Research Article

Optical Properties and In Vitro Biological Studies of Oligonucleotide-Modified Quantum Dots

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Water-soluble semiconducting nanocrystals or quantum dots (QDs) have attracted much interest in recent years due to their tuneable emission and potential applications in photonics and biological imaging. Fluorescence resonance energy transfer (FRET) processes are very important for elucidating biochemical mechanisms in vitro, and QDs constitute an excellent substrate for this purpose. In this work, new oligonucleotide-functionalised CdTe-based QDs were prepared, characterised and biologically tested. These QDs demonstrated interesting optical properties as well as remarkable in vitro behaviour and potential for a range of biological applications.

1. Introduction

Quantum dots (QDs) are semiconductor nanoparticles where the electrons and holes are quantum-confined, which translates into strong photoluminescence (PL) in the visible range. The emission wavelength is directly related to the particle size as larger particles will absorb lower energy, that is, longer wavelength photons [1]. Water-soluble CdTe-based QDs have attracted much interest in recent years for their potential applications in biological imaging especially, which consists in illuminating the cell membrane, the cytoplasm, or selected organelles using a fluorophore [2–14]. Choosing an appropriate combination of staining agents enables one to elucidate cellular structures or mechanisms by visualising them with a fluorescence or confocal microscope. In this area, QDs have a number of advantages over organic dyes. QDs have a continuous absorption spectrum for wavelengths that are shorter than the emission wavelength and a narrow and symmetrical emission which can be tuned to a particular wavelength by changing the particle size. It is therefore possible to excite QDs of various colours with the same source and to spectrally resolve their emissions [15].

The aqueous synthesis of thiol-stabilised CdTe QDs has been reported and optimised over the last years and adapted for different types of ligands [2, 4, 16]. Recently, Tikhomirov and coworkers published a method for producing oligonucleotide-modified CdTe QDs using phosphorothioate-functionalised oligonucleotide sequences [17]. They also demonstrated how the number of strands to be immobilised on a single QD is highly dependent on the QD-binding domain of oligonucleotides as well as the particle diameter. On the other hand, QDs are excellent candidates for the observation of fluorescence resonance energy transfer (FRET) phenomena [1, 17–32] owing to their narrow and symmetrical emission spectra. The sensitive distance dependence of FRET makes it a powerful tool for resolving bio-chemical processes in vitro and biosensing [23, 33–41]. In this work, a range of oligonucleotide-modified QDs were produced and fully characterised. The role of hybridisation and melting of double-stranded (ds)-DNA in QD interactions was investigated, and the occurrence of energy transfer was studied with various degrees of spectral overlap between donor and acceptor. Finally, the potential of the
new bionanocomposites for in vitro biological imaging was assessed.

2. Materials and Methods

2.1. Materials. The oligonucleotides with the sequences (5’ NH2-A10 TAG GAA TAG TTA TCA (T6) 3’ and 5’ NH2-A10 TGA TAA CTA TTC CTA (T6) 3’ were prepared according to standard on-support oligonucleotide synthesis. T6 phosphorothioate tail was prepared by using Beaucage reagent during oxidation steps and 5-amino group was introduced at the last step of DNA synthesis by using the corresponding phosphoramidite C6-amino linker from Eurogentec. Al3Te3 was purchased from Cerac Inc. All other chemicals for QD synthesis were purchased from Sigma-Aldrich.

2.2. Synthesis of Oligonucleotide-Modified CdTe QDs. We used a modification of the method developed by Gaponik et al. [16] to produce CdTe QDs stabilised with Thioglycolic acid (TGA). Briefly, cadmium perchlorate hydrate Cd (ClO4)2·6H2O (5.5 g) and TGA (1.2 mL) were added to 150 mL degassed Millipore water. The pH of the solution was adjusted to 11 by dropwise addition of a 2 M NaOH solution. 30 μL of 100 μM aqueous solution of oligonucleotide (oligo-1: 5’ NH2-A10 TAG GAA TAG TTA TCA (T6) 3’ or oligo-2: 5’ NH2-A10 TGA TAA CTA TTC CTA (T6) 3’) was added to the solution. To 0.5 g of Al3Te3 15 mL of H2SO4 was added to generate H2Te gas which then bubbled through the Cd/thiol solution. The resulting nonluminescent solution was then heated under reflux. Once the QDs reached the desired size, the reflux was stopped and different fractions were obtained via size-selective precipitation using isopropanol. The final samples were further purified on a Sephadex-G25 column.

