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Glyco-quantum dots: a new luminescent system with multivalent carbohydrate display

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Abstract—Nanoclusters of cadmium sulfide covalently bound to biologically significant neoglycoconjugates (glyco-QDs) have been synthesized by means of a single step solution procedure. The neoglycoconjugates confer the QDs water solubility, stability, and biological activity. The nanometer-sized glyco-QDs have been characterized by TEM, EDAX, ^{1}H NMR, UV–vis, and fluorescence spectroscopy. The carbohydrate conjugate nanocrystals can be used as bioactive fluorescence probes for specifically labeling carbohydrate cell receptors.

1. Introduction

Semiconductor nanoparticles of nanosized dimensions (quantum dots) are powerful fluorescence probes, which emit light at a variety of precise wavelengths with the exact color mainly depending on the dot size. Moreover, quantum dots are about 100 times more stable against photo-bleaching as organic dyes, and often reveal a long fluorescence lifetime.[1] These properties have been a challenge for producing QD-bioconjugates as fluorescent labels for in vitro and in vivo cellular imaging.[2]

There are several approaches for the coupling of biomolecules to semiconductor QDs.[1b] Most commonly QDs are first prepared at high temperature in the presence of protective additives to prevent crystal aggregation and to regulate the growing rate. Alternatively, the protection process can be performed in solution using thiols with hydrophilic end-groups to confer stability and water solubility to the QDs. In the second step, the biomolecules are attached to the protected nanocrystals for cell labeling. Quantum dots bio-conjugated to peptides and proteins,[2b,d,h,k,3] antibodies,[2i,j,3] DNA,[4,2g] and other molecules[5,2a,f] have recently been prepared mainly by coupling the biomolecules to the thiol protected QDs and tested as biological markers. However, QDs conjugated to carbohydrate antigens for specific cell targeting have not yet been prepared.

Our laboratory has recently been successful in preparing gold nanoclusters functionalized with carbohydrate antigens (glyconanoparticles)[6,7] using a simple procedure. These carbohydrate functionalized gold nanoclusters have been used as polyvalent tools for studying carbohydrate–carbohydrate interactions[7] and for interfering carbohydrate mediated cell–cell adhesion processes.[8] Using gold nanoclusters functionalized with the trisaccharide antigen Lewis X (Le^X), we have provided conclusive evidence of the Ca^{2+}-mediated self-interaction of this antigen, which is involved in morula compaction and aggregation of teratocarcinoma cells.[7,9] We also explored the potential of these tools for cell labeling and imaging of carbohydrate-mediated biological processes. To this end, hybrid glyconanoparticles incorporating the antigen Le^X and a fluorescence probe were prepared.[6] However, attempts to label F9 teratocarcinoma cells with these glyconanoparticles proved unsuccessful. Herein we explore the preparation of carbohydrate functionalized CdS nanocrystals.

2. Results and discussion

We used a single step procedure to prepare CdS nanocrystals functionalized with the disaccharide maltose (Glcα1-4Glc) and the trisaccharide antigen Le^X (Galβ1-4[Fucα1-3]GlcNAc) as tools for targeting specific cell sites. Neoglycoconjugates **1** or **2** (Scheme 1) were synthesized by glycosidation of the conveniently protected maltose or Le^X derivatives with 11-thioacetate

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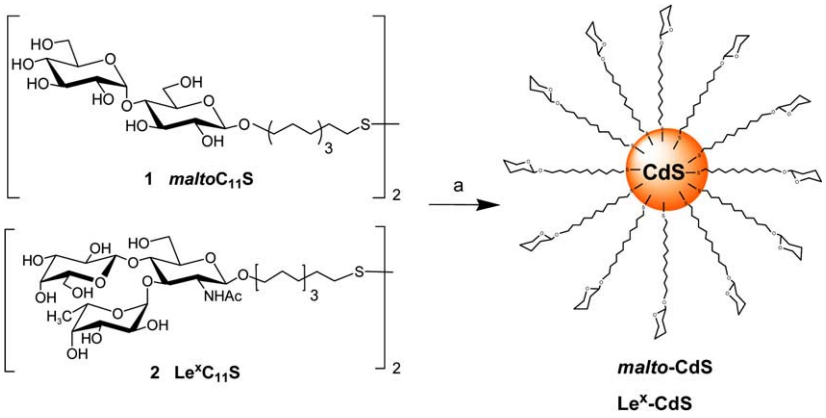

