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Quantum dot-based assay for Cu^{2+} quantification in bacterial cell culture

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ABSTRACT

A simple and sensitive method for quantification of nanomolar copper with a detection limit of 1.2×10^{-10} M and a linear range from 10^{-9} to 10^{-8} M is reported. For the most useful analytical concentration of quantum dots, 1160 μg/ml, a $1/K_{sv}$ value of 11 μM Cu^{2+} was determined. The method is based on the interaction of Cu^{2+} with glutathione-capped CdTe quantum dots (CdTe–GSH QDs) synthesized by a simple and economic biomimetic method. Green CdTe–GSH QDs displayed the best performance in copper quantification when QDs of different sizes/colors were tested. Cu^{2+} quantification is highly selective given that no significant interference of QDs with 19 ions was observed. No significant effects on Cu^{2+} quantification were determined when different reaction matrices such as distilled water, tap water, and different bacterial growth media were tested. The method was used to determine copper uptake kinetics on *Escherichia coli* cultures. QD-based quantification of copper on bacterial supernatants was compared with atomic absorption spectroscopy as a means of confirming the accuracy of the reported method. The mechanism of Cu^{2+}-mediated QD fluorescence quenching was associated with nanoparticle decomposition.

Fluorescent semiconductor nanoparticles (NPs)[1] or quantum dots (QDs) are emerging as a powerful tool in nanotechnology, and the applications based on their properties are growing day by day [1].

During recent years, a variety of NPs have been used to develop analytical methods, some of them based on fluorescence quenching in the presence of different chemical species [2,3]. In particular, QDs are good candidates for analytical assays because they exhibit a Stern–Volmer quenching behavior [2–4]. This phenomenon has been related to a series of factors such as the chemical nature of the metal core and especially the capping agents surrounding the NP [2–5], both factors mostly determined by the synthetic method used. Most methods of QD synthesis involve high temperatures, anaerobic solutions, toxic reagents (beyond the metals and metalloids involved), large pH adjustments, and/or organic solvents—all conditions that complicate the procedure, affect NP toxicity, and increase production costs [6].

Considering these problems and the relevance of synthesis on QD properties, we recently developed a biomimetic synthetic method involving mild conditions resembling those found in biological systems [7,8]. This method uses the biological thiol glutathione (GSH) as capping and reducing agent to produce water-soluble QDs at low temperatures, aerobic conditions, and close to neutral pH. GSH as a capping agent is a well-documented molecule that enhances biocompatibility and solubility of different QDs such as CdTe, CdSe, and CdS [9–12]. The low production costs and unique properties of biomimetic QDs enhance their possible use for analytical assays in complex matrices such as culture media.

In this context, QD-based methods for quantification of different chemical species have been reported previously. Gattás-Asfura and Leblanc [13] reported the use of CdS QDs capped with different peptides for Cu^{2+} and Ag^{+} detection in aqueous solutions. They also tested the interference of different soluble cations on QD fluorescence, and no effects on fluorescence were observed. In addition, Zhang and coworkers [14] used cysteine-capped CdSe/CdS QDs for Cu^{2+} detection in vegetable samples; in this case, quenching was detected only with Cu^{2+} and no interferences were reported. Finally, Wang and coworkers [15] developed an arsenic quantification with GSH or mