Fluorescent Polysaccharide Nanoparticles for pH-Sensing

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The exploration of chemical microenvironments in organisms as well as on the cellular level is of great interest in medical and biological research. Therefore, dextran nanoparticles, which are labelled with both a pH-indicator dye (fluorescein isothiocyanate, FITC) and a reference dye (sulforhodamine B acid chloride) as an internal standard, were developed as biocompatible nanosensors that can be used for ratiometric pH measurements. The ratio of the fluorescence dyes can easily be tuned during the nanoparticle formation by choosing the appropriate mixture of the labelled derivatives prior the nanoprecipitation. The fluorescence intensity of FITC increases with increasing pH value, whereas the signal of the reference dye remains constant. The polysaccharide based pH-nanosensors are characterized in terms of fluorescence lifetime, autoclaving stability, response to ionic strength, oxidation and photobleaching. The resulting pKa of 6.45 is appropriate for most of the measurement purposes. Fluorescence lifetime measurements indicate that energy transfer between the dyes takes places. However, no negative influence on the performance of the pH value measurements could be observed.

Keywords: dextran, nanoparticles, pH-sensors

1. Introduction

The exploration of the microenvironments in organisms, but also on the cellular level, is of great interest in medical and biological research. Every organism carefully controls an enormous list of physiological data including the pH value and the concentration of ions[1]. Therefore, there is a significant interest to measure the pH value in the extra- and intracellular space in order to understand physiological and pathological mechanisms on the molecular level. In particular fluorescent pH indicators play an important role. However, there are several shortcomings associated with this approach because the dyes may redistribute or aggregate[2]. One solution is the embedding of the dyes in a polymer matrix[3]. Therefore, dextran was functionalized with fluorescein isothiocyanate (FITC) as pH-sensitive and sulforhodamine B acid chloride (SRB) as reference dye[4]. Moreover, the hydrophobic moiety propionate was introduced in order to form water-unsoluble dextran derivatives for the defined nanoparticle formation via nanoprecipitation. By combining FITC and SRB, the ratiometric detection of the pH value is possible even if there are fluctuations in the sensor concentration or in the light source intensity. The preparation of the particles, their characterization as well as cell studies are included in the present contribution[4,5].

2. Experimental

Dextran (\(M_w = 54,800\) g/mol; PDI = 1.56) is produced by Leuconostoc mesenteroides strain no. NRRL B-512(F). The dextran derivatives and dextran particles thereof were produced as
described in ref. [4]. The particle size of the nanospheres was determined by dynamic light scattering (DLS) studies using a laser beam at 633 nm and a scattering angle of 173° (Zetasizer Nano ZS, Malvern Instruments, UK). For SEM studies, one droplet nanoparticle suspension was placed on a mica surface, lyophilized and covered with gold. The images were obtained with a scanning electron microscopy (SEM, LEO-1530 VP Gemini, LEO, Oberkochen, Germany) operating at 10 kV. The fluorescence spectra were recorded in a 10 mm cuvette on a Fluorolog 3 from Jobin Yvon-Spex at a temperature of 25 (± 1) °C. The fluorescence kinetics were measured with a time-correlating single photon counting spectrometer (CD900, Edinburgh Instruments). The wavelength for excitation of the dyes in solution as well as for the nanosensors was 489 nm for fluorescein isothiocyanate and 543 nm for SRB, respectively. For characterization of the particles with fluorescence spectrometry, 0.1 mL of a dialyzed particle suspension (1 mg nanoparticles/mL) was added to 2 mL of phosphate buffer solution in a cuvette. Confocal images were acquired with a Zeiss LSM 510 (Carl Zeiss GmbH, Oberkochen, Germany) inverted laser scanning microscope (LSM) using a C-Apochromat × 63 water immersion objective lens (Zeiss).

3. Results and Discussion

The biologically well-established polyglucan dextran was functionalized with hydrophobic groups as well as dyes yielding multifunctional derivatives capable for the formation of tailored sensor particles for intracellular pH measurements. The desired ratio of dyes and hence the desired fluorescence absorption of the particles can be easily adjusted during dialysis of a N,N-dimethylacetamide solution against water (Figure 1).

Generally, the adjustment of fluorescence intensities in particle systems for ratiometric measurements represents a challenging task. However, it is possible to adjust the optimal peak intensities only by mixing the two dextran derivatives in the desired ratio. Reasonable spectra were obtained in the ratios 7/1 and 3/1 for SRB-/FITC-dextran propionate. The particles are ~500 nm in size and of spherical shape as determined by DLS and SEM studies (Figure 2).

The effective pKa value of the ratiometric dextran propionate particles at both SRB excitation wavelengths is 6.45, which is exactly in the range of the transition of the mono- and dianionic state of unbound fluorescein dissolved in aqueous solution[6]. The sensitivity of the sensor particles was evaluated and lies between pH 5.7 and 7.1, the relevant biological measuring conditions.

The efficiency of a fluorescence resonance energy transfer (FRET), which might limit the application of the sensor particles, was evaluated using mathematical and experimental methods. Therefore, the Förster radius between the dyes and the distance of the dyes inside the particle were calculated leading to a FRET efficiency of 23%[7]. In addition, the fluorescence lifetime of FITC inside the particles containing only FITC was measured as well as the FITC lifetime in particles containing additionally the acceptor fluorophore SRB[ 8 ]. The experimentally determined transfer efficiency (E = 30 %) is in the same range as the calculated one.

For applications of the sensor particles in vitro or in vivo, the effect of intracellular substances like enzymes has to be taken into consideration. Enzymes are capable of catalyzing oxidation processes. Experiments using hydrogen peroxide have shown that the oxidation of the dyes is prevented via immobilization inside the polymer particles. The particles can further be used at varying excitation caused, e.g., by fluctuations in light source intensity.
To evaluate if the particles can be incorporated into cells, human foreskin fibroblasts were incubated with FITC/SRB-dextran propionate nanoparticles. Confocal laser scanning microscopy shows that the cells easily incorporate the particles in a high amount. The particles located in the more acidic cytosol have an orange color, whereas particles in the more alkaline surrounding solution exhibit a more greenish color. Long-term experiments over a period of 22 days evidence the absence of cytotoxic effects. No leaching of the fluorescence dyes was observed, which indicates that the particles are not degraded during the time.

4. Conclusion
The presented experiments show the potential of fluorescence labelled dextran nanoparticles in biological experiments because they are non-toxic and incorporated by the cells without any additional additives. However, the photostability of the FITC limits the lifetime of the nanosensors. By using other indicator dyes, analogue nanosensors can be easily developed also for other analytes with other dyes of interest. The preparation technique provides a potentially widely applicable tool for varying the ratio of sensor- and reference fluorescence dextran derivatives.

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