Scheme 1. Preparation of glyco-QDs. Reagents and conditions: (a) $Cd(NO_3)_2{\cdot}4H_2O$, Na_2S, pH 10, H_2O.

undecanol, as previously described.[6,10] The neoglycoconjugates were obtained as disulfide derivatives and in this form directly attached to the CdS nanocrystals. The length of the neoglycoconjugates was 3 nm including the spacer and carbohydrate moieties. The disulfide neoglycoconjugates acted as the stabilizer controlling particle size and aggregation and provided, at the same time, water solubility and active groups for specific labeling. This procedure allows the preparation in aqueous solution of fluorescent nanocrystals covalently attached to a variety of different neoglycoconjugates in one step. In a similar fashion, as previously reported for gold glyconanoparticles,[6] hybrid glyco-QDs with differing carbohydrate density and different spacers can also be prepared.

The functionalized CdS nanocrystals were obtained by adding sodium sulfide to a water solution of **1** or **2** and cadmium nitrate at room temperature using a modification of the procedure of Spanhel et al.[11] The malto-CdS and Le^X–CdS glyco-QDs (Scheme 1) thus prepared gave yellow solutions and emitted light in the green region (550 nm) when observed under an ultraviolet lamp at $\lambda = 360$ nm (Fig. 1).

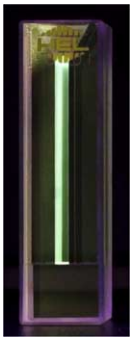

Figure 1. Photograph of a malto-CdS solution under an ultraviolet lamp ($\lambda = 360$ nm).

These were purified by centrifugal filtration and characterized by TEM as well as by ^1H NMR, UV–vis, and fluorescence spectroscopy. After lyophilization the glyco-QDs were water soluble and stable for months in the absence of light at 4 °C.

The ^1H NMR spectra in D_2O of the prepared glyco-QDs (Fig. 2) differed from the corresponding neoglycoconjugates in the line broadening typical for macromolecules in solution. However, the signals were narrower than those of the corresponding gold glyconanoparticles.[6] The transmission electron micrographs show dispersed nanoparticles, spherical in shape, of a diameter between 2 and 5 nm (Fig. 3a and b). The Cd to S ratio was, as determined by EDAX, 1:3 and 1:1 for the malto-CdS and Le^X–CdS, respectively (Fig. 3c and d).

The UV–vis absorption spectra and the fluorescence emission spectra for the glyco-QDs are shown in Figure 4. The UV–vis spectra of these glyco-QDs exhibited surface plasmon bands at 440 nm typical for 2.5 nm nanoclusters.[12] A band of lower intensity was also observed at 500 nm for the Le^X–CdS revealing distinct population of clusters. The emission spectra of the malto-CdS particles presented bands at 490 and 545 nm when the excitation wavelength was 400 nm. The Le^X–CdS nanocrystals showed two intense bands at 525 and 550 nm when the excitation wavelength was 400 nm. The Le^X–QDs are now being used as polyvalent and luminescent tools to observe aggregation of F9 teratocarcinoma cells and the malto-QDs as control system.

3. Conclusion

In conclusion, we have prepared for the first time water soluble CdS nanoclusters functionalized with biologically significant oligosaccharides by means of a straightforward one step procedure. These glyco-QDs present the carbohydrate antigens in a three dimensional and polyvalent array mimicking glycosphingolipid presentation at the cell surface and providing a convenient tool for studying and interfering carbohydrate mediated cell–cell adhesion processes. Recently, the synthesis of luminescent glyconanospheres stabilized with carboxymethyldextran by electrostatic interactions has been reported.[13] However, further functionalization of these 160 nm dextran protected nanospheres would be neces-

