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Dextran-coated CdSe quantum dots for the optical detection of monosaccharides by resonance light-scattering technique

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A resonance light-scattering (RLS) detection method for saccharides was developed using dextran-coated CdSe quantum dots (dextran-CdSe-QDs) optical probes. The dextran-CdSe-QDs can be aggregated with concanavalin A (Con A), and the change in RLS intensity is used to monitor the extent of aggregation. The presence of glucose competitively binds with Con A, dissociating the Con A/dextran-CdSe-QDs complexes, affording the RLS intensity change and hence determining glucose concentrations in the range from a few to about 90 mM. Transmission electron microscopy was used to investigate the competitive interaction between glucose and dextran-CdSe-QDs with Con A. The competitive strategy could also be used to detect similar types of saccharides and the affinities of various monosaccharides for Con A increased in the order galactose $\ll$ glucose $<$ fructose $<$ mannose. The proposed method was successfully applied to determine glucose in the human serum.

Keywords: water-soluble quantum dots; dextran; concanavalin A; monosaccharide; resonance light scattering

Introduction

Owing to its advantages, such as high sensitivity and selectivity, good reproducibility, and easy performance with an ordinary spectrofluorimeter, resonance light-scattering (RLS) technique has become an important tool for biochemical analysis. RLS occurs when an incident beam is close in energy to an absorption band. Pasternack et al. (*1*, *2*) first used the RLS technique to study the biological macromolecules by means of a common fluorescence spectrometer. Later on, the method was widely used for the detection and determination of inorganic ions (*3*), pharmaceuticals (*4*, *5*), nucleic acid (*6*, *7*), proteins (*8*, *9*) and so on. Metal nanoparticles are known to enhance light scattering, which makes them well suited for use in biochemical analysis. The application was typically based on the analyte-induced aggregation events that result in measurable changes in the RLS signal. Colloidal gold and silver nanoparticles are the most widely utilised RLS probes (*10–14*).

Semiconductor quantum dots (QDs) are inorganic nanoparticles with unique photophysical properties, such as broad-absorption spectrum, size-controlled fluorescence, high-fluorescence quantum yield and good stability against photobleaching. These properties make them very attractive for many applications in different areas (e.g. medicine, biology, technology and, most recently, in analytical chemistry (*15–19*)). In recent years, water-soluble QDs have been extensively used as fluorescent probes of biologically important ions and molecules, and most of them are based on the fluorescence changes induced by the direct interaction between the analytes and the QDs surface (*19*). Exhibiting unique light-scattering signals, QDs are also likely to be used as effective light-scattering probes just like gold nanoparticles. Compared to the fluorescence measurements, the RLS detection has no blinking inherent to QDs. However, very few reports of chemical sensing of small molecules and biomolecules with QDs, via analyte-induced RLS changes, have been reported so far (*20*, *21*). Here, we report the use of water-soluble CdSe QDs as a new kind of RLS probes for the detection of monosaccharides.

It was previously reported that Con A, a jack-bean globulin, contains sites that can bind with sugars such as α-D-mannose and α-D-glucose. A large number of sensors have been developed based on this principle (*22–25*). Recently, dextran-modified metal nanoparticles have been used for the detection of glucose (*11*, *26*). These methods were based on the absorption or scattering changes induced by the competitive glucose binding to the Con A/nanoparticle–dextran complexes. Thiol-related molecules, including thiolated carbohydrate and mercapto-containing bifunctional ligands, were previously used to prepare the carbohydrate-conjugated nanoparticles. However, the thiol-based methods have some limitations and disadvantages, such as difficulty in preparation and instability. In a recent report (*27*), a novel

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dextran-functionalisation scheme for various nanoparticles and QDs was developed. Moreover, the method was based on the disuccinimidyl carbonate-mediated dextran conjugation to amine-functionalised nanoparticles. Herein, we present a convenient method for the preparation of water-soluble dextran-coated CdSe quantum dots (dextran-CdSe-QDs) by partially replacing the original oleic acid (Ole) chains on the CdSe QDs with dextran. Based on the dissociation of Con A-aggregated dextran-coated CdSe QDs, we have developed a simple and efficient RLS method for the detection of glucose as well as other similar monosaccharides. The proposed method was applied to the determination of glucose in human serum samples with satisfactory results.

