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ORIGINAL PAPER

Luminescent Detection of ATP in Aqueous Solution Using Positively Charged CdSe–ZnS Quantum Dots

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Abstract Commercially available CdSe–ZnS Quantum Dots (QDs) have been modified by exchanging the hydrophobic surface ligands with (2-mercaptoethyl)-trimethylammonium chloride. The resulting water soluble conjugate was titrated with solutions of adenosine triphosphate (ATP), adenosine diphosphate, adenosine monophosphate, guanosine triphosphate (GTP), guanosine diphosphate and guanosine monophosphate in 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.4). A strong fluorescence quench of about 80% was observed for ATP, a quench of 25% was observed for GTP while the others had virtually no effect. The quenching effect of ATP and GTP was attributed to the high negative charge density associated with these substrate's resulting in a strong attraction to the QD surface enabling them to engage in electron transfer with the excited QD. The lack of fluorescence quenching associated with the other nucleotides was most likely due to their reduced charge density resulting in a lower affinity for the QD surface.

Keywords Quantum dots · Fluorescence · ATP · Nucleotides

Introduction

Semiconducting nanocrystals, also known as Quantum Dots (QDs), have recently emerged as a new class of luminophore and offer many advantages over traditional organic dyes, such as broader absorption spectra, narrow size dependent emission spectra and a reduced susceptibility to photobleach [1–4]. Although commercially sold as fluorescent tags for biomolecules, QDs have struggled to compete with their organic counterparts in a sensing arena. Our research is focussed on utilising QDs as the signalling unit in fluorescent sensors. Previously, we have demonstrated that a CdSe/ZnS QD, surface functionalised with a thiourea receptor, was effective at measuring anion concentration, such as acetate, by a quenching of fluorescent intensity [5]. Thioureas are charge neutral entities that bind anions through strong hydrogen bonds but tend to perform poorly in aqueous solution. Other common organic dye based anion receptors include positively charged species such as guanidinium groups or polyamine-Zn^{2+} complexes that tend to be more effective in aqueous solution [6]. Here, we design a simple, water soluble sensor based on a QD fluorophore for the detection of adenosine triphosphate (ATP). A charged quaternary ammonium group was used not only as a receptor but also to ensure aqueous solubility and was coordinated to the surface of a CdSe/ZnS QD via a thiol group. The oxidisability of guanine and adenine is well known as they have the lowest oxidation potentials among the nucleic acid bases [7]. CdSe QDs can be oxidised and reduced at relatively moderate potentials and can thus participate in electron transfer processes in a similar manner to organic dyes [8]. Therefore, our present design attempts to discriminate between nucleotides based on differences in their surface charge, which in turn modulates their attraction to the surface of the QD. Electron transfer efficiency is known to be distance dependant [9] and so only those nucleotides that are attracted close to the surface of the QD can participate in effective electron transfer.

The fluorescent discrimination of nucleotides by targeting differences in their charge is one approach that has been investigated [10–12]. For example, Kanekiyo et al. developed a "two component" approach which required the initial addition of a polycation to the nucleotide solution to enable the attraction of ATP to the polymer surface. A boronic acid functionalised pyrene sensor was then added

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and formed a boronate ester with ATP at the polymer surface. This facilitated the self assembly of the pyrene units leading to a change from monomer to excimer emission upon binding. Addition of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) resulted in reduced excimer formation as they did not bind as strongly to the polycationic surface. Progressing from this, we have designed a one component system where the cationic surface is inherent within the sensor design. This is the first reported example of a QD based fluorescent sensor for ATP and offers potential benefits over organic dye based ATP sensors due to the superior optical properties QDs offer.

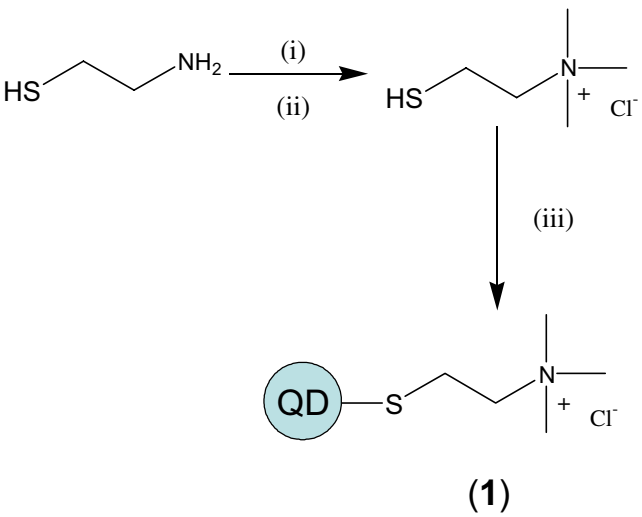
Experimental

Reagents and chemicals

All reagents were purchased from Aldrich at the highest quality available. CdSe-ZnS QDs were purchased from Evident technologies, New York (Product No. ED-C10-TOL-0545).

