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Preliminary analysis of the interactions between CdTe quantum dots and human metallothionein

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ABSTRACT

Metallothionein (MT) plays the important role in the detoxification of heavy metals, protection against oxidative compounds and as a prognostic marker in the development of tumors. It is important to find selective, stable and sensitive tools and probes to evaluate the presence of MT in biological fluids or tissues. QDs linked with ligands such as peptides or small molecules are a promising tool for selective, fast, and sensitive tagging and imaging in medicine. In previous findings, the authors proved the possibility of interaction with QDs (particularly with CdTe) and analyzed the stability of the formed complexes between CdTe and MT during incubation over time. Following that, an initial analysis of the interactions between CdTe quantum dots (QDs) and human metallothionein (MT) was performed. Complexes of mercaptosuccinic acid-covered CdTe QDs + MT were investigated using fluorescence intensity changes along a timeline, quenching analysis, stability interpretation based on zeta potential, and quenching intensity. Based on the preliminary results, it appears as though the possible interactions depend on the size of the CdTe QDs. Additionally, the formation of complexes between CdTe and human MT likely depends mostly on structural changes and conformational reorganization rather than on electrostatic interactions. Both types of interactions are responsible for complex creation and stabilization.

1. Introduction

Quantum dots (QDs) are nanocrystals ranging in size from 2 to 10 nm, depending on their method of synthesis, type of attached ligand, temperature, and time of preparation. Their atypical properties – behaving as something between semiconductors and quantum particles – give them the ability to absorb light across a wide spectral range [1,2]. Additionally, during the process of molecular and cellular imaging, QDs can be repeatedly excited without a noticeable loss of fluorescence. Because of their optical properties of light emission and their broad range of absorption, QDs are used as biomedical, chemical and biological probes [3,4]. In comparison to organic fluorophores, QDs have broad excitation spectra, narrow emission spectra and a 4- to 10-fold greater fluorescence spectra lifetime. Additionally, their high quantum yield (40–90%) and high molar extinction coefficient (10–100 times higher than commonly used fluorophores) lead to a brighter light emission spectra without degradation or photobleaching [5–7]. Their unique optical properties usually remain unaffected during the process of conjugation with biomolecules [8,9], although most of their applications require a water-soluble environment. To achieve monodispersity of QDs, two main approaches were proposed: (1) encapsulation, surface coating, or double-layer incorporation using proteins, liposomes or peptides [2]; ligand exchanging and cross-linking conjugation of QDs with small molecules [1,10,11].

Nevertheless, the dissolvability of QDs could cause polydispersion and thus low or passive involvement in the coupling processes. The presence of stabilizing surfaces increased QDs' hydrodynamism and, hence, increased their energy emission [11]. The formed fluorescent QD probes allowed for long-term monitoring (dynamics, kinetics, and stability along a reaction timeline) of the formed complexes. Additionally, the surface changes of QDs led to a reduction in cytotoxicity, which is caused by the presence of cadmium ions in the nanoparticle core [8,11–14]. Using tripeptides (e.g., GSH) [15], proteins [16], nucleic acids, or antibodies [17] as dispersity stabilizers significantly increased the size of QDs and thus eliminated the usage of the nanoparticles in biological or biomedical systems.

The other way was to use water-soluble "agents". To give the QDs solubility in water, the following monothiol ligands have been proposed: mercaptosuccinic acid (MSA), mercaptopropionic acid (MPA), and mercaptoundecanoic acid (MUA), which attach to the QD surface

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providing monodispersity without a meaningful change in size [11,13].

Among the different types of QDs proposed as biological probes, carbon-based dots (CDs) have also been pointed out as effective biomarkers. CDs' low toxicity, good aqueous loading capacity, and high biocompatibility have made them applicable in a variety of systems, including sensing, catalysis, photovoltaics, and optoelectronics [18,19].

Quantum dots' (QDs) unique properties give them the possibility of being used as selective and sensitive biomarkers in molecular imaging. Modifications of QDs' size or surface allow them to be used as accurate probes [3,4]. Among other possibilities, QDs might be used for atypical (non-standard) protein marking with high accuracy [20–23].

Metallothionein (MT) is a low molecular weight protein (6–7 kDa) belonging to a group of cysteine-rich proteins that were first isolated in 1957 from horse kidney by Margoshes and Vallee [24]. The coding genes are located at chromosome 16; in mammals, four isoforms have been identified: MT-1, MT-2, MT-3 and MT-4 [25,26].

One MT molecule consists of 60–68 amino acids (depending on the isoform). The most well-known isoforms are MT-1 and MT-2, which are located in most of the tissues, with particularly high concentrations in the liver and kidney. Ten subisoforms of MT-1/2 have been identified, nine of them contain 61 amino acids, and one, MT-1 G, which contains 62 amino acids [27,28].

Because of the high content of thiol groups (-SH) from the cysteine residues, MT is available for binding metals [26,29]. Structurally, MT has two domains, alpha (α) and beta (β), containing 11 and 9 cysteine residues, respectively. In the α domain, four metal ions can be bound; in the β domain, three metal ions from the II group can be bound. Due to the higher reactivity of the β domain, metal exchange takes place quicker there than in the α domain [30,31].