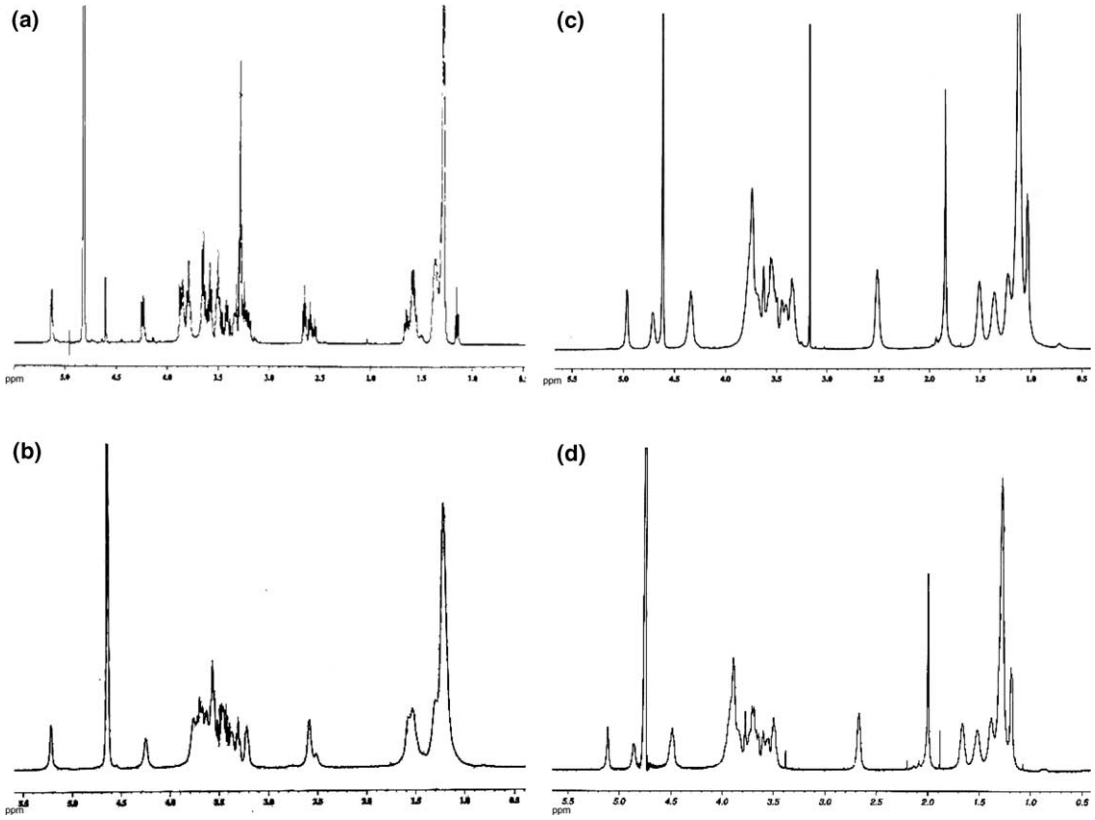

Figure 2. ^{1}H NMR in D_2O of: (a) **1**; (b) malto-CdS; (c) **2**; (d) Le^{X}–CdS.

