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Bacterial detection using carbohydrate-functionalised CdS quantum dots: a model study exploiting *E. coli* recognition of mannosides

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ABSTRACT

Mannose-coated CdS quantum dots (Man-QDs) were prepared in a facile aqueous, one-pot process that exploits the self-assembly of thiolated mannose in the presence of CdS under reducing conditions. The resulting ~15 nm diameter nanoparticles produce an intense, broad luminescence emission centred at 550 nm. These Man-QDs induce luminescent aggregates of *Escherichia coli* which can be used to detect bacteria in cell suspensions containing as few as 10^4 *E. coli* per mL. The aggregation process is dependent on the *E. coli* cell surface FimH mannose-specific lectin. The recognition and subsequent detection of the *E. coli* using the Man-QD has been shown to be specific as aggregation does not occur either with an *E. coli* strain defective in the FimH lectin or with galactose-coated QDs.

1. Introduction

Numerous human pathogens use cell surface carbohydrates as receptors to facilitate cell adhesion. Many examples have been described, for instance: *Escherichia coli* binds to host mannosides while the influenza virus binds to host sialic acids.[1] By virtue of the copy number of both the carbohydrate-binding protein on the pathogen and the cognate glycan ligand on host cells, such interactions are multivalent, which imparts higher binding avidity than simple monovalent interactions.[2] Not surprisingly, therefore, methods for the detection of bacteria that exploit this effect have been developed. For instance, such sensor systems have employed fluorescent,[3] optically[4] or thermally responsive[5] synthetic glycopolymers. Magnetic glyconanoparticles[6] have been used to specifically detect the FimH mannose-specific lectin present in *E. coli* pili,[7] as have carbohydrate-coated quartz crystal microbalance biosensors.[8] The FimH lectin has also been targeted with mannose-coated gold nanoparticles, albeit in imaging mode with transmission electron microscopy.[9] The design and application of sensors based on nanoparticles and quantum dots have been reviewed,[10] as has the use of carbohydrate-coated (glyco)nanoparticles.[11] In a 'Homeland security' context, there is a need for cheap, fast sensor systems[12] for the detection of a number of agents, including a range of bacterial species.[13] Given the potential health risks, and the challenges of designing quantitative, species-specific devices, qualitative or semi-quantitative sensors would be useful. Previously, we have successfully developed colorimetric assays based on the aggregation of metal nanoparticles for the detection of lectins, such as concanavalin A,[14] cholera toxin[15] and RCA_{120}/ricin.[16] In the current study, we have a particular interest in devising a bacterial detection system that is cheap and straightforward to prepare, that could be used for rapid, qualitative analyses, and that is sufficiently robust for potential field use. We therefore considered approaches based around intensely luminescent semiconductor quantum dots (QDs).[17] The use of sugar-coated[18] and glycopolymer-coated[19] quantum dots for soluble lectin detection has been reported, as has the use of CdSe/ZnS core–shell QDs for luminescence imaging of the location of lectins on the surface of sea urchin sperm.[20] However, to the best of our knowledge, the use of carbohydrate-coated QDs for bacterial detection has not previously been reported. The cost of commercial QDs can be prohibitive and the technical complexity of their preparation can put off all but the expert synthetic materials chemist. Recently, de la Fuente and Penadés have developed a low-tech aqueous self-assembly procedure for the preparation of glyco-QDs.[21] Herein, we demonstrate the synthesis and application of this type of self-assembly procedure for the facile generation of mannose-conjugated QDs (Man-QDs). Whilst synthetically simple, such functionalisation provides for a multivalent display of carbohydrates on the QD surface. The Man-QDs have been used for the selective detection of an *E. coli* strain expressing the cell surface mannose-specific lectin FimH.[7]

The simple one-pot aqueous self-assembly procedure for the preparation of the Man-QD (**1**) is shown in Scheme 1. The carbohy-

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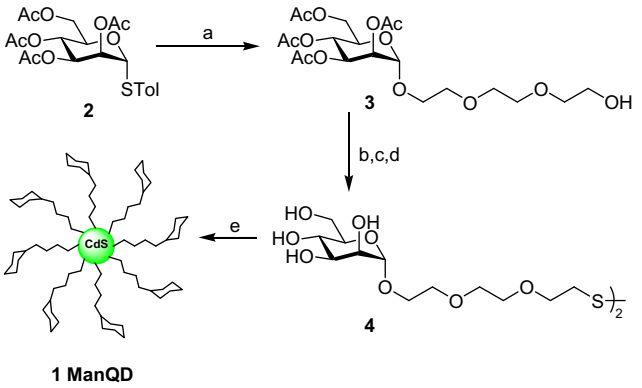

Scheme 1. Synthesis of Man-QD (**1**). Reagents and conditions: (a) triethylene glycol, NIS, TfOH, CH_2Cl_2, 65%. (b) MsCl, Py, 98%. (c) KSAc, 2-butanone, reflux, 96%. (d) NaOMe, MeOH, 98%. (e) $Cd(NO_3)_2{\cdot}4H_2O$, Na_2S, H_2O, pH 10 (NaOH), 30 min, rt.

drate-functionalised quantum dots prepared in this manner exhibited an average diameter of ca. 15 nm, as determined by transmission electron microscopy (Fig. 1A) and dynamic light scattering measurements (supporting information). Conjugation of the mannosyl-thiol ligand to the QD surface was confirmed by ^{1}H NMR spectroscopy, which showed a profile for the Man-QD (**1**) that was similar to that of the precursor disulfide, but broadened by virtue of the longer relaxation time of the nanoparticle construct (Supplementary data).

