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Studies on the structure of coumarin-modified dextran nanoparticles by fluorescence spectroscopy

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ABSTRACT

The photophysical and photochemical characteristics of nano-scaled particles obtained via solvent displacement from coumarin-modified dextrans were studied by means of absorption- and fluorescence-spectroscopy. The environment-dependent fluorescence emission of the pendant 4-methyl-7-alkoxy coumarin groups was exploited as a probe to gain information about the inner structure of the polysaccharide based nanoparticles. Time-resolved fluorescence measurements showed that the particles had two domains of different polarity and it could be confirmed that the core of the nano-assemblies contained water. Moreover, preliminary experiments were carried out demonstrating the possibility to control the morphology of the nanoparticles by the light induced $2\pi + 2\pi$ cycloaddition of the coumarin substituents.

1. Introduction and scope

Recently, polymeric nanoparticles based on polysaccharide derivatives with high degree of substitution (DS) have received much attention due to their biocompatibility, enormous structural diversity, and functional versatility (Bachelder, Beaudette, Broaders, Dashe, & Frechet, 2008; Bachelder et al., 2010; Beaudette et al., 2009; Broaders, Grandhe, & Frechet, 2011; Cohen et al., 2010, 2011; Cui, Cohen, Broaders, Beaudette, & Frechet, 2011; Hornig et al., 2008; Hornig & Heinze, 2008; Hornig, Heinze, Hesse, & Liebert, 2005; Liebert, Hornig, Hesse, & Heinze, 2005; Wondraczek, Elschner, & Heinze, 2011). However, it is obvious that knowledge of the structure and of the local microenvironment inside the polysaccharide particles is of fundamental importance for any of the desired applications. Therefore, fluorescent probe molecules, whose fluorescence properties characterize their distinct environment, have been commonly employed to investigate the structure of organized assemblies. In earlier studies on nanoparticles of hydrophobic polysaccharide derivatives with low DS, pyrene has been incorporated into gel-particles and it has been concluded that the particles contain hydrophobic domains in the core and a hydrophilic shell (Akiyoshi, Deguchi, Moriguchi, Yamaguchi, & Sunamoto, 1993; Lee, Jo, Kwon, Kim, & Jeong, 1998; Nishikawa, Akiyoshi, & Sunamoto, 1994; Vieira, Moscardini, Tiera, & Tiera, 2003; Yinsong, Lingrong, Jian, & Zhang, 2007). In the case of polysaccharide derivatives with a high DS, hydrophobic domains have been also confirmed in fluorescence probe studies (Hornig & Heinze, 2007). Although fluorophores can be entrapped in nanoparticles, their covalent attachment to the particle forming polymer would offer various advantages: the fluorophores are fixed to the particles, preventing leaching, the polysaccharide matrix can prevent fluorescence quenching often caused by high concentration of fluorophores, and the fluorophores are uniformly distributed in the interior of the macromolecular assembly. Moreover, the inner structure of the particles will not be affected by the incorporation of the additional probe itself.

Recently, 4-methyl-7-alkoxy coumarin molecules were used as hydrophobic modifiers for nanoparticle-forming polysaccharide derivatives in order to control the particle properties utilizing the photo-active character of this type of chromophores (Wondraczek & Heinze, 2008). However, coumarins are also applicable as fluorescent probes since their fluorescence properties are sensitive to the polarity of their microenvironment (Wagner, 2009). 7-Alkoxy

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coumarins in particular exhibit a higher fluorescent quantum yield and fluorescence lifetime in a polar environment compared to a less polar one (Aaron et al., 1995; Heldt, Heldt, Ston, & Dieh, 1995; Muthuramu & Ramamurthy, 1984). In the present study we focus on the absorption and fluorescence properties of nanoparticles prepared from coumarin-modified dextran derivatives with different DS. Fluorescence lifetime measurements were applied to get more information about the particle structure and morphology.

2. Experimental

2.1. Materials

N,N-Dimethylacetamide (DMAc) was purchased from Sigma–Aldrich (purity 99%). Deionized water was obtained from a Millipore Milli-Q system. Photoactive dextran derivatives (dextran [(4-methyl-2-oxo-2H-chromen-7-yl)oxy] acetate) were synthesized as described elsewhere(Wondraczek & Heinze, 2008). Nanoparticles were prepared by dialysis. Briefly, the dextran derivative (20 mg) was dissolved in DMAc (5 mL) and dialyzed against deionized water (400 mL) using a regenerated cellulose dialysis membrane (Spectra/Por, molecular weight cut off of 3500 g/mol). The water was first renewed after at least 12 h and then subsequently four times after at least 3 h.

