Hydroxylated Quantum Dots as Luminescent Probes for in Situ Hybridization

Srikant Pathak,[†] Soo-Kyung Choi,[‡] Norman Arnheim,*[,‡] and Mark E. Thompson*[,†]

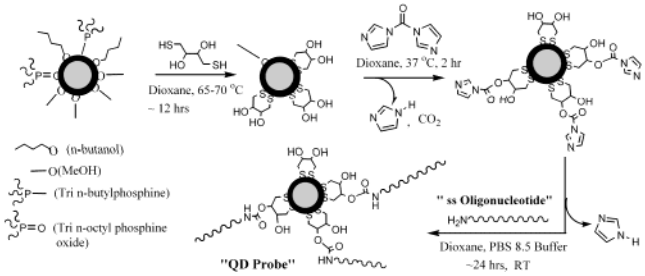
Department of Chemistry and Molecular Biology Program
University of Southern California
Los Angeles, California 90089

Received November 28, 2000

Semiconductor quantum dots (QDs) have been used in a range of optoelectronic applications.[1] It has recently been shown that QDs can be used as nonisotopic biolabels, by binding proteins, antibodies, etc., to the surface of the QD.[2] In a related study, oligonucleotide derivatized quantum dots were used as building blocks to form extended networks.[3] In these luminescence-based applications, it has proven beneficial to use QDs with core-shell architectures.[4] Core-shell quantum dots are prepared by capping an emissive semiconductor dot core (CdSe, CdTe, etc.) with a thin shell of higher band gap material, for example, ZnS, CdS, ZnSe, etc., to prevent surface quenching of excitons in the emissive core and hence increase the quantum yield for emission, as well as the photostability of the dots as compared to that of the core alone.[5]

Our goal is to use multicolor QD-oligonucleotide-based probes for detection of chromosome abnormalities or mutations using common fluorescence in situ hybridization (FISH) procedures.[6] FISH analysis using quantum dot probes requires the QDs be soluble and stable in physiological buffer conditions, and that the dot surface be efficiently derivatized with oligonucleotides. Reliably attaching oligonucleotides to the quantum dot surface by the reported techniques (via surface carboxylic acid groups)[7] proved to be problematic, leading to inefficient oligonucleotide loading on the QDs and poor long-term stability.[8] We were also concerned that under the hybridization conditions (pH = 7−8.5), the free carboxylic acid groups on the QD surface lead to nonspecific binding to the oligonucleotide probe backbone[9] as well as the target cells, making them far less useful than traditional organic fluorophore probes in FISH detection. In the present communication we report an approach to solve this problem, which involves making water-soluble QDs by modifying them to have surface hydroxyl groups. Surface modification of quantum dots with hydroxyl groups leads to solubility properties very similar to those of carboxylic acid derivatized dots. In addition, it has been shown that protein adsorption to hydroxylated surfaces has reduced nonspecific binding.[10] Hydroxyl-terminated quantum dots have been coupled with different oligonucleotide sequences via a carbamate linkage, which does not hydrolyze under FISH conditions, leading to probe−dot conjugates that are stable for months and useful luminescent probes in FISH assays.

Scheme 1



CdSe/ZnS (core/shell) quantum dots were prepared using the literature procedure reported by Alivisatos et al.[11] The resulting *n*-butanol clad core−shell dots are precipitated, washed several times with methanol, and dried under argon. The QDs were then redispersed in anhydrous dioxane to which dithiothreitol (DTT) was added. The solution was refluxed overnight, followed by centrifugation and washings to remove excess DTT. The DTT-derivatized dots can be stored for an extended period of time without decomposition. The hydroxyl-terminated QDs were activated by treating them with 1,1′-carbonyl diimidazole (CDI) forming imidazole−carbamate groups at the QD surface (Scheme 1). CDI activation of dots can easily be followed by the IR spectroscopy. Hydroxyl-terminated quantum dots show a broad $\nu_{OH} \approx 3388$ cm^{-1} band that decreases markedly in CDI-activated dots. Furthermore, CDI-activated dots show a prominent carbonyl absorption band at $\nu_{C=O} \approx 1790$ cm^{-1}. The CDI-activated quantum dots precipitate out of the dioxane solution, making them readily separable from excess CDI and other reagents. The CDI-activated QDs are coupled to 5′-aminated oligonucleotides, forming a carbamate linkage between the QD and the oligonucleotide (Schemes 1 and 2). CDI-activated QDs, which are only sparingly soluble in dioxane/water, become highly soluble once coupled to the oligonucleotides with no significant aggregation, suggesting an efficient coupling of oligonucleotides to the CDI-activated dots. The inherent stability of DTT bound to the QD surface[8] may be due to a chelation effect, since the S−S distance in DTT is close to the expected Zn−Zn distance in ZnS.[12]