2.3. In Vitro Cellular Assay. HT1080 cells were cultured in a medium (500 mL Minimum Essential Medium (MEM) supplemented with 0.055 g of sodium pyruvate, 5 mL of a solution of penicillin (2 mM) and streptomycin (2 mM), 5 mL of 1 mM gentamicin, and 100 mL of fetal bovine serum (FBS) at 37°C and in a 5% CO2 atmosphere. Cells were plated in 35 cm² glass-bottomed petri dishes (from iBidi) at a final concentration of 10⁵ cells per dish and left to adhere overnight. Half of the cell medium was then removed from all dishes and replaced by serum-free medium. Cells were incubated for another 4 hours. QDs were added to a final concentrations of 10⁻⁷ mol/L in Dulbecco’s modified phosphate buffered saline (DPBS) with sodium chloride and magnesium chloride, and cells were incubated for another 4 hours. The QD-containing medium was aspirated out of the dishes and the cells were washed three times with DPBS. The cell cultures were finally imaged using an Olympus FV-1000 confocal microscope.

3. Results and Discussion

Complementary sequences of oligonucleotides bearing a T6 phosphorothioate tail at their 5’-end and an amino function at their 5’-end (5’ NH2-A10 TAG GAA TAG TTA TCA (T6) 3’ and 5’ NH2-A10 TGA TAA CTA TTC CTA (T6) 3’) were used to synthesise three series of modified QDs. The procedure was adapted from the work of Tikhomirov et al. [17]. A range of QD sizes were thus produced and enabled to interact through DNA hybridising. The optical properties of the new composites were investigated, as well as their behaviour in in vitro cell cultures.

The presence of oligonucleotides on the surface of QDs and their ability to hybridise with the complementary sequence immobilised on other QDs were confirmed by several instrumental techniques. First, circular dichroism (CD) spectra were recorded for QDs modified with complementary oligonucleotides as well as a one-to-one mixture of those. They exhibited a weak signal in the 500–800 nm range, which corresponds to the position of the exciton peak. The corresponding spectra are shown in Figure 1. It was previously reported that plasmonic nanoparticles may induce CD in that region when a chiral molecule, such as an oligonucleotide, is immobilised on their surface and aggregation occurs [42]. It was also demonstrated that QDs did not produce such an effect when oligonucleotides were coupled to the TGA ligand [42]. In the present case, however, oligonucleotides were bound directly to the surface of QDs, which allowed for closer interactions. The T6 phosphorothioate tail was assumed to lay on the surface, and as a result, the chiral moiety was in direct contact with the particle surface. When bound to the ligand, however, electrostatic repulsion between the carboxylic acid groups and the DNA backbone prevented such contact. Furthermore, it may be assumed that CD signal was generated through local chiral distortion of the crystal structure by chiral ligands, such as previously demonstrated for penicillamine-stabilised CdS and CdSe QDs [43–45]. The CD signal was also not significantly altered by the mixing of complementary-modified
Table 1: Characterisation of oligonucleotide-modified QDs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oligonucleotide</th>
<th>Absorption (nm)</th>
<th>Emission (nm)</th>
<th>Quantum yield (%)</th>
<th>Diameter (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor (a)</td>
<td>Oligo-2</td>
<td>525</td>
<td>558</td>
<td>27</td>
<td>2.7</td>
<td>-44</td>
</tr>
<tr>
<td>Acceptor (a)</td>
<td>Oligo-1</td>
<td>556</td>
<td>592</td>
<td>25</td>
<td>3.7</td>
<td>-29</td>
</tr>
<tr>
<td>Donor (b)</td>
<td>Oligo-1</td>
<td>535</td>
<td>560</td>
<td>50</td>
<td>2.9</td>
<td>-35</td>
</tr>
<tr>
<td>Acceptor (b)</td>
<td>Oligo-2</td>
<td>601</td>
<td>644</td>
<td>51</td>
<td>5.2</td>
<td>-40</td>
</tr>
<tr>
<td>Donor (c)</td>
<td>Oligo-2</td>
<td>531</td>
<td>567</td>
<td>6</td>
<td>2.9</td>
<td>-47</td>
</tr>
<tr>
<td>Acceptor (c)</td>
<td>Oligo-1</td>
<td>556</td>
<td>592</td>
<td>25</td>
<td>3.7</td>
<td>-29</td>
</tr>
</tbody>
</table>

QDs, thus confirming the predominant role of surface fragments.

For further assessment of the binding effect of oligonucleotides, a 1:1 mixture of QD-oligo 1 and QD-oligo 2 was scanned by dynamic light scattering (DLS) in order to measure the changes of the hydrodynamic diameter over time. Figure 2 displays the graph of hydrodynamic diameter distribution from 0 to 55 min of incubation.