Experimental

Reagents

Cadmium oxide (99.99%), selenium (powder, 100 mesh, 99.99%), octadecane, Ole and anhydrous methanol were obtained from Aldrich (Milwaukee, WI, USA). 4-(2-Hydroxyethyl)-1-piperazine ethanesulphonic acid (HEPES) was purchased from Alfa Aesar (Karlsruhe, Germany). Dextran (MW = 70,000) was obtained from Pharmacia Fine Chemicals Co. (Beijing, China) and concanavalin A (Con A) from Sigma (St Louis, MO, USA). Monosaccharides (mannose, glucose, galactose and fructose) and all other chemicals were of analytical grade and used without further purification. All four sugars were dissolved in doubly distilled water. The Con A solution was prepared in phosphate-buffered saline (PBS), pH 7.4.

Apparatus

All the fluorescence and RLS measurements were carried out on a LS-55 luminescence spectrometer (Perkin-Elmer Co., Waltham, MA, USA). Fluorescence-emission spectra were measured at an excitation wavelength of 390 nm. The excitation and emission slits were set at 10 and 15 nm, respectively. RLS spectra were obtained by scanning simultaneously the excitation and emission monochromators from 200 to 700 nm, with $\Delta\lambda = 0$ nm. Both the excitation and emission slit widths were set at 15 nm. The RLS intensities were measured at 390 nm. Fourier transform infrared spectra (FT-IR) were obtained with a Shimadzu 8400S infrared spectrometer (Shimadzu, Kyoto, Japan). Transmission electron microscopy (TEM) images were acquired on a JEM-2010 transmission electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 200 kV. Specimens for TEM were prepared by placing a drop of the suspension onto carbon-coated formvar films on copper grids. The grids were dried completely before imaging.

Synthesis of dextran-CdSe-QDs

Nearly monodispersed Ole-stabilised CdSe QDs (Ole-CdSe-QDs) were synthesised following the procedure that we already described (*28*). After purification by methanol precipitation, centrifugation and decantation, the synthesised Ole-CdSe-QDs were redispersed in hexane.

The CdSe QDs were then modified with dextran as follows: dextran was dissolved in doubly distilled water and 0.1 M NaOH was added to adjust the solution pH between 8 and 9. A 2 ml aliquot of as-prepared aqueous solution of dextran was mixed with 1 ml of CdSe QDs suspension dispersed in hexane, and then the mixture was stirred for 24 h at room temperature. The resulting colloidal solutions of QDs were centrifuged at 10,000 rpm for 15 min to remove the free dextran. After discarding the supernatant, the precipitates of the modified nanoparticles were resuspended in water, and centrifuged for two more circles to obtain water-soluble dextran-CdSe-QDs. The aqueous solution of QDs was found to be stable for at least one month when stored at 4°C in the dark.

General procedure

All photoluminescence and light-scattering measurements were performed in 0.01 M HEPES buffer, pH 7.4. An aliquot of the dextran-CdSe-QDs working solution was pipetted into a 1 × 1 cm quartz cell and the titration was carried out by successive injections of Con A and glucose into the cell. Before making measurements, the mixture was incubated at room temperature for about 5 min to allow complexation of dextran-CdSe-QDs with Con A or disaggregation of QDs–dextran/Con A complexes by glucose. Fructose, galactose and mannose were also tested in a similar manner.

Human serum sample preparation

Human serum samples were obtained from the Second Affiliated Hospital of Shanxi Medical University (Taiyuan, China). The sample preparation procedure is as follows: 5.0 ml aliquots of serum samples were deproteinised with 2 volumes of acetonitrile. After centrifugation (16,000 rpm for 30 min), the supernatant was evaporated to remove acetonitrile. The deproteinised serum samples were then used for glucose determination.