Scheme 2 shows the oligonucleotide sequences used in our experiments. The "Y-specific" sequence is a human, alphoid-repeated sequence,[13] whose complementary pairs are found only

* Authors for correspondence. M.E.T.: E-mail: met@usc.edu. Telephone: (213) 740-6402. N.A.: E-mail: arnheim@usc.edu. Telephone: (213) 740-7675.

[†] Department of Chemistry.

[‡] Molecular Biology Program.

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(7) QDs are treated with thio-carboxylic acids, i.e., meracaptoacetic acids, mercaptopropionic acids, etc., making them water soluble and giving free carboxylic acid groups for binding biomolecules (see ref 2).

(8) The thio-carboxylic acids are hydrolyzed over 3−4 weeks, leading to aggregation and precipitation of the QDs from water.

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(12) Zn−Zn distance (using $a = 5.4093$ Å for bulk ZnS) of 3.82 Å is comparable to the S−S distance ~3.6 Å (AM1 level calculation) in DTT.

(13) For a description with references, see Supporting Information.

10.1021/ja0058334 CCC: $20.00
Published on Web 04/07/2001

Scheme 2

C6: six carbon spacer

'Y-Specific' (5') NH_2-(C6)-TCC ACT TTA TTC CAG GCC TGT CC, (3')

'Random' (5') NH_2-(C6)-GGA TAA CAT TGG ATT CGG ATG GCA CA, (3')

on the Y chromosome. A "random" sequence, which is not identical to any known in the human genome, was used as a control. Fluoroscein end-group labels (added at the 3′ end of the oligonucleotide) were used to examine the binding stability of the oligonucleotides to the QDs over time. Fluorescein emission was observed for the labeled oligonucleotides in solution but is completely quenched when the oligonucleotide is bound to the QD. Fluorescein emission is not observed for solutions of the oligonucleotide-derivatized QDs after two months at room temperature. The oligonucleotide can be readily removed from the QD, however, by treating the derivatized QDs with an excess of thioethanol, leading to recovery of intense fluoroscein emission. The details of these experiments are given in the Supporting Information. No appreciable loss of the oligonucleotide from the QD probes was observed for either random or Y-specific samples (after two months).

To examine whether these DNA-derivatized QDs give specific hybridization, we carried out FISH assays using each of the QD probe conjugates (Y-specific and random), with human sperm cells. Half of the cells are expected to contain a Y chromosome and therefore hybridize to the alphoid probe. Cell counts over multiple images gave ∼41% (255/625) strong positive signals against a theoretical total of 50%.[14] The other half of the cells contains the X chromosome and should not hybridize with the probe. Consistent with this proposal, remaining cells show effectively no binding (Figure 1). The controls, utilizing (a) QDs with a random oligonucleotide sequence and (b) hydroxyl-terminated QDs (no oligonucleotide sequence attached) showed very little (less than ∼5%) binding to the sperm cells (Supporting Information). The highly specific probe binding and lack of nonspecific binding for either bare or random oligonucleotide-derivatized QDs demonstrated here indicate that oligonucleotides attached to hydroxylated quantum dots can be used as efficient probes in FISH assays. It is also pertinent to mention that multiple signals in a cell may be observed in some cases because of uneven cell de-condensation, chromosomal splitting due to nonuniform nuclear swelling, or cell overlap. Signal intensity variation among sperm cells could be due to nonspecific binding or probe aggregation.