MT is one of the proteins that affects processes working in the maintenance of homeostasis, which require the participation of heavy metals such as cadmium, zinc and copper. At the same time, MT can influence the metabolism of those metals and their role in biological processes. Due to its structure, MT is also highly active in response to reactive oxygen species (ROS), thus protecting the organism against the harmful effects of oxidative stress and damage to the DNA structure [25,32].

QDs' interaction with proteins [20–23], including metallothionein (MT), is still of great interest to researchers [10,12,33–35]. It is known that in the process of conjugating rabbit MT with QDs, electrostatic interactions and structural effects might have an influence on the formed complexes. Several authors have pointed out that formed complexes for both human and rabbit MT with CdTe QDs are dependent on CdTe size [10,12,33]. Yet, the mechanisms underlying the coupling for human MT have not yet been identified.

The possibilities of interaction between human MT and CdTe QDs have been confirmed and analyzed [33]. Researchers have also analyzed the stability of human MT + CdTe QDs complexes [12]. Based on the literature regarding the types of interactions between MT and QDs, three potential approaches have been identified (Table 1).

Due to the great potential of QDs as probes and markers for MT, understanding the type of interaction is needed. The effectiveness, conditions and process of targeting human MT by CdTe QDs depends on how well the experiment is planned, and it is critical that the type of possible interactions be taken into account. In this article, the authors attempt to investigate the preferred types of interaction during the process of bond formation between human MT and CdTe QDs. To our knowledge, analysis of the type of interaction between human MT and CdTe QDs has not been published to date.

Table 1
Identified potential types of interactions between MT and QDs [3,10,33–35].

Type of interaction	Possible mechanism
by -SH groups	due to the presence of -SH groups, metals in the QDs (e.g., Cd, Zn) can bind or attach to the MT molecule
by electrostatic interactions	the electrostatic interactions arising from the pI of MT indicate that the molecules in this protein exist in a positively charged state in a water solution, supported by weak interactions like hydrogen bonds and van der Waals forces
by mixed interactions	a combination of these two types of interactions

2. Materials and methods

2.1. Chemicals

All necessary chemicals for QD preparation and MT were purchased from Sigma Aldrich (St. Louis, MO, USA). High-purity deionized water was used throughout the study.

2.2. QD preparation and characterization

CdTe QDs were prepared according to the Duan et al. method [36]. The full protocol for the CdTe QD preparation method, with modifications, was published in Guszpit et al [33]. The average hydrodynamic parameters and diameter distribution of the CdTe nanoparticles were determined by a Zetasizer (Malvern-zetasizer Nano ZS, Malvern, UK); size as hydrodynamic diameter was modeled by the Stokes-Einstein equation [37]. The increase in temperature during preparation caused increases in the QDs' hydrodynamic diameter as follows: 3.4 nm (blue QDs), 3.8 nm (green QDs), 4.5 nm (yellow QDs), and 5.2 nm (red QDs). Fluorescence and absorbance spectra of CdTe QDs have already been published by the authors [33]. For more information, please see Fig. 2A and B in the referred-to paper. For those data, the extinction coefficient was also calculated (see Supplementary material 1).

2.3. Human MT

Isolation and verification procedures for the fractions containing human MT were previously described by the authors [33]. In brief, pieces of human liver were washed several times with PBS and homogenized in a buffer (10 mM Tris/HCl, pH 8.6, 10 mM β-mercaptoethanol and 25 mM sucrose) at a ratio of 1:4 (v/v). The cytosol fraction obtained after a three-step centrifugation at 4 °C was precipitated with acetone and centrifuged again. Precipitates were dissolved in Tris-HCl pH 8.6 and centrifuged again. After isolation on a Sephadex G-75 column, the fractions were collected and concentrated using a YM3 membrane. Next, absorbance at λ = 220, 250 and 280 nm was measured. Fractions with a 250/280 ratio between 7.4–9 were combined and applied to a DEAE-cellulose (DE-52) column (2.6 cm × 120 cm) equilibrated with 10 mM Tris/HCl, pH 8.6. Peaks corresponding to MT-1 and MT-2 were obtained and the fractions with a high absorbance at 220 and 250 nm were collected, combined and concentrated using a YM3 membrane (Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-3 membrane, Millipore, ref. No UFC9-003, USA) [29].

Next, the concentrated samples containing both isoforms were analyzed using the Bradford method [28], SDS-PAGE, Western blot, ELISA [29], capillary gel electrophoresis (CGE), capillary zone electrophoresis (CZE) and capillary isoelectric focusing (CIEF) analysis [28]. Additionally, in previous articles, the authors analyzed the MT sample using: MALDI-TOF/TOF, chip-electrophoresis and the Brdicka reaction [12,33]. The results from MALDI-TOF/TOF are available in the supplementary material in [33], where a single peak with *m/z* 6189.806 was identified.

2.4. Fluorescence measurement of MT + QD complexes

The fluorescence intensity of the complexes of MT + QDs was