The initial mixing of both types of QDs resulted in an average diameter of around 10 nm with a polydispersity index (PDI) of 0.431. As incubation time was prolonged, the PDI did not significantly vary; the average diameter, however, slightly decreased to around 7 nm after 20 min and remained in that range afterwards. This indicated that QDs in initial clusters which were formed in the first 20 min interacted more strongly than and packed more tightly with time. The cluster size of 7 nm was consistent with dimeric structures. Subsequently, the role of oligonucleotide hybridisation in the process was ascertained by measuring the hydrodynamic diameter of the same one-to-one mixture with increasing temperature. Results are displayed on Figure 3. For temperatures up to 65 °C, the cluster size fluctuated between 10 and 35 nm while the PDI rose to 0.510 on average. These two effects were mainly imputable to thermal agitation. Further heating, however, led to a dramatic increase in average diameter. The measured values were 41 (±5) nm at 75 °C, 174 (±15) nm at 85 °C and 326 (±50) nm at 90 °C. It was therefore concluded that the nanoclusters dissociated when the temperature was higher than the oligonucleotides melting temperature. The QDs moved further apart but remained grouped together as there was no mechanical agitation. Diffusion would lead over time to a reduction in measured size as QDs could eventually be seen as individual particles. The experiment also demonstrated that hybridisation was possible at physiological temperature (37 °C), which is important for potential biological applications.

Having ensured the presence and hybridisation ability of complementary oligonucleotides on QD surfaces, we investigated the energy transfer processes in the nanocomposites. Three sets of donor-acceptor pairs were considered. Their full characterisation data are summarised in Table 1.

In the first pair, the donor emission and acceptor absorption perfectly overlapped which was expected to produce very efficient energy transfer. In the second case, the PL emissions were well separated which enabled good discrimination between them. In the third pair, there was near complete overlap between both absorption and emission spectra, allowing for mutual energy transfer. In each pair, the donor and acceptor were functionalised with oligonucleotide sequences complementary to each other.

Energy transfer in each donor-acceptor pair was investigated by measuring the PL emission of various donor-acceptor solutions of different compositions. The donor quenching and acceptor enhancement were plotted against the donor-to-acceptor ratio (Figures 4–6). The donor quenching is defined as the ratio of the donor intensity in the mixed solution to that of the pure donor at the same concentration. Similarly, the acceptor enhancement is defined as the ratio of the acceptor intensity in the mixed solution to that...
of the pure acceptor at the same concentration. It should be noted that donor and acceptor QDs can bind to each other in multiple configurations through oligonucleotide hybridisation and that the complementary domain is approximately 5 nm in length (15 base pairs) [46]. In Tikhomirov et al. work [17], the valency of oligonucleotide-modified QDs was measured for QDs prepared with the same method that was followed here. It was shown to depend on the length of the QD-binding domain of the oligonucleotide and the QD diameter. In our case, the QD-binding domain was a T6 tail; therefore, all QDs are estimated to have a valency of two to three for the donors and three to four for the acceptors. In the first pair (donor (a)–acceptor (a)), as shown in Figure 4, the donor was rapidly quenched for ratios up to 0.5:1 where it reached 30% of the original value and remained stable for ratios up to 1:1. When the acceptors was in excess, all donor QDs could be assumed to bind to acceptor QDs and to be quenched by them. For these lower ratios, clusters of one donor and two or more acceptor could also form. It led to a slight degree of acceptor quenching through acceptor-to-acceptor intraensemble energy transfer due to spectral overlap of the acceptor absorption and emission. Enhancement of the acceptor PL was thus not observed as long as it was in excess. When the donor-to-acceptor ratio was further increased, the donor emission slightly recovered and plateaued at around 40% of the original value. This was attributed to the possibility of donor-to-donor energy transfer due to partial overlap of donor absorption and donor
emission spectra [18]. Meanwhile, the acceptor emission was gradually enhanced up to 160% of its original value, thus confirming the occurrence of the energy transfer.

Considering the donor-acceptor pair (b) with well-separated absorptions and emissions, the interaction pattern appeared slightly different as presented in Figure 5. At low donor-to-acceptor ratios, a similar trend of rapid donor quenching was observed, down to around 20%. The acceptor emission, however, was immediately enhanced to 120% and remained around that level independently of the amount of donor. The high FRET efficiency at low donor-to-acceptor ratio was due to the high quantum yield of both sets of QDs, while the rapid saturation of the acceptor enhancement was attributed to the spectral separation. As more donor was added its emission slightly recovered due to donor-to-donor intraensemble energy transfer but remained below 50%.

Figure 6 shows that in the pair (c), both sets of QDs had almost completely overlapping spectra. This means that either could actually be donor or acceptor. At low donor-to-acceptor ratio, the so-called “acceptor” was quenched and the “donor” emission was enhanced. The species in excess was most likely to surround the other one through oligonucleotide hybridisation, thus transferring more energy than it received. When the ratio reached one-to-one, the “acceptor” emission had fully recovered, while the donor was still 140% of its original value. It should be noted that the “donor” had a rather low quantum yield of 6% and any energy transfer would therefore translate into a high percentage of variation. Its emission then plateaued at 120% while the acceptor emission continuously rose up to 180% as the donor-to-acceptor ratio was increased. Because of the spectral overlap, it became difficult to distinguish the donor emission at high ratio. It is therefore very likely that the donor was quenched but the emission at that particular wavelength was compensated by the acceptor.