Results and discussion

Characterisation of dextran-CdSe-QDs

The formation of dextran-CdSe-QDs was confirmed by FT-IR spectroscopy. Figure 1(A) and (C) shows the FT-IR spectra of neat dextran and dextran-coated CdSe QDs, respectively. Both spectra exhibit very similar peak shape

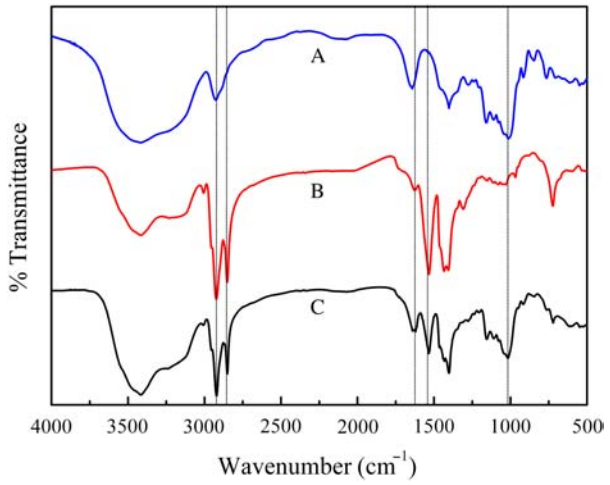

Figure 1. FT-IR spectra of (A) neat dextran, (B) Ole-modified CdSe QDs and (C) dextran-coated CdSe QDs.

and positions in the 1200–761 cm^{-1} region. The strong band at 3413 cm^{-1} (O—H group stretching vibration) plus those 1155, 1110 and 1018 cm^{-1} in Figure1(C), which are the characteristic absorption peaks of dextran (*29*, *30*), clearly indicate the formation of the dextran–QDs complex. It should be noted that the FT-IR spectrum of dextran-CdSe-QDs also shows the characteristic bands of Ole (the carbonyl peak at 1533 cm^{-1} and the intense adsorption peaks at 2921 and 2852 cm^{-1} attributed to the alkyl chain), indicating that Ole ligands still exist at the surface of the dextran-coated CdSe QDs. We may therefore suppose that dextran partially replaced Ole on the QDs surface by donating its non-bonding electron pairs on the oxygen atoms to coordinate with the Cd^{2+} ion and the hydrophilic hydroxyl groups render QDs water-soluble. The dextran-CdSe-QDs complex is illustrated schematically in Figure 2.

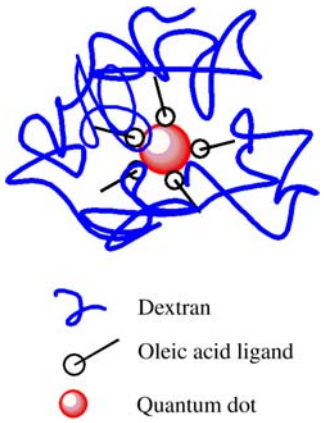

Figure 2. Schematic representation of the dextran-coated CdSe QDs.

The synthesised QDs were characterised and analysed by TEM. Nearly monodispersed spherical Ole-CdSe-QDs are produced with an average diameter of 5 nm (*31*). An obvious aggregation of nanoparticles occurred after the formation of dextran-CdSe-QDs conjugates. As presented in Figure 3(A), the dextran-CdSe-QDs self-assembled to form a spherical network structure, having an average aggregate diameter of c. 50 nm. The TEM image also indicated a clear boundary between individual QDs, that is, well-separated QDs are clearly observed. We suppose that dextran containing hydroxyl groups can self-associate and form an intermolecular interaction, which induced the aggregation of QDs nanoparticles. The aggregation, however, does not induce precipitation of the particles, which is probably due to the existence of dextran-bridge-induced hydrophilic interactions. The good water solubility of dextran-CdSe-QDs makes them ideal for certain bioassays.

Further aggregation of dextran-CdSe-QDs induced by Con A

As previously reported, Con A molecule contains binding sites for dextran (*32*) and can cause aggregation of dextran-conjugated nanoparticles including QDs (*11*, *26*, *27*). Figure 4 shows the fluorescence spectra of dextran-CdSe-QDs before and after the addition of Con A. It can be seen that dextran-CdSe-QDs exhibited a fluorescent band centred at 595 nm with a non-zero tail towards longer wavelength, suggesting the presence of surface-energy traps. This characteristic fluorescence emission is generally attributed to the recombination of the charge carriers immobilised in traps of different energies (*33*, *34*). Apart from the non-zero tail, the fluorescence spectrum of dextran-CdSe-QDs also shows a non-zero start towards shorter wavelength. This indicates, in agreement with the TEM result, that CdSe QDs aggregated after the modification with dextran. The addition of Con A led to a red shift of the peak position (c. 6 nm), a broadening of emission band accompanied by an increase of emission intensity. The observed fluorescence changes indicated the aggregation of dextran-CdSe-QDs nanoparticles to larger aggregates promoted by Con A, which can link with dextran molecules on the surface of QDs.