2.2. Characterization methods

The hydrodynamic diameter of the nanoparticles was determined by dynamic light scattering using a Malvern zetasizer Nano ZS instrument (He–Ne laser 633 nm, scattering angle: 173°). The mean particle size was approximated as the effective (*Z*-average) diameter and the width of the distribution as the polydispersity index (PDI). Both parameters were achieved using the cumulants method for data analysis, assuming spherical particle shape and log-normal size distribution. The effective zeta-potential of the nanoparticles was determined by electrophoretic light scattering (ELS) using a Malvern zetasizer Nano ZS instrument. It was calculated using the Smoluchowski equation. The measurements were repeated three times for each sample. The intrinsic error of the measured zeta-potential was ±5 mV.

Extinction (absorption + scattering) spectra of the aqueous particle suspensions were measured using a Perkin-Elmer λ900 spectrophotometer equipped with an integrating sphere (PELA-1000). The sample cuvette (optical path 10 mm) was placed at the beam entrance of the integrating sphere. This setup reduces the effect from scattering of light by the nanoparticles significantly and thus the measured spectra are referred to as absorption spectra (Merzlyak & Naqvi, 2000), even though this is not the absorption in the physical correct sense. Fluorescence spectra of the aqueous nanoparticle suspensions were measured using a Perkin-Elmer LS50B spectrofluorometer. A time-correlated single photon counting (TCSPC) system consisting of a PicoHarp 300 controller and a PDL 800-B driver was used for the time resolved fluorescence measurements. The excitation wavelength was 340 nm from a pulsed diode laser head PLS-8-2-295. The fluorescence signal was detected with a micro channel plate photomultiplier (Hamamatsu R2809U). The time resolution of the instrument was approximately 320 ps (full width, half maximum). The fluorescence decays were fitted to a multiexponential model, from which an amplitude-weighted fluorescence lifetime was calculated. The decay associated spectra were obtained by measuring the decay curve at each monitoring wavelength with a constant data accumulation time and fitting the decays globally. The amplitudes were corrected by taking into account the wavelength sensitivity of the photomultiplier tube. The amplitudes at <390 nm may appear lower compared to the actual values, because an optical filter with a sharp change in transmittance at 380 nm was used for excluding the excitation light from the detector. The stability of the samples during DAS measurements was monitored by measuring the absorption spectra before and after the experiments.

Table 1
Size, polydispersity index (PDI), and zeta-(ζ)-potential of nanoparticles prepared from dextran [(4-methyl-2-oxo-2H-chromen-7-yl)oxy] acetate depending on the degree of substitution (DS).

DS[a]	Diameter [nm]	PDI	ζ-potential [mV]
0.44	314	0.135	−25
0.94	218	0.145	−31
1.63	182	0.124	−30
2.32	131	0.093	−38

[a] Determined by ^{1}H NMR spectroscopy (Wondraczek & Heinze, 2008).

3. Results and discussion

Coumarin-modified dextran was prepared by the reaction of the biopolymer with [(4-methyl-2-oxo-2H-chromen-7-yl)oxy]acetic acid activated by *N,N′*-carbonyldiimidazole. It was transferred to nanoparticles by an exchange of the organic solvent DMAc against the non-solvent water (Fig. 1) (Wondraczek & Heinze, 2008). The diameter of the coumarin-modified dextran nanoparticles was in the range from 131 to 314 nm and it decreases with increasing DS (Table 1). All particles obtained possess a negative zeta-(ζ)-potential and thus the stability of the suspension can be attributed to electrostatic repulsion.

The suspensions of the particles obtained by dialysis (estimated concentration of particles 1 mg/mL) were adjusted to contain approximately equal amounts of polysaccharide derivative by diluting them to 2% (v/v), thus making it possible to compare the

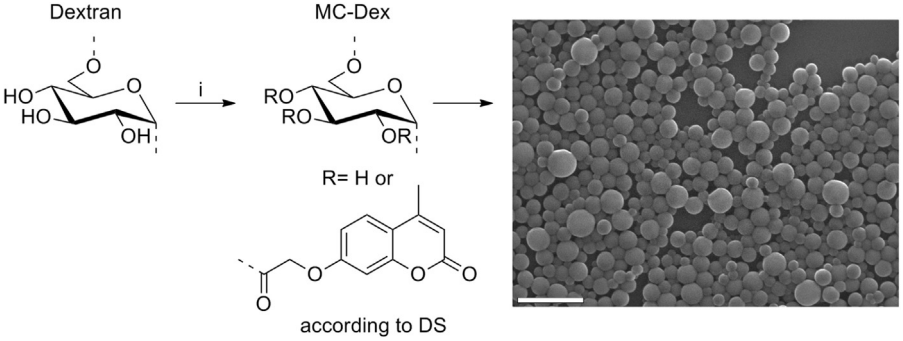

Fig. 1. Synthesis of 4-methyl-7-alkoxy coumarin functionalized dextran (MC-Dex) and SEM picture of nanoparticles obtained via solvent exchange (scale bar is 1 μm): (i) [(4-methyl-2-oxo-2H-chromen-7-yl)oxy]acetic acid, *N,N*-carbonyldiimidazole, DMSO, 16 h, 80°C.