In all cases, energy transfer was observed between QDs modified with complementary oligonucleotide sequences, and there was always a range of ratio for which one was quenched and the other was enhanced. Both donor quenching and acceptor enhancement plateaued between 3 and 4 donors per acceptor, consistently with the calculated valency of the acceptors.

To be suitable for in vitro FRET experiments, donor and acceptor must have well-separated emission spectra in order to be detected independently, and FRET should result in nearly full quenching of the donor and maximum enhancement of the acceptor. Only the donor-acceptor pair (b) was found to satisfy these two conditions and was therefore tested in live cell cultures. Two cultures of HT-1080 cells from human fibrosarcoma were incubated with donor (b) and acceptor (b), respectively. Both types of QDs were readily uptaken and could be observed as isolated spots in the cytoplasm, green for the donor and red for the acceptor. A third culture was then incubated with donor and acceptor simultaneously, with no prior mixing. This resulted in the partial colocalisation of donor and acceptor, but isolated green and red QDs could still be seen. Preincubation of donor and acceptor in equal concentrations for an hour and subsequent addition to the cell culture, however, resulted in the donor luminescence completely disappearing and the acceptor emission being enhanced. Thus, our QD-based donor-acceptor system clearly demonstrated fluorescent resonant energy transfer (FRET) inside live HT-1080 cells. Example confocal images of all four types of cultures are displayed in Figure 7. It was therefore concluded that preformed assemblies of QDs persisted in cell cultures but that binding did not spontaneously occur in that biological medium. The disappearance of all green emissions confirmed the occurrence of FRET in the system with near complete quenching of the donor QDs.
FIGURE 7: Confocal microscope images of HT-1080 cells exhibiting internalised QDs. First row: negative control; second row: donor (b); third row: acceptor (b); fourth row: donor (b) and acceptor (b) added simultaneously; fifth row: donor (b) and acceptor (b) with preincubation. First column: excitation 543 nm, emission 603 nm; second column: excitation 633 nm, emission 668 nm; third column: overlay of green and red channels with bright field. Arrows indicate green QDs.
Figure 8: Confocal microscope close-up images of HT-1080 cells exhibiting internalised green and red QDs. (a) Without preincubation. (b) With preincubation. Overlay of green and red channels with bright field. Excitation 543 nm, emission 603 nm; excitation 633 nm, emission 668 nm.

Figure 9: Histograms representing the number (b) and size (a) of QD aggregates internalised by cells depending on QD type: donor, acceptor, and 1:1 mixture with or without preincubation.

Prehybridisation of QDs also resulted in a higher degree of aggregation and accumulation in individual cells as illustrated in Figure 8. This was quantified by both the number and size of QD aggregates visible inside cell cytoplasms and represented in Figure 9. The overall higher internalisation was imputable to the formation of larger aggregates. Larger objects tend to be better internalised by cells due to a thermodynamically favoured attachment to the cell membrane [47].

This observation opened to another possible type of application, where DNA-hybridisation processes could be monitored through QD aggregation using a system where FRET does not occur. Similar cellular testing of the donor-acceptor pair (c) provided an example of such system. As predicted by the fluorescence titration described above, neither species was quenched at 1:1 ratio, thus allowing visualising them simultaneously in cells. The presence in cytoplasms of large aggregates containing both red and green QDs confirmed the occurrence of oligonucleotide binding after preincubation. Confocal microscopy images of cells treated with these QDs are displayed in Figure 10.

Thus, the results above demonstrated that our composites were suitable for studying energy transfer processes or DNA binding/digesting processes in cell cultures in vitro.

4. Conclusions

In this work, a series of new oligonucleotide-modified CdTe QDs have been prepared, characterized, and tested in vitro. Hybridisation and separation of complementary strands of oligonucleotides were assessed as a function of time and temperature. It was found that the hybridised systems demonstrated variable FRET efficiencies depending on spectral overlap, which could be observed in vitro in HT-1080 cell cultures. Further work will include an investigation of specific intracellular oligonucleotide-modified QD interactions in various cell cultures to understand and explain the biological behaviour of these nanomaterials in detail. We believe that
Figure 10: Confocal microscope images of HT-1080 cells exhibiting internalised QDs. (a) donor (c), (b) acceptor (c), (c) donor (c) + acceptor (c) added simultaneously, (D) donor (c) + acceptor (c) after a one-hour preincubation. Overlay of bright field with green and red channels.

these QD-oligonucleotide conjugates might find important applications for \textit{in vitro} biological imaging and sensing.

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References