The interaction between dextran-CdSe-QDs and Con A was further investigated by monitoring the RLS spectra. Figure 5 shows the RLS spectra of Con A and dextran-CdSe-QDs in the absence and presence of Con A. As can be seen from Figure 5, the main RLS spectral peaks of the QDs are at 390 and 448 nm with a maximum peak located at 390 nm. It is clearly seen that Con A has a rather weak RLS signal, even if its concentration is 1 μM, but can enhance the RLS signal of the QDs. As the RLS intensity is related to the aggregate particle size, the enhanced RLS

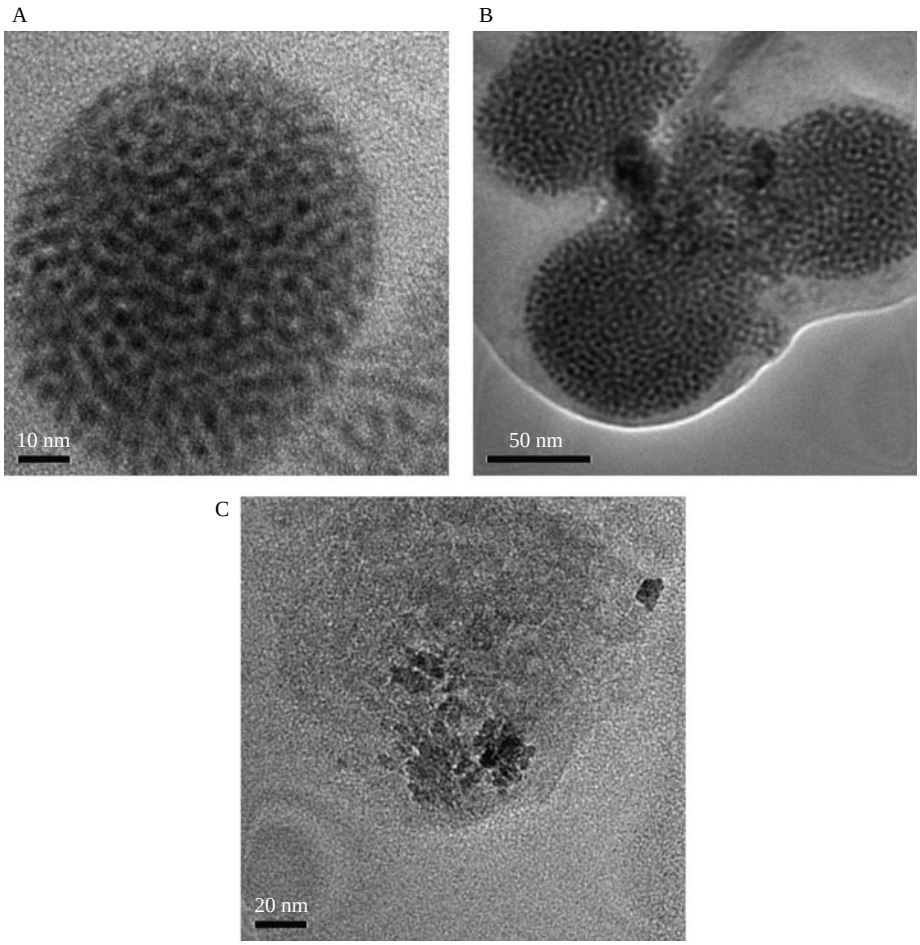

Figure 3. TEM micrographs of (A) dextran-CdSe-QDs, (B) Con A-induced aggregation of dextran-CdSe-QDs and (C) dissociation of Con A/dextran-CdSe-QDs complexes by glucose.