The Man-QDs absorb light at 360–380 nm, giving rise to a broad luminescence emission centred around 550 nm (180 nm, full width at half height) (Fig. 1B). Similar broad emission has previously been reported for quantum dots prepared in this fashion.[22]

Two strains of *E. coli*, namely ORN178 and ORN208, that differ in their mannose-binding properties were used to assess whether Man-QDs can bind to the cell surface mannose-specific adhesin FimH.[7] The ORN178 strain expresses wild-type type 1 pili, whereas ORN208 is mutated in the fimH gene such that it expresses pili that fail to bind mannose. The two bacterial strains were incubated separately with Man-QDs in PBS at 25 °C, and the binding was visualised by confocal fluorescence microscopy. Luminescent aggregates of the order of 100 μm across were found with ORN178 (Fig. 2A), while only individual cells were found in the case of ORN208 (Fig. 2B), confirming specific binding of Man-QD (**1**) to the *E. coli* expressing functional FimH. When similar experiments were performed with Gal-QD (galactose is not a FimH ligand), neither ORN178 nor ORN208 showed aggregation confirming that the Man-QD specifically binds to the FimH adhesin on the *E. coli* bacterium.

To determine the sensitivity of the Man-QD, serially diluted suspensions of mannose-binding *E. coli* ORN178 were incubated separately with Man-QDs. A wash step was used to remove unbound Man-QDs from QD-bacterial aggregates, and the resuspended

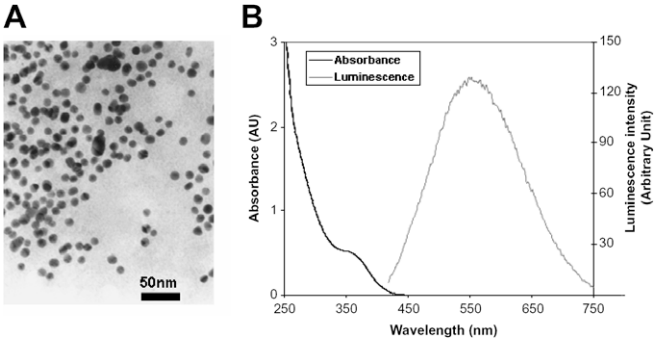

Figure 1. (A) Transmission electron microscopy image and (B) absorption/luminescence emission spectra for the Man-QD (**1**) (50 μM; λ_{ex} 365 nm).

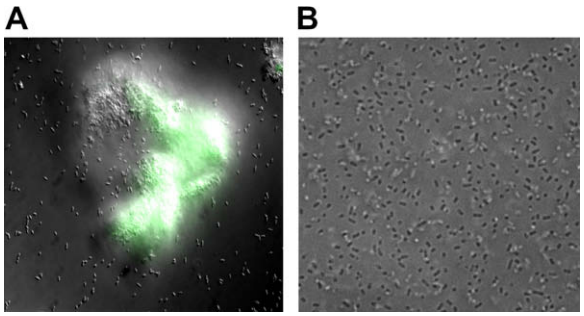

Figure 2. Confocal fluorescence microscopy images of (A) bacterial aggregates due to Man-QD-mediated aggregation of *E. coli* ORN178 (10^8 cells/mL) and (B) the same experiment, but with *E. coli* ORN208, showing no aggregation.

aggregates were imaged using the confocal fluorescence microscope. The images obtained are shown in Figure 3. Luminescent aggregates were observed with as few as 104 bacteria per mL whilst the size of the bacteria-QD aggregate decreased with decreasing bacterial load. The soluble FimH ligand D-mannose could be used to inhibit the bacteria-QD aggregation process: whilst no effect was seen for D-mannose up to 10 μM concentration, 10 mM D-mannose was required for complete inhibition of aggregation.

In summary, we have demonstrated the utility of a simple aqueous one-pot self-assembly procedure for the generation of intensely luminescent, mannose-coated CdS quantum dots. Particles prepared in this manner have been used for the detection of *E. coli* expressing functional FimH mannose-specific lectin. Even without optimisation of ligand presentation, as few as 10^4 *E. coli* per mL can be detected, which is competitive with more elaborate detection systems (vide supra). Further work is required to understand and exploit the impact of ligand presentation on bacteria recognition. The potential in addressing this point has been amply demonstrated in work on optimising soluble lectin recognition through changes in glycan density, linker composition and linker length.[14b,16,23] The simplicity of the glyco-QD preparation reported herein offers scope for targeting other glycan–lectin interactions in order to extend the repertoire of bacteria that can be detected using this system; such studies are ongoing.