Synthesis of (2-mercaptoethyl)-trimethylammonium iodide

To a solution of 2-dimethylamino-ethanethiol (1 g, 9.52 mmol, 1 eq) in anhydrous acetonitrile (10 mL) was added iodomethane (5.4 g, 38 mmol, 4 eq). The reaction was allowed to stir at room temperature for 18 h after which time the product precipitated from solution. The product was collected by filtration, washed with cold acetonitrile and dried in vacuo at 50 °C. Yield=2.3 g, 49.7%. ^{1}H nuclear magnetic resonance (NMR; D_2O) δ ppm=3.62 (m, 2H, NCH_2), 3.08 (s, 9H, 3 x CH_3), 3.06 (m, 2H, HSCH_2). ^{13}C NMR (D_2O) δ ppm=66.2 (HSCH_2), 53.0 (CH_3), 29.7 (CH_2N).



Scheme 1 Synthesis of QD-Receptor *1* (*i*) methyl iodide, dry DCM, 18 h, 25 °C. (*ii*) amberlite ion exchange resin. (*iii*) CdSe/ZnS QDs, MeOH, pH 10.0, 6 h

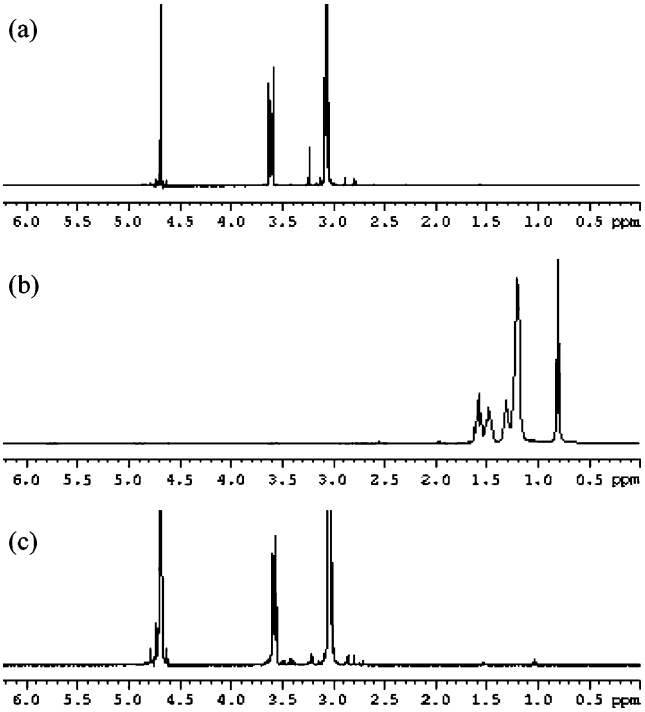


Fig. 1 ^{1}H NMR spectra of **a** receptor **b** CdSe/ZnS QDs and **c** QD-receptor conjugate. Spectra **a** and **c** were recorded in D_2O and spectrum **b** in $CHCl_3$, 400 MHz

Ion exchange

(2-Mercaptoethyl)-trimethylammonium iodide (1 g, 4.0 mmol) was dissolved in deionised water (100 mL). Amberlite resin (30 g) was paced into a separating funnel and washed with 2 M HCl (~200 mL). When all the HCl had drained from the resin, deionised water was flushed through until the pH had neutralised. The (2-mercaptoethyl)-trimethylammonium iodide iodide solution was washed through the resin a total of three times. The collected solution was concentrated to about one-half its original volume on a rotary evaporator. The product was then obtained by freeze drying. Yield=0.49 g, 77.7%.

Synthesis of *1*

To a solution of (2-mercaptoethyl)-trimethylammonium chloride (0.06 g, 386 μmol), dissolved in anhydrous methanol (45 mL) was added CdSe/ZnS QDs (2 mL, 108 nmol). The pH was adjusted to 10.5 with the aid of tetrabutylammonium hydroxide and the reaction heated under reflux for 5 h. After cooling, the solution was concentrated to about one third of its original volume and treated with diethyl ether (100 mL) to precipitate the product. The supernatant was carefully decanted off and the remaining solution centrifuged for 5 min at 13,500 rpm.