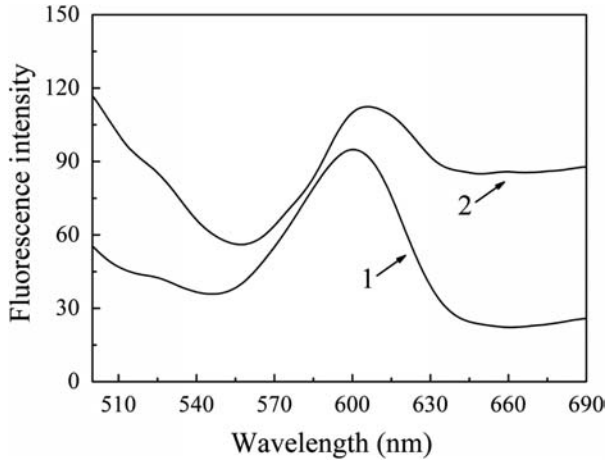

Figure 4. Fluorescence spectra of dextran-CdSe-QDs (1) before and (2) after the addition of Con A: [dextran-CdSe-QDs] = 78 nM; [Con A] = 3.7 μM.

signal of the system reflects an interaction between QDs and Con A, i.e. aggregation of dextran-CdSe-QDs could be induced by Con A. Again, it was found that the aggregation was dependent on the Con A concentration. As shown in Figure 6, the RLS intensity increased with the increase in the concentration of Con A. We suppose that the observed RLS enhancement was the result of aggregation of dextran-CdSe-QDs induced by Con A. This was further supported by TEM studies. Figure 3(B) clearly shows that further aggregation of dextran-CdSe-QDs occurs after the addition of Con A.

Dissociation of Con A/dextran-CdSe-QDs aggregates by glucose

Monosaccharides such as glucose, mannose and fructose have higher affinity to Con A than dextran (*32*). We therefore investigated the competitive interaction between glucose and dextran-CdSe-QDs with Con A by RLS and TEM.

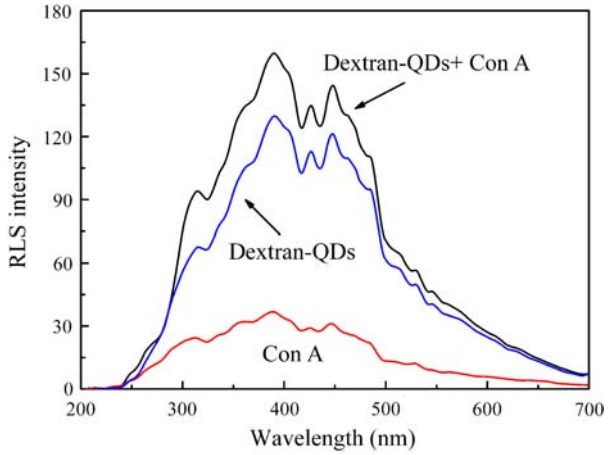

Figure 5. RLS spectra of Con A and dextran-CdSe-QDs in the absence and presence of Con A: [dextran-CdSe-QDs] = 31 nM; [Con A] = 1.0 μM.

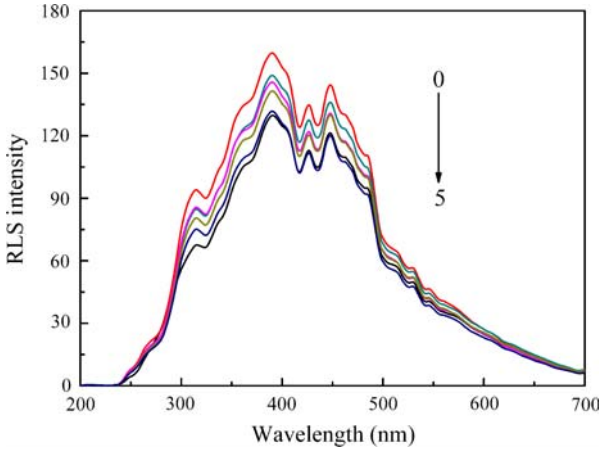

Figure 7. RLS spectra of Con A/dextran-CdSe-QDs upon titration with glucose. [dextran-CdSe-QDs] = 31 nM; [Con A] = 1.0 μM; [glucose]/mM: (0–5) 0, 10, 20, 30, 60 and 95.

