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Surface modified fluorescent quantum dots with neurotransmitter ligands for potential targeting of cell signaling applications

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ABSTRACT

The possibility of combining nanotechnology with nanomedicine opens a broad field of research which may truly revolutionize our society. The neural system plays a crucial role in the human body, and most related diseases can dramatically change the quality of life. Thus, the present study reports a novel approach for using neurotransmitters as ligands in the synthesis of surface-modified fluorescent nanocrystals for potential use in cell labeling applications. Briefly, CdS quantum dots (QDs) were prepared using L-glutamic and L-aspartic as surface capping agents *via* a one-step chemical processing method, which resulted in stable aqueous colloidal systems at room temperature and ambient pressure. UV–visible spectroscopy, photoluminescence spectroscopy (PL), Fourier transform infrared (FTIR) spectroscopy, and transmission electron microscopy (TEM) were used to characterize the synthesis and relative stability of peptide-capped CdS nanocrystals. The results demonstrate that both ligands were effective in nucleating and stabilizing CdS quantum dots in colloidal aqueous suspensions, with an estimated dimension below 3.3 nm and with fluorescence activity. Thus, novel nanohybrids were developed based on QDs bioconjugated to surface-active neurotransmitter moieties suitable for investigation as potential biomarkers in cell targeting and signaling applications.

1. Introduction

Interest in developing novel biomedical applications using quantum dots (QDs) has grown dramatically over the past two decades. This intriguing class of materials is characterized on a nanoscale dimension and has unique electronic, optical and biological properties. QDs have been considered very promising nanomaterials for a myriad of biomedical applications, like cell targeting, immunolabeling, biosensing, drug delivery and therapeutics, bio-imaging and outer-inner cellular tracking [1–6]. The significantly enhanced photochemical stability, brightness, and narrower emission spectra of QDs compared to organics dyes or to fluorescent proteins result in an important improvement in the sensitivity and duration of bio-imaging at lower concentrations of analyte [1,2]. Moreover, the emission color of QDs may be tuned over a wide range of wavelengths, from ultraviolet to visible and infrared, by controlling the size and chemical composition of the nanocrystals. Furthermore, these semiconductor nanoparticles can be surface modified by applying biocompatible shells, such as polymers, peptides, carbohydrates, DNA and RNA sequences and other molecules, thus providing a convenient way to provide novel functionalities for biomedical applications [2,3,6–9].

Among several feasible alternatives of natural biomolecules, peptides and proteins (for instance, enzymes, immunoglobulins, Arg-Gly-Asp sequences or RGD peptides) have been the most common choice as biofunctional molecules mainly due to their specificity, biological affinity, and relative chemical and thermal stability in physiological environments, associated with industrial production scale at coherent costs. Despite the relative success of developing QDs and their bioconjugates, most methods usually involve hydrophobic media using organic solvents at high temperatures, which would exclude them from being used effectively in aqueous biological environments [10–12]. In that sense, recent studies of water-based synthesis routes of semiconductor nanoparticles have been published, but with the large majority using complex procedures and/or expensive precursors [13]. It should be highlighted that regardless of the route one may choose, when designing systems for targeting the interior of cells and other living organisms, the final dimension must be kept within a very limited range referred to as the "hydrodynamic diameter" (HD)

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[12,14]. As a consequence, research of innovative methods for producing water soluble QDs with long-term stability, narrow size distributions and biocompatibility, associated with the smallest possible HD is still a challenge that must be overcome [15,16]. Hence, amino acids may be considered an interesting choice for developing bioconjugates with QDs because they exhibit some important characteristics, such as biocompatibility, water solubility, chemical stability in physiological medium, bio-affinity and the "smallest" possible molecular size among peptides and proteins. However, due to the complexity of performing both quantum dot synthesis and their bioconjugation at the same time, only a few reports can be found in the literature that use amino acids as capping agents using aqueous processing routes. Moreover, the majority of these studies rely on cysteine (either as an amino acid or as a residue present in the protein sequence) as a stabilizing ligand due to the strong interactions between the thiol groups (sulfhydryl, R-SH) and transition metal cations (M-SH) [17,18]. Other authors have suggested producing semiconductor nanocrystals using amino acids, peptides or dendrimers based on the amine groups (R-NH_2) in the side-chains due to a chelating effect with metal ions (M-NH_2) [19,20]. Nevertheless, no study has been found that addresses the synthesis and characterization of CdS quantum dots directly nucleated and stabilized by negative polar amino acids residues, aspartic and glutamic acids (R-COOH) designed especially for neuronal and biomedical applications.