2. Experimental

2.1. Reagents

$Cd(NO_3)_2{\cdot}4H_2O$, Na_2S and triethylene glycol were purchased from Sigma–Aldrich (Gillingham, UK). All aqueous solutions were prepared using analytical reagent grade water purchased from Fisher Scientific UK Ltd (Loughborough, UK). *E. coli* strains ORN178 and ORN 208 (Ref. 7a) were kindly provided by Professor P. E. Orndorff, North Carolina State University, United States.

2.2. Preparation of Man-QD (1)

The known mannosylated disulfide (**4**)[16] was prepared as outlined in Scheme 1. Briefly, the thioglycoside **2**[24] was used to mono-glycosylate triethylene glycol in the presence of NIS/TfOH.[25] The resulting alcohol was then converted via the mesylate to the corresponding thioacetate derivative, which was deprotected to give mannosylated disulfide **4**. The preparation of Man-QD (**1**) employed the previously reported method[21] for the preparation of related glyco-QDs (Scheme 1). A solution of $Cd(NO_3)_2{\cdot}4H_2O$ (9.3 mg, 0.03 mmol) and mannosylated disulfide **4** (26 mg, 0.04 mmol) in degassed (argon) water (50 mL) was adjusted to a pH of 10 with 0.1 M NaOH. A solution of Na_2S (2.4 mg, 0.03 mmol) in degassed

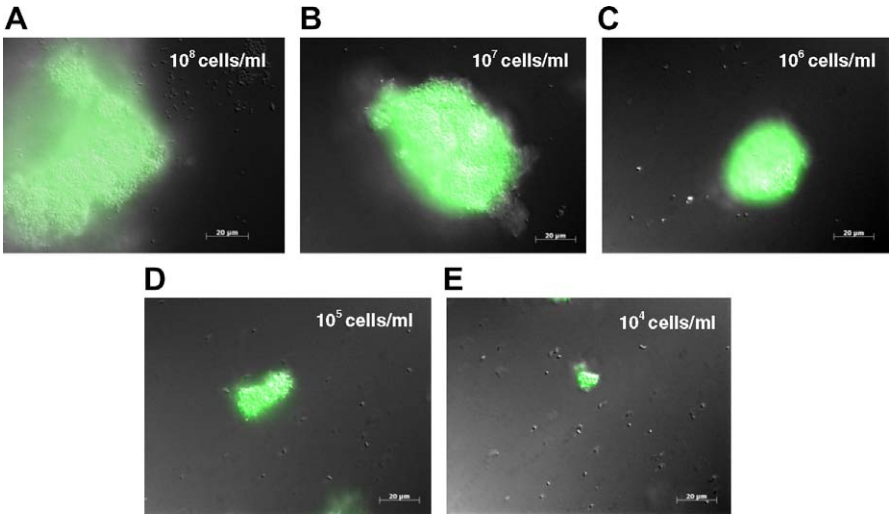

Figure 3. Confocal fluorescence microscopy images of Man-QD-mediated aggregation of *E. coli* ORN178 following serial dilution of bacterial load (A–E).

water (5 mL) was added drop-wise at room temperature under a continuous flow of argon with vigorous stirring. The stirring was continued for 30 min while maintaining the pH at 10 to yield a yellow-orange suspension. The glyco-QDs were purified by repeated centrifugal filtration (10,000*g*) using a Microcon spin filter (m.w. cut-off 30,000). The process was repeated 3 times by washing with Tris buffer (100 mM, pH 8.8) to remove any unbound sugar derivative. The resulting residue was resuspended in water (2 mL) and lyophilised to give a yellow-orange solid. This solid was then resuspended in phosphate-buffered saline (PBS; 20 mL) and was used as a stock solution for analyses. A protocol similar to that used to prepare α-linked Man-QD (**1**) was also used to prepare β-linked galactose-QD (Gal-QD) for use as a control in binding studies (see Ref. 16a for the synthesis of the *galacto*-isomer of disulfide **4**).

2.3. Bacterial detection experiments

E. coli ORN178 and ORN208 were grown overnight at 37 °C in LB medium in order to attain an optical density measured at 600 nm (OD_{600}) of approximately 1.0 ($\sim 10^8$ cells/mL). The culture was centrifuged at 30,000*g* for 20 min, washed with PBS buffer and spun down twice, and finally suspended in PBS. Aliquots of the Man-QD stock solution (0.5 mL each) were mixed with aliquots of 10-fold serial dilution of bacterial cells (1 mL each) in PBS containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM). The cell suspensions were incubated at room temperature for 30 min with gentle shaking. After incubation, the mixture was centrifuged to pellet the QD-cell aggregates, the supernatant was discarded and the pellet was resuspended in the same buffer. The process was repeated twice to remove all unbound Man-QD from the mixture (visual inspection of trial experiments demonstrated that this type of processing did not disrupt the QD-cell aggregates). Finally, the pellet was suspended in PBS and was visualised using a UV light box. For confocal fluorescence microscopy studies, a drop of the cell suspension was spotted onto a microscope slide and fixed with a drop of cold ethanol at 4 °C overnight prior to analysis.

NMR data showing soluble and QD-immobilised glycan; DLS data for determination of size and dispersity of Man-QDs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.12.029.

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