Figure 7 shows the RLS spectra of Con A/dextran-CdSe-QDs aggregates in the presence of glucose. The observed decrease in RLS intensity indicated that the Con A/dextran-CdSe-QDs complexes are partially dissociated into smaller particles in the presence of glucose, which was also verified by the TEM image (Figure 3(C)). As shown in Figure 8, the RLS intensity decreased sharply with the addition of glucose, and this change became slow and tended to reach a plateau when the concentration of glucose was more than 30 mM, indicating the saturation of Con A binding sites with the addition of glucose. The RLS intensity change observed can be used for the determination of glucose. It should be noted that the RLS response of the system was much more sensitive to the change in glucose concentration than to fluorescence. In fact, only a ~10% decrease in fluorescence intensity with 60 mM glucose was observed, whereas a ~ 6% change in the RLS signal was observed with just 10 mM glucose.

As a blank control, the RLS response of the Con A solution to glucose was measured in the absence of dextran-CdSe-QDs. As can be seen from Figure 9, the RLS response of the Con A solution at the maximum peak of 390 nm was insensitive to the glucose concentration over a wide range. Hence, the observed decrease in RLS intensity in the presence of dextran-CdSe-QDs could be attributed to the dissociation of Con A/dextran-CdSe-QDs complexes by glucose.

The RLS response of Con A/dextran-CdSe-QDs to the addition of glucose was measured at various concentrations of Con A. As can be seen in Figure 8, the extent of the RLS intensity change increased with the increasing Con A concentration. At the higher concentration of Con

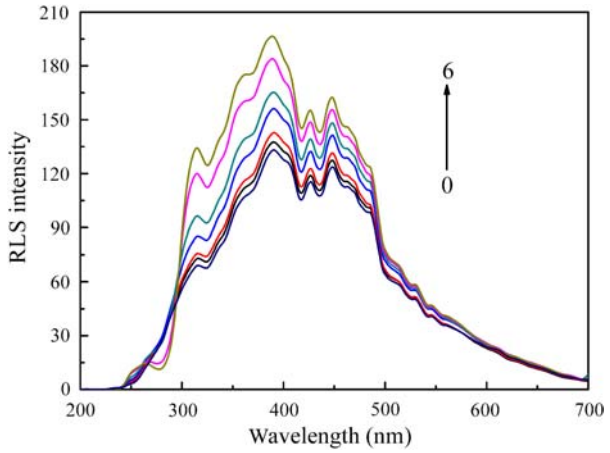

Figure 6. RLS spectra of dextran-CdSe-QDs with different concentrations of Con A. [dextran-CdSe-QDs] = 31 nM; [Con A]/μM: (0–6) 0, 0.10, 0.23, 0.46, 1.0, 2.8 and 3.7.

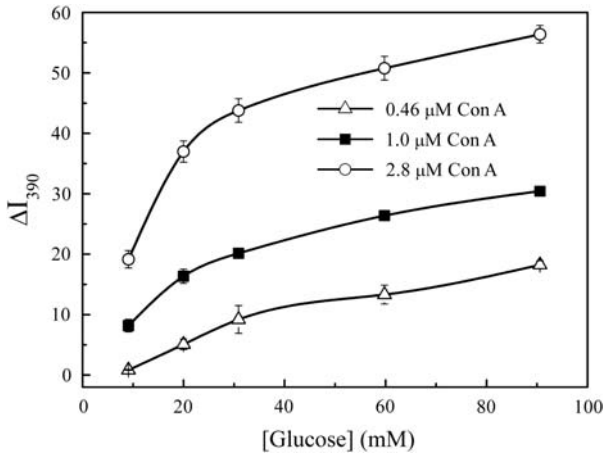

Figure 8. Change in RLS intensity of Con A/dextran-CdSe-QDs at the maximum peak of 390 nm vs. the concentration of glucose. [dextran-CdSe-QDs] = 31 nM; [glucose]/mM: (1–5) 10, 20, 30, 60 and 95.