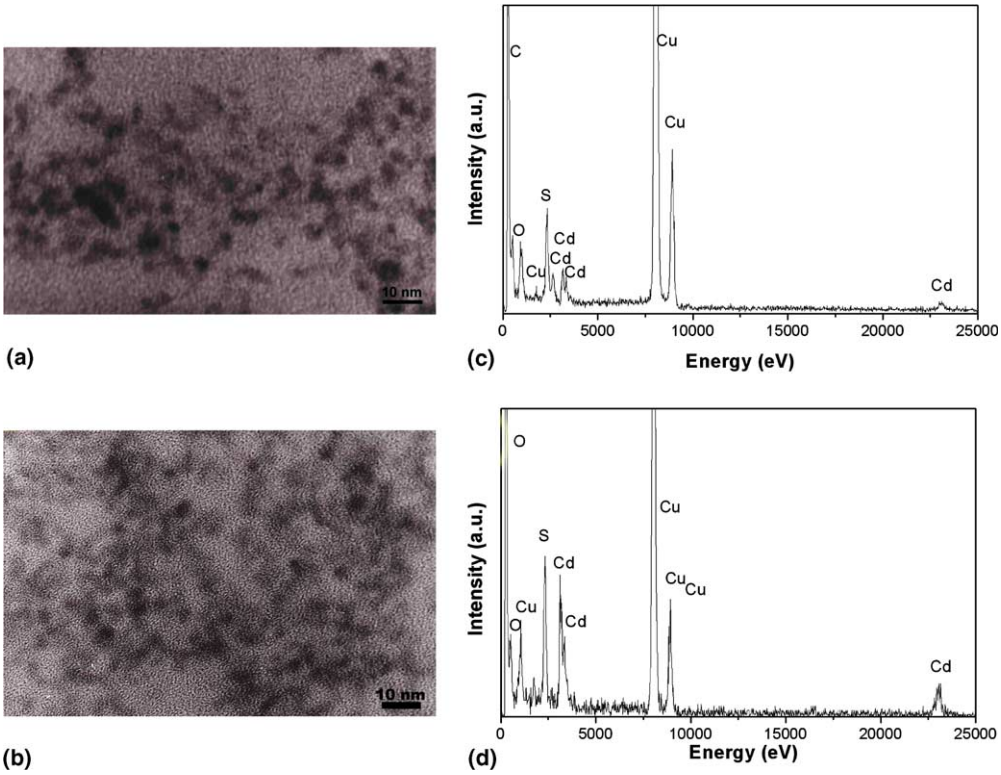

Figure 3. Transmission electron micrographs of: (a) malto-CdS; (b) Le^{X}–CdS and (c,d) of their corresponding EDAX spectra.

sary to obtain bio-conjugated QDs for specific labeling of cell surfaces. The glyco-QDs approach presented here open the way to a straightforward preparation of a great variety of 2–5 nm sized, multivalent, and fluorescent

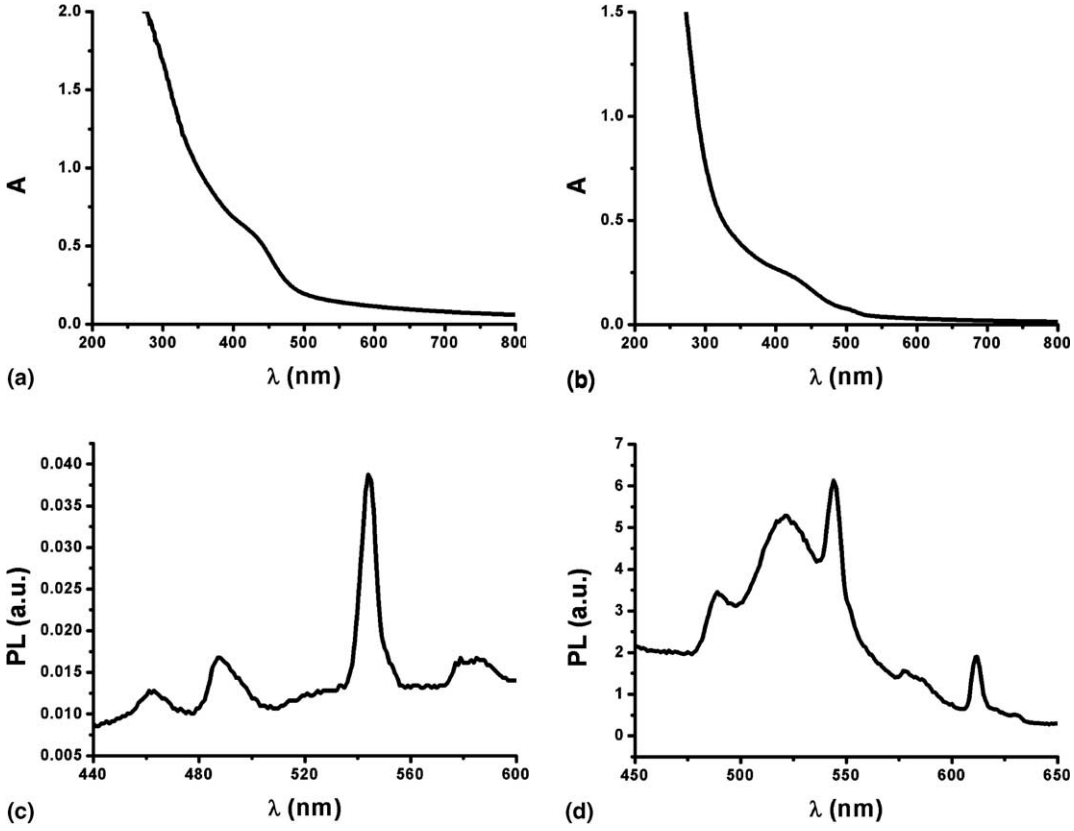

Figure 4. UV–vis spectra in water of: (a) malto-CdS; (b) Le^X–CdS; and fluorescence spectra (λ_{exc} = 400 nm) in water of: (c) malto-CdS; (d) Le^X–CdS.

nanocrystals labeled with synthetic carbohydrate antigens. To the best of our knowledge, this is the first example of QDs specifically functionalized with biologically significant glycoconjugates for which no equivalent exists.