Very little nonspecific binding was observed in FISH experiments done with the hydroxyl-terminated quantum dots. This is in stark contrast to QDs with carboxyl groups on their surface, which lead to strong nonspecific binding to oligonucleotides and other biological tissue, when used in a FISH assay. These hydroxylated QD probes have strong emission, are photostable, and can be stored for long periods and resuspended in various buffers without significant loss of activity or aggregation. The application of multicolor quantum dots to other assays dependent upon DNA probes is currently being explored in our laboratory. In addition to oligonucleotides, hydroxylated quantum dots can be derivatized with other biomolecules and used to study the properties of cells, tissues, and proteins. Examples of such applications include studying the absorption of biomaterials on both natural and artificial substrates,[15] and selective fluorescent labeling of biological tissue.[2]

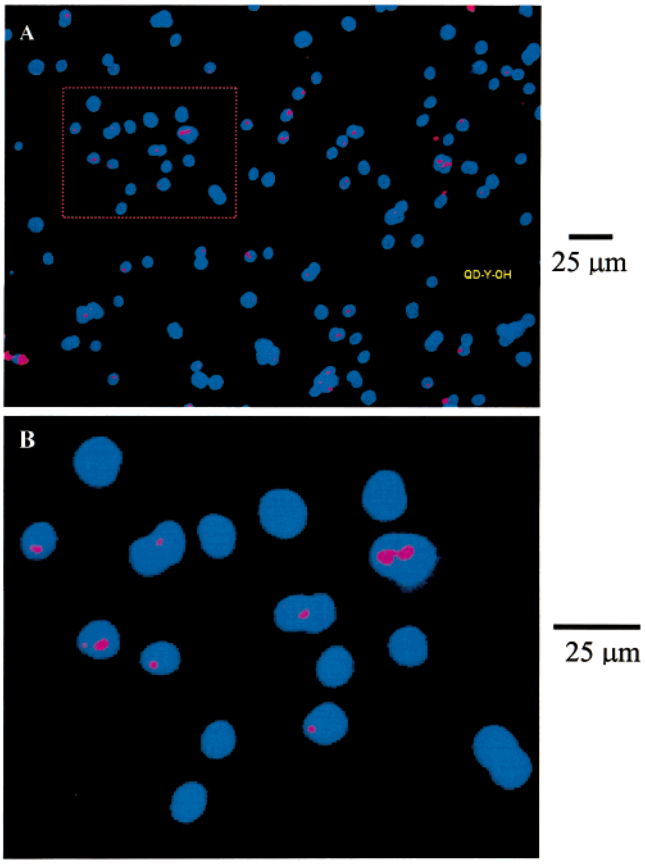

Figure 1. (A) Fluorescence micrograph of in situ hybridization of red quantum (λ_{max}= 609 nm, fwhm = 38 nm) dot probe(s) for the Y chromosome in human sperm cells (B) magnified image of the selected area in A. Preparation of the sperm cells on the slide used the free chromatin method, carnoy fixation, and denaturation with 3 M NaOH, which swells the cell nuclei, making it more accessible to QD probes. The hybridization mixture (20% formamide, 10% dextran sulfate, 200 ng/mL BSA, 10 mM DTT, 1 μg/μL HSD, 4 × SSC, pH 7.0) containing the QD probe was placed over the cells on the slide and incubated at 37 °C overnight in a humidified chamber. After hybridization the slides were washed in 50% formamide/2 × SSC−0.1%Tween 20 for 10 min followed by two additional washes of 10 min each in 2 × SSC−0.1% Tween 20 at 42 °C. The nuclei were then counterstained with DAPI (4′,6-diamidino-2-phenylindole) in antifade solution. Fluoroescence microscopy (at 200×) was carried out using an Olympus BX60 microscope with excitation at 572 nm (QD) and 360 nm (DAPI). The average particle size of the probes (TEM) was 7 nm. (BSA: bovine serum albumin, SSC: saline−sodium citrate, HSD: herring sperm DNA).

Acknowledgment. We thank the Air Force Office of Scientific Research (AFOSR-MURI Program) and NIH (Grant No-R37GM36745) for the funding. We also thank Dr. A. P. Alivisatos and Dr. D. Gerion for helpful discussions.

Supporting Information Available: Detailed procedures for QD derivatization, stability studies, FT-IR spectra, and FISH results with the controls are given (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0058334

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