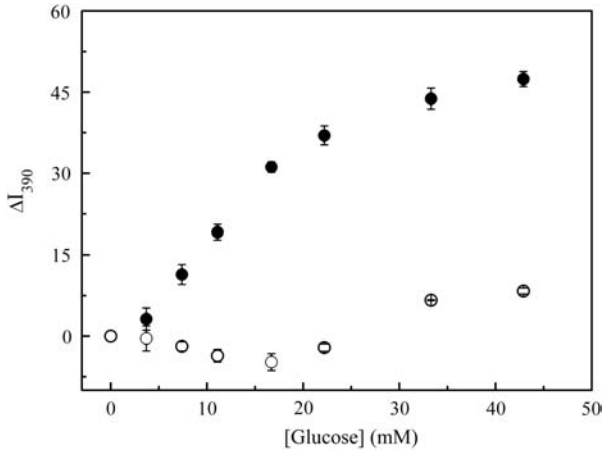

Figure 9. Change in RLS intensity of Con A or Con A/dextran-CdSe-QDs at the maximum peak of 390 nm vs. the concentration of glucose. [dextran-CdSe-QDs] = 31 nM; [glucose]/mM: (0–7) 0, 3.7, 7.4, 11.1, 16.7, 22.2, 33.3, 42.9. ●: dextran-QDs + 2.8 μM Con A; ○: 2.8 μM Con A.

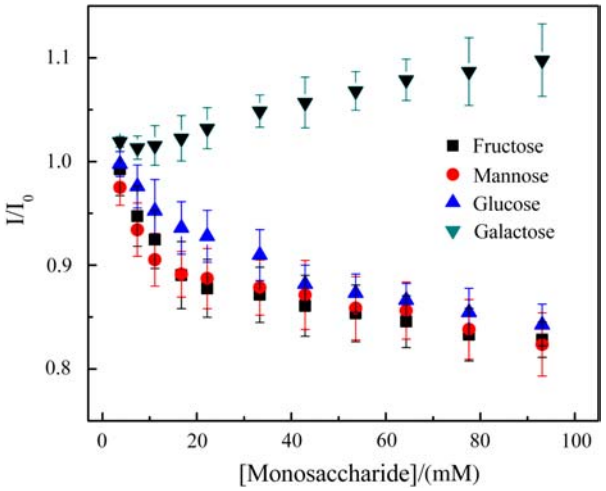

Figure 10. Change in RLS intensity of Con A/dextran-CdSe-QDs upon titration with monosaccharides. [Con A] = 1.0 μM; [monosaccharide]/mM: (0–11) 0, 3.7, 7.4, 11.1, 16.7, 22.2, 33.3, 42.9, 53.6, 64.3, 77.6, 93.1; [dextran-CdSe-QDs] = 31 nM.

A, however, precipitation could occur due to the specific binding of Con A with dextran (*32*). The huge aggregates will result in a loss of stability of colloidal dextran-coated nanoparticles. Therefore, a suitable Con A concentration of 1.0 μM was chosen for further studies.

A quantitative analysis of the RLS data was made as a function of the glucose concentration. The decreased RLS signal of the Con A/dextran-CdSe-QDs system was represented as $\Delta I_{390} = I_0 - I$, in which I_0 and I are the RLS intensities at the maximum peak of 390 nm in the absence and presence of glucose, respectively. The obtained experimental data could be well fitted to the following equation (*26*):

$$\Delta I_{390} = \Delta I_{390(\text{final})} \times (1 - e^{-k[\text{sugar}]}), \qquad (1)$$

where $\Delta I_{390(\text{final})}$ is the maximum scattering intensity change at 390 nm, and k is the rate of scattering intensity change, which is thought proportional to the rate of dissociation of the Con A/dextran-CdSe-QDs aggregates.

The kinetic data obtained are listed in Table 1. It shows that k increased with the increase in Con A concentration, indicating that the dissociation rate of the Con A/dextran-CdSe-QDs aggregates with the addition of glucose depended largely on the concentration of Con A. Increasing the concentration of Con A could increase binding sites for both dextran and glucose, and result in significant aggregation and dissociation of the particles.

Table 1. Kinetic parameters obtained from the plots given in Figure 8.