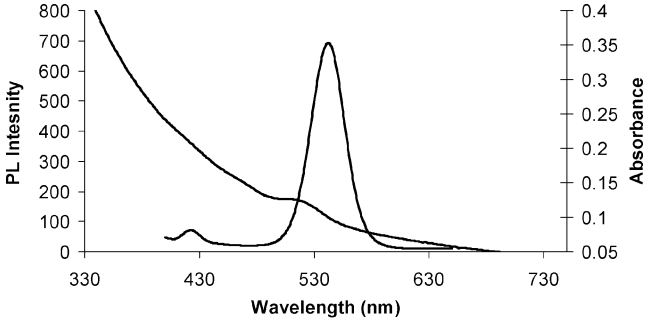


Fig. 2 Absorbance and fluorescence spectra of *1*. Fluorescence spectra recorded after excitation at 370 nm. [*1*]=1.0×10^{-5} M for absorbance measurements and 3.5×10^{-6} M for fluorescence measurements

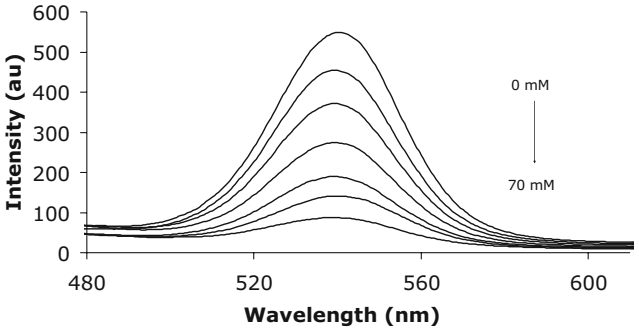


Fig. 4 Plot of fluorescence intensity against wavelength for *1* upon increasing addition of ATP. [1]=3.5×10^{-6} M, excitation wavelength= 370 nm

The pellet was redissolved in methanol, precipitated from ether and centrifuged a further two times. The resulting product was dried in vacuo for 18 h.

UV-vis and fluorescence analysis

Absorbance measurements were recorded on an Agilent UV-Vis Spectrometer using 10 mm quartz cuvettes. Fluorescence measurements were recorded on a Perkin Elmer LS55 Luminescence Spectrometer using 10 mm quartz cuvettes. Excitation wavelength unless otherwise stated was set at 370 nm. Excitation slit size was 10 nm and emission slit size was 10 nm. Scan speed was set at 500.

^{1}H NMR analysis

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer. Chemical shifts are reported downfield of trimethylsilane.

Zeta potential determination

Zeta potential measurements were recorded in aqueous solution at 25 °C on a Malvern NanoZS zetasizer calibrated against polystyrene latex.

Results and discussion

Synthesis of receptor ligand and incorporation onto CdSe–ZnS surface

The synthetic scheme adopted for the synthesis of the charged receptor ligand and its attachment to the surface of the QD is shown in Scheme 1. The receptor was synthesized in one step by the direct *N*-alkylation of 2-dimethylamino-ethanethiol with iodomethane. An aqueous solution of the receptor was then passed through an ion exchange resin to exchange the iodide for chloride to prevent potential quenching by the heavy atom effect. Surface attachment of the ligand to the QD surface was performed at pH 10 to ensure generation of the thiolate. The ^{1}H NMR spectra of the free ligand, the free QD and the QD-ligand conjugate (*1*) are shown in Fig. 1. The S-CH_2-protons of the receptor were observed at 3.08 ppm while the $-CH_2$–N– and N–$(CH_3)_3$ protons were observed together at 3.60 ppm (Fig. 1a). The spectrum of the free QD shows the methyl and methylene protons of trioctyl-phosphine oxide (TOPO), the strongly coordinating surfactant used in the synthesis of the core QD, at 0.8 and 1.2 ppm respectively (Fig. 1b). After the ligand exchange reaction, these signals disappear almost completely as