4. Experimental

4.1. General procedures

^1H and ^{13}C NMR spectra were acquired on a Bruker DRX-500 spectrometer with chemical shifts are given in ppm (δ) relative to the residual signal of the solvent used. UV spectra were carried out with a UV/vis Perkin–Elmer Lambda 12 spectrometer. Fluorescence spectra were carried out in an Aminco–Bowman Series 2 Spectronic Instruments luminescence spectrometer in miliQ water. For TEM examinations, a single drop (20 μL) of the aqueous solution (ca. 0.1 mg mL^{-1}) of the gold glyco-quantum dots was placed onto a copper grid coated with a carbon film. The grid was left to dry in air for several hours at room temperature. TEM analyses were carried out in a Philips CM200 microscope working at 200 kV. EDX analyses were carried out in a Philips DX4 apparatus.

4.2. Malto-CdS

$Cd(NO_3)_2{\cdot}4H_2O$ (6.2 mg, 0.02 mmol, 1 equiv) and **1**[6] (13 mg, 0.013 mmol, 1.3 equiv) were dissolved in 50 mL of Ar degassed water and the pH adjusted to 10 with 0.1 M NaOH. Then a solution of Na_2S (1.6 mg, 0.02 mmol, 1 equiv) in degassed water (2 mL) was added dropwise at room temperature under Ar with vigorous stirring. The stirring was continued for 20 min while maintaining the pH at 10 to yield a yellow-orange solution. The glyco-QDs were purified by centrifugal filtering (Microcon MW30000, 10 min, 10,000*g*). The process was repeated by washing with Tris (100 mM, pH 8.8) until the nanocrystals were free from the starting materials. The residue in the Microcon filter was dissolved in water (2 mL) and lyophilized to give a pale yellow substance soluble in water. EDX (ratio Cd/S): 1:3; ^1H NMR (500 MHz, D_2O): δ 5.33 (s, 1H, H-1′), 4.37 (s, 1H, H-1), 3.87–3.33 (m, 15H), 2.70 (s, 2H CH_2S), 1.65–1.33 (m, 18H); UV/vis (H_2O): λ = 450 nm; fluorescence (H_2O) λ_{exc} = 400 nm, λ_{em} = 463, 488, 544, 583 nm.

4.3. Le^X–CdS

$Cd(NO_3)_2{\cdot}4H_2O$ (1.7 mg, 0.005 mmol, 1 equiv) and **2**[10] (5 mg, 0.0035 mmol, 1.3 equiv) were dissolved in 25 mL of Ar degassed water and the pH adjusted to 10 with 0.1 M NaOH. Then a solution of Na_2S (0.4 mg, 0.005 mmol, 1 equiv) in degassed water (1 mL) was added dropwise at room temperature under Ar with vigorous stirring. The stirring was continued for 20 min while maintaining the pH at 10 to yield a yellow-orange solution. The glyco-QDs were purified by centrifugal filtering (Microcon MW30000, 10 min, 10,000*g*). The pro-

cess was repeated by washing with Tris (100 mM, pH 8.8) until the nanocrystals were free from the starting materials. The residue in the Microcon filter was dissolved in water (2 mL) and lyophilized to give a pale yellow substance soluble in water. EDX (ratio Cd/S): 1:1; ^{1}H NMR (500 MHz, D_2O): δ 5.12 (s, 1H, H-1′), 4.86 (m, 1H, H-5′), 4.50 (m, 2H, H-1, H-1″), 4.10–3.50 (m, 17H), 2.67 (s, 2H, CH_2S), 2.00 (s, 3H, NHAc), 1.60–1.10 (m, 20H); UV/vis (H_2O): λ = 430 nm; fluorescence (H_2O) λ_{exc} = 420 nm, λ_{em} = 490, 520, 548, 613 nm.

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