Con A (μM)	$\Delta I_{390(\text{final})}$	k_1 (s^{-1})	r^2
0.46	23.1	0.0184	0.9991
1.0	31.5	0.0316	0.9934
2.8	55.7	0.0537	0.9915

Effect of other monosaccharides

The other three monosaccharides, including mannose, galactose and fructose, were also tested in the competition assays. As shown in Figure 10, mannose and fructose exhibited RLS responses similar to that of glucose, indicating that both sugars have comparable binding affinities for Con A as with glucose. Therefore, the competitive strategy could also be used to detect these similar types of saccharides. The addition of galactose, however, did not induce any decrease in RLS intensity of the Con A/dextran-CdSe-QDs aggregate. On the contrary, the RLS intensity of the Con A/dextran-CdSe-QDs system increased slightly with the addition of galactose. Previous studies have indicated that the hydroxyl groups at the C-3, C-4 and C-6 positions of the monosaccharides are essential for binding to the active sites of Con A (*32*). Galactose is the C-4 epimer of glucose. So, compared to glucose, galactose cannot fit into the Con A binding sites as the hydroxyl group on its C-4 is axial. Although the C-2 hydroxyl group is not essential for binding, it may also participate in the binding of saccharides to Con A. Therefore, the observed slight increase

Table 2. Kinetic parameters obtained from the plots given in Figure 10.

Monosaccharides	$\Delta I_{390(\text{final})}$	k (s^{-1})	r^2
Glucose	36.1	0.0293	0.9960
Mannose	32.8	0.0663	0.9532
Fructose	37.3	0.0568	0.9472
Galactose	–	–	–

Table 3. Effect of coexisting substances on the determination of glucose (glucose = 7.5 mM).

Coexisting substance	Concentration ($mol l^{-1}$)	Change in RLS intensity (%)	Coexisting substance	Concentration ($mol l^{-1}$)	Change in RLS intensity (%)
Ca^{2+}	1.0×10^{-2}	3.1	Fe^{3+}	2.0×10^{-6}	5.3
Mg^{2+}	1.0×10^{-4}	2.3	Cu^{2+}	1.0×10^{-5}	6.1
Al^{3+}	6.0×10^{-5}	−1.5	Uric acid	1.0×10^{-5}	2.8
K^{+}	1.0×10^{-3}	2.7	L-Ascorbic acid	1.0×10^{-5}	2.5
Na^{+}	1.0×10^{-3}	3.0	L-Cysteine	2.0×10^{-5}	4.7
Zn^{2+}	5.0×10^{-5}	−1.2	L-Lysine	1.0×10^{-5}	2.7
Co^{2+}	5.0×10^{-6}	4.2	Tyrosine	5.0×10^{-5}	−3.0

Table 4. Analytical results of glucose in human serum samples.

Samples	Original[a] (mM)	Added (mM)	Found (mM)	Recovery (%)	RSD (%, $n = 5$)
1	4.43	5.50	10.19 ± 0.33	104.7	3.2
2	3.85	5.50	9.60 ± 0.26	104.5	2.7
3	4.51	5.50	9.79 ± 0.36	96.0	3.7

[a] Measured value with the biochemical analyser.

in scattering intensity suggested that galactose could be weakly adsorbed to the surface of Con A rather than occupy the Con A binding sites competitively.

For mannose and fructose, the experimental data were also well fitted to Equation (1). As shown in Table 2, the binding affinities deduced from the magnitudes of the RLS response increased in the order galactose ≪ glucose < fructose < mannose, which are in agreement with the findings from previous reports in different measuring systems (*32*, *35*).

Interference analysis and determination of glucose in serum samples

The influence of some coexisting substances such as metal ions, amino acids and ascorbic acid was tested at a fixed glucose concentration of 7.5 mM. As shown in Table 3, all the tested metal ions and small organic molecules can be allowed at relatively high concentrations with a tolerance level of 10%, indicating that the present method has good selectivity.

To check the validity of the proposed method, it was applied to the analysis of glucose in human serum samples. Accuracy was assessed by investigating the recovery of glucose spiked into three different serum samples (five replicates for each sample) (Table 4). The average recovery ranged from 96.0 to 104.7% with RSD less than 3.7%, indicating good accuracy and precision. From these results, it seems that the proposed method is applicable to the determination of glucose in human serum samples.

Conclusions

In summary, we have succeeded in developing water-soluble dextran-coated CdSe QDs as sensitive RLS probes for the detection of monosaccharides. The dextran-CdSe-QDs were coupled to Con A to facilitate the aggregation of nanoparticles and the Con A/dextran-CdSe-QDs complexes were dissociated by a competitive complexation of monosaccharides. The resulting RLS changes can be used to detect these monosaccharides. The proposed method has been successfully used for the determination of glucose in the human serum samples, showing great promise in monitoring blood glucose levels.

Acknowledgements

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