Synthesis and Characterization of Water Soluble Choline Labeled Cadmium Selenide/Zinc Selenide/Zinc Sulfide Luminescent Quantum Dots

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ABSTRACT. Water soluble choline-labeled CdSe/ZnSe/ZnS quantum dot (QD) bioconjugates were synthesized by attaching a thiolated choline analogue to the core-shell QD surface. Characterization was conducted by absorption and luminescence spectroscopy and scanning electron microscopy. QDs with diameters of 5-6 nm resulted and exhibited a luminescence maximum at 663 nm in aqueous solution.

Keywords. CdSe/ZnSe/ZnS quantum dots, neurotransmission, choline, Alzheimer's disease, choline uptake

Neurodegenerative diseases are debilitating progressive disorders with no known cure. For example, Alzheimer's disease (AD) is the sixth leading cause of death in the United States [1] affecting 5.4 million, mainly elderly Americans. [2] Although AD was first described in 1906, [3] the cause of neuronal breakdown is complicated and the subject of significant research. Indications of AD have been associated with accumulation of β -amyloid proteins in the region between neurons, [4] the buildup of insoluble twisted tau fibers in neurons, [5] and the breakdown of the myelin sheath surrounding neurons. [6] Degradation of various neurotransmitter systems, including selective degradation of cholinergic neurons, also has been observed with a decrease in brain concentrations of the neurotransmitter acetylcholine (ACh), its precursor (Ch, Figure 1a), and proteins that mediate their concentrations such as acetylcholinesterase, the high affinity choline transporter (CHT), and choline acetyltransferase.[7]

Normal cholinergic function requires synthesis of ACh in the neuron from Ch imported by CHT. [8] Therefore, Ch transport is a critical process and studies have focused on understanding the structure and function of CHT through the synthesis of Ch analogues with cholinomimetic features. [9] Even so, structural information for Ch transporters is limited [10] and often inferred from other biogenic amine transport proteins. [9] Since Ch is both hydrophilic and a positively charged ion, passive diffusion across the cell membrane is unlikely. Studies have shown that recognition of Ch by CHT was dependent upon the presence of both the quaternary ammonium and hydroxyl groups among other subtle factors. [11] Such studies have relied extensively on radiochemical methods in the presence and absence of CHT inhibitors. [9] An alternate non-radiochemical approach was developed in our laboratory using capillary electrophoresis with electrochemical detection to measure small time-dependent changes in Ch concentrations in vitro and in vivo under conditions of neuronal degradation with attomole detection limits and nL-sized samples. [12]

Quantum dot (QD) semiconductor nanocrystals are known to have numerous advantages over organic dyes [13] as novel luminescent imaging probes for investigating biological systems. In fact, core-shell QDs of CdSe/ZnSe/ZnS have been designed for site-specific targeting of neurotransmitter uptake and transport. Examples include probes for sites of interaction and function of biogenic amine transporters for serotonin [14] and dopamine. [15] To target and investigate cholinergic sites, we reported the first example of water-soluble CdSe/ZnSe/ZnS QDs with an aminated analogue of hemicholinium-15 (HC-15), a well-known inhibitor of CHT, attached to the surface of the QDs. [16] In this manuscript a new bioconjugate with a thiolated Ch analogue (Figure 1b) was synthesized and coupled to QDs to mimic the natural Ch substrate for CHT. The thiolated Ch derivative was designed with the important recognition elements for CHT, the quaternary ammonium and hydroxyl groups, and a terminal thiol to function as the tether to anchor the recognition label to the outer ZnS layer of the QDs. Together, QDs conjugated to Ch and a known inhibitor provide new chemical diagnostic probes for investigating choline transport.