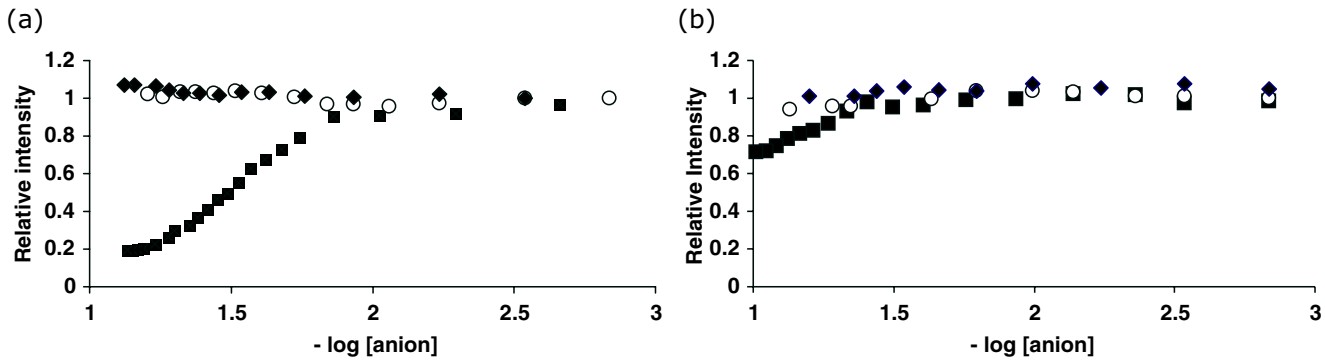


Fig. 3 Plot of relative fluorescence intensity against −log [anion] for **a** *1* titrated with AMP (*empty circle*), ADP (*filled diamond*) and ATP (*filled square*) and **b** *1* titrated with GMP (*empty circle*), GDP (*filled diamond*), GTP (*filled square*). [*1*]=3.5×10^{-6}, 0.1 M HEPES at pH 7.4, excitation wavelength=370 nm

shown in the spectrum of *1* (Fig. 1c). As expected however, the signals from the receptor remain and are relatively unaffected in terms of chemical shift. This confirms an almost complete exchange of the TOPO ligands for (2-mercaptoethyl)-trimethylammonium chloride. The zeta potential of an aqueous solution of the QD-receptor conjugate was +56 mV/mol, which was similar in magnitude but opposite in sign to a solution of QDs whose surface TOPO groups were exchanged for dihydrolipoic acid (zeta potential −61 mV/mol; Callan JF et al., unpublished work).

Photophysical properties of the QD-ligand conjugate

The UV-Vis and fluorescent spectra for *1* are shown in Fig. 2. The position of the first exciton peak, centred at 519 nm, enables calculation of the particle diameter which was found to be 2.55 nm [13]. When excited at 370 nm, *1* displayed an emission maxima centred at 542 nm, highlighting the large Stokes shifts possible with these nanoparticles.

Figure 3a shows a plot of relative fluorescence intensity against –log[anion] for ATP, ADP and AMP recorded in 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid solution at pH 7.4. Addition of ATP causes a 80% reduction of the original fluorescence intensity over about one log unit, while ADP and AMP has virtually no effect. The binding constant Log β for ATP, calculated from Eq. 1 was found to be 1.87 [14, 15].

$$-\log\ (F_{\mathrm{MAX}} - F)/F - F_{\mathrm{MIN}} = \log[\mathrm{Anion}] + \log\ \beta \qquad (1)$$

where F_{MAX} is the maximum fluorescence intensity, F_{MIN} is the minimum fluorescence intensity and F the observed fluorescence intensity.

Figure 3b shows a similar plot for guanosine triphosphate (GTP), guanosine diphosphate (GDP) and guanosine monophosphate (GMP). Again the mono- and di-phosphates produce virtually no quenching of the original fluorescence intensity. Surprisingly, addition of GTP resulted in only a 25% quench over the same concentration range as ATP. This quenching of fluorescence is most likely due to an electron transfer from the nucleotides to the excited QD, as has been observed before when other QD-ligand conjugates were exposed to electron rich analytes [16]. The reason for the enhanced quenching efficiency of ATP with respect to GTP is unclear and was not expected, as guanine has a lower oxidation potential than adenine (1.29 and 1.42 V vs normal hydrogen electrode respectively) [7]. The overall trend of increasing fluorescence intensity with decreased analyte charge suggests the extent of attraction between nanoparticle surface and analyte is responsible for modulation of the fluorescence output within each series of nucleotide.

A plot of fluorescence intensity of *1* against increasing concentrations of ATP is shown in Fig. 4. No change in the fluorescence λ_{MAX} was observed upon the addition of ATP, indicating that the substrate is neither affecting the nanoparticle surface nor causing sample aggregation.

Conclusions

We have produced the first reported example of a water soluble QD based fluorescent probe for ATP. By targeting differences in substrate charge the sensor was able to identify ATP among other nucleotides with good selectivity. The attractive interaction between the negatively charged ATP and the positively charged QD was sufficient to bring the two close enough so that an electron transfer mediated quench of fluorescence was observed. The reduced negative charge associated with ADP and AMP resulted in reduced attraction with the nanoparticle surface and a reduced quenching of the QD fluorescence. Similarly GTP displayed greater quenching than GDP or GMP but markedly less than ATP. More work is ongoing in this laboratory to gain a fuller understanding of this interaction.

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