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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.

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 choline (Ch, Fig. 1a), and proteins that mediate their concentrations such as acetylcholinesterase, the high affinity choline transporter (CHT), and choline acetyl transferase [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 hemicholium-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 (Fig. 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

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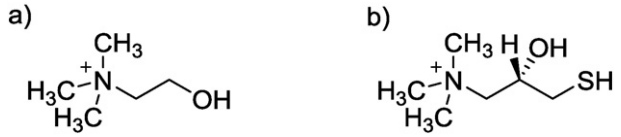


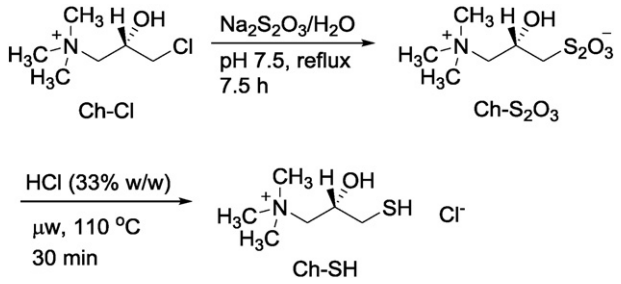
Fig. 1. Structure of a) choline and b) thiolated choline derivative (Ch–SH).

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–S_2O_3, (*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–S_2O_3 (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 was 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 excess salt. Remaining salt did not affect formation of the Ch–S-QDs. It is also important to note the reaction time for conversion of Ch–S_2O_3 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.



Scheme 1. Synthesis of Ch–SH.

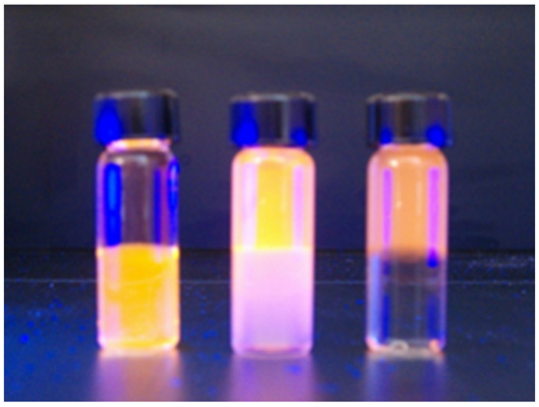


Fig. 2. Left: CdSe/ZnSe/ZnS QDs, Center: Ch–S-QDs after initial cleaning with $CHCl_3$, Right: Ch–S-QDs after thorough cleaning with $CHCl_3$. Illumination by a UV lamp in 1:1 PBS:$CHCl_3$.

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 $CHCl_3$ 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:$CHCl_3$ mixture. Fig. 2 depicts from left to right the fluorescence of the unmodified CdSe/ZnSe/ZnS QDs in the $CHCl_3$ phase, the Ch–S-QDs in the aqueous phase after initial removal of impurities with $CHCl_3$, and the water soluble Ch–S-QDs after cleaning with sufficient $CHCl_3$ to completely remove all organic impurities. Fig. 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 ^{1}H 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

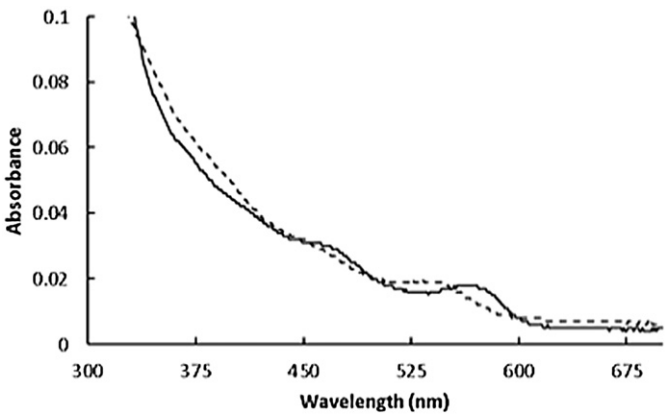


Fig. 3. UV–visible spectra of (—) annealed CdSe/ZnSe/ZnS core-shell QDs in $CHCl_3$ and (— — —) Ch-S-QDs in 0.1 M PBS pH 7.3.