The synthesis of (*S*)-2-hydroxy-3-mercapto-*N,N,N*-trimethylpropan-1-aminium chloride (Scheme 1) was developed from a method reported by Lewis and Sun for the synthesis of a mercaptoethyl quaternary ammonium derivative. [17] To produce Ch-S₂O₃, (*S*)-(-)-(3-chloro-2-hydroxypropyl)-trimethylammonium chloride (Ch-Cl) (11.3 g, 0.0601 mol) and sodium thiosulfate (14.9 g, 0.0942 mol) were dissolved in deionized water (160 g, 8.89 mol). The pH was adjusted to 7.5 with NaOH and the mixture was refluxed for 7.5 h. Water was removed under reduced pressure and the residue was stored under vacuum overnight. The solid product was rinsed twice with acetone and dissolved in as little water as possible. Acetone was added to precipitate the solid. The mixture was stirred and the precipitate was left to settle at the bottom. The supernatant was decanted off, and the solid was again dried under vacuum. A white solid was recovered and stored in a desiccator for further use.

The final product, Ch-SH, was prepared by sealing Ch-S₂O₃ (0.4 g, 1.39 mmol) in a microwave test tube purged with Ar. A solution of HCl (37%, 5.36 g, 14.7 mmol) in water (2.64 g, 146 mmol) was deoxygenated by an Ar purge and added to the sealed vial. The solution was heated by microwave irradiation for 30 min at 110 °C. After cooling, the solution was transferred to a round bottom flask, and the water was removed under reduced pressure.

Anhydrous and deoxygenated ethanol was added to the dried powder, and allowed to stir for 30 min under Ar. The supernatant was collected and run through a pipette packed with cotton. The ethanol wash was repeated three times. The combined ethanol washings were either used immediately to make QD bioconjugates or concentrated to obtain a cloudy white honey-like substance that was stored under Ar for further use. Yields for both reaction steps were variable due to the presence of inorganic residues.

Ch-SH was successfully synthesized and the identity confirmed by NMR and mass spectrometry. CHN analyses however indicated purification was complicated by an inorganic byproduct in the reactions, most likely NaCl. Not surprisingly, this resulted in variable yields for each reaction step. [17] Incorporation of additional rinsing steps with ethanol increased the purity of the product. Since the amount of Ch-SH used for the preparation of the labeled QDs (Ch-S-QDs) was in excess, this procedure was sufficient to remove the majority of the excess salt. Remaining salt did not affect the formation of the Ch-S-QDs. It is also important to note the reaction time for conversion of Ch-S₂O₃ to Ch-SH was reduced from days to under an hour by heating the reaction mixture by microwave irradiation in a sealed tube under Ar. NMR spectroscopy also revealed complete conversion to Ch-SH was achieved.

The choline-labeled QDs were prepared by first synthesizing the CdSe/ZnSe/ZnS core-shell QD [16] followed by ligand exchange of TOPO coated QDs with Ch-SH. Ch-SH was dissolved in ethanol and filtered through a cotton filled pipette. QDs (0.1 g) were added to the filtrate and this mixture was allowed to stir for 30 min, followed by centrifugation for 2 min. The ethanol containing unreacted ligand was finally decanted off. The precipitate was dissolved in 0.1 M phosphate buffer (PBS) pH 7.3 to which CHCl₃ was added to extract residual organics from the aqueous solution. The exchange process was monitored by following the QD fluorescence under UV illumination in a 1:1 PBS:CHCl₃ mixture. Figure 2 depicts from left to right the fluorescence of the unmodified CdSe/ZnSe/ZnS QDs in the CHCl₃ phase, the Ch-S-QDs in the aqueous phase after initial removal of impurities with CHCl₃, and the water soluble Ch-S-QDs after cleaning with sufficient CHCl₃ to completely remove all organic impurities. Figure 2 clearly demonstrates the formation of water soluble Ch-S-QDs by transfer to the aqueous phase following exchange of the organic capping groups with Ch-SH.

The Ch-S-QDs stored on the bench top in PBS or dried by rotary evaporation and stored in the dark in the vacuum oven maintained their solubility and fluorescence for over two months.