L-Glutamate (L-glu) and L-aspartate (L-asp), the ionized forms of glutamic acid and aspartic acid, respectively, are very important amino acid neurotransmitters in the mammalian central nervous system (CNS) [21,22]. Essentially, upon release, the actions of L-glutamate are terminated by a class of proteins called glutamate transporters, which are located in the plasma membranes of neurons and glial cells. L-Asp has a potential function in the depolarization-induced release from cerebral synapses, but its real contribution has not yet been entirely elucidated [21,23]. Thus, these neurotransmitters play major roles in the development of the brain, including neurogenesis, migration, and synapse formation. They are also involved in many aspects of normal brain function, including learning and memory. However, excessive glutamate stimulation acts also as a neurotoxin (glutamate-related cell injury or death) and may contribute in part to a large number of neurodegenerative diseases and disorders, including stroke, head and spinal cord trauma, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, human immunodeficiency virus-associated dementia, glaucoma, epilepsy, and others [24,25]. Hence, in the neurobiological and medical fields, several studies have been focused on the development of biomarkers to target specific receptors in neuronal synapses to better understand the complex phenomena involved in cellular signaling [26]. In that sense, recently researchers have been investigating the possibility of using biomolecule-capped QDs for detecting single-molecules involved in the cellular signaling and neuronal synapses on a nanoscale dimension (10–50 nm) [27–29]. For that reason, it is exceptionally important to develop new ultrasmall QD bioconjugates designed for potentially assessment of neuronal events such as synapses and the biological receptors involved [2,3,6–10,27–29]. Thus, in the present study, it is reported the synthesis and characterization of CdS quantum dots stabilized and biochemically functionalized using L-glutamic acid (L-glu) and L-aspartic acid (L-asp) as direct capping agents by means of one-step aqueous colloidal processing route. Despite being a very dynamic area of research, the novelty of this work relies on the use of active neurotransmitters (L-glu and L-asp) as ligands for the "one-pot" synthesis of CdS quantum dot bioconjugates at room temperature in aqueous media, a study that had not yet been explored. This strategy may be potentially applied to innumerous opportunities and challenges faced in the field of neuroscience nanotechnology.

2. Materials and methods

2.1. Materials

All reagents and precursors were used as-received: thioacetamide (Sigma–Aldrich, USA, ≥99%, CH_3CSNH_2), cadmium perchlorate hydrate (Aldrich, USA, $Cd(ClO_4)2{\cdot}6H_2O$), sodium hydroxide (Merck, USA, ≥99%, NaOH), L-glutamic acid (Sigma, USA, ≥99%, $HO_2CCH_2CH_2CH(NH_2)CO_2H$), and L-aspartic acid (Sigma, USA, ≥98%, $HO_2CCH_2CH(NH_2)CO_2H$). Deionized water (DI water, Millipore Simplicity™) with a resistivity of 18 MΩ cm was used to prepare the solutions.

2.2. Preparation methods for CdS precursor solutions

The precursors for the synthesis of CdS quantum dots were prepared according to similar procedures previously reported by our group [7,8,30]. Briefly, approximately 0.0601 g of CH_3CSNH_2 was added to 75 mL of DI water in a 100 mL flask and mixed under moderate stirring for 10–15 min. The total volume was then filled to 100 mL using DI water. This sulfur precursor stock solution was referred to as "SOL_S".

Approximately 0.4193 g of $Cd(ClO_4)_2{\cdot}6H_2O$ was added to 75 mL of DI water in a 100 mL flask and mixed under moderate stirring for 10–15 min. The total volume was then filled to 100 mL using DI water. This Cd^{2+} stock solution was referred to as "SOL_Cd".

2.3. Preparation of ligand solutions

Capping ligand solutions (45 mmol L^{-1}, referred to as "SOL_L-glu" or "SOL_L-asp") were prepared by dispersing the powder (0.669 g of L-glutamic acid or 0.611 g of L-aspartic acid) in a 80 mL of DI water and heating the mixture to 40 ± 5 °C under slow magnetic stirring for 1 h until a clear solution was reached. Then, the total volume was increased to 100 mL using DI water. Additionally, a mixture of both these solutions was also prepared (molar ratio1:1) and referred to as "SOL_L-asp:L-glu").

2.4. Synthesis of CdS quantum dots

CdS bioconjugates were synthesized *via* an aqueous route in a reaction flask using the stock solutions detailed in previous sections ("SOL_S" and "SOL_Cd") and using peptides, L-aspartic acid (L-asp), L-glutamic acid (L-glu) and a mixture (L-asp:L-glu), as capping ligands.

A typical quantum dot synthesis was carried out as follows: 47 mL of "SOL_L-asp", "SOL_L-glu" or "SOL_L-asp:L-glu" were added to the reaction flask. Under moderate magnetic stirring, the pH was adjusted to 11.5 ± 0.2 with NaOH (1.0 mol L^{-1}). Then, 4.0 mL of the cadmium precursor solution ($Cd(ClO_4)_2$, "SOL_Cd", 10 mmol L^{-1}) and 2.5 mL of the sulfur source solution (CH_3CSNH_2, "SOL_S", 8.0 mmol L^{-1}) were added to the flask (the S:Cd molar ratio was 1:2). The solution turned yellowish almost instantaneously, and sampling aliquots of 3.0 mL were collected to evaluate the colloidal stability, which was measured using UV–visible spectroscopy. After preparation, QD dispersions were stored at 6 ± 2 °C.

The schematic representation of the experimental procedure used to synthesize CdS_L-asp, CdS_L-glu and CdS-L-asp:L-glu systems is shown in Fig. 1S.