moieties, avoiding partial restoration of the hydrogen-bond-based superstructure of the backbone, which usually results in metastable nanostructures. The polymer is completely bio-based and contains no impurities, making it a valuable tool for applications in the biological or medical field. The synthesis path allows perfect control of the amphiphilicity and solubility. Investigations are in progress toward the influence of the hydrophilic−hydrophobic balance of the polysaccharide derivative on the nanoparticle size and morphology, which can be efficiently controlled by different amounts of the two ester moieties. Moreover, we can study the release of different substances, such as fluorescent dyes, as a model from these nanospheres.

Acknowledgment. The authors gratefully acknowledge the financial support of the "Deutsche Forschungsgemeinschaft", Project He 2054/8-1. They are also indebted to Dr. W. Günther and I. Herrmann (EMZ, FSU Jena) for their contributions.

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JA052594H