The ¹H NMR spectra for the Ch-SH ligand and the Ch-S-QDs are found in the supplementary materials in Figures S1-S3. Two distinct sets of peaks were observed indicating the presence of both free and bound forms of the Ch-SH ligand in the spectrum for the Ch-S-QDs (Figure S3). The peaks for H_c at 4.34 – 4.21 and H_d at 2.71 – 2.60 correspond to the free ligand in solution. The peaks moved downfield in the bound form with H_c appearing at 4.57-4.44 ppm and H_d at 2.97- 2.79 ppm. No such shift was observed for H_a and H_b, which are further away from the surface of the QDs. The signal for H_a was split into two peaks consistent with two ligand species present in solution. Peaks in the ³¹P NMR spectra for the core-shell QDs were consistent with a TOPO and TOP coated surface. [16] Upon reaction with Ch-SH the characteristic TOPO and TOP peaks disappeared providing further evidence that ligand exchange occurred.

Figure 3 shows the comparison of the UV-visible spectra for the CdSe/ZnSe/ZnS core-shell QDs in CHCl₃ to the Ch-S-QDs in 0.1 M PBS pH 7.3. A well-defined peak maximum centered at 564 nm is observed consistent with the formation of core-shell CdSe/ZnSe/ZnS QDs. Ligand-exchange and water solubilization of the Ch-S-QDs yields a broad absorption for the first excitonic transition from 525 to 535 nm, which was blue-shifted in comparison to the organic capped QDs.

Fluorescence spectra before and after ligand exchange with Ch-SH are shown in Figure 4. A slight red shift in the wavelength of maximum fluorescence intensity was observed from the core-shell CdSe/ZnSe/ZnS QDs (645 nm) to the Ch-S-QDs (663 nm) due to the coordination of the thiol to the QD periphery. [18] Ligand exchange also led to a substantial decrease in fluorescence. The relative peak intensity fell from 6.8 to 0.2. Previous literature reports indicate a reduction of the fluorescence intensity results upon thiol coordination [16,19] and solubilization in water. [20] Some additional intensity may be regained through photobrightening although potentially accompanied by surface structural changes. [21]

A STEM image of the Ch-S-QDs is shown in Figure 5. The Ch-S-QDs exhibited diameters of approximately 5 to 6 nm with a range from 1.5 to 7.5 nm. Close examination of the image reveals some elongation of particles resulting from asymmetric growth during the synthesis. As a result there exists some variation in particle morphology with the measured polydispersity of 21%.

In summary, to the best of our knowledge we report the first choline-labeled CdSe/ZnSe/ZnS bioconjugate. The Ch-S-QDs are nanometer in size and exhibit the characteristic spectroscopic features in aqueous solution for CdSe/ZnSe/ZnS QDs. Their water solubility and high level of luminescence potentially make them a valuable tool to be used as a probe in cholinergic assays.

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Figure 1. Structure of a) choline and b) thiolated choline derivative (Ch-SH).

Figure 2. Left: CdSe/ZnSe/ZnS QDs, Center: Ch-S-QDs after initial cleaning with CHCl_3 , Right: Ch-S-QDs after thorough cleaning with CHCl_3 . Illumination by a UV lamp in 1:1 PBS: CHCl_3 .

Figure 3. UV-visible spectra of (—) annealed CdSe/ZnSe/ZnS core-shell QDs in CHCl_3 and (---) Ch-S-QDs in 0.1 M PBS pH 7.3.

Figure 4. Fluorescence spectra of (—) annealed CdSe/ZnSe/ZnS core-shell QDs in CHCl_3 and (---) Ch-S-QDs in 0.1 M PBS pH 7.3. Inset: Expanded version of the Ch-S-QD luminescence spectrum. The absorbance for each sample was matched at the excitation wavelength of 420 nm.

Figure 5. STEM image of Ch-S-QDs.

Scheme 1. Synthesis of Ch-SH.

Graphical Abstract - Pictogram

Graphical Abstract - Synopsis

Water soluble choline labeled CdSe/ZnSe/ZnS core-shell quantum dots were synthesized and exhibit strong luminescence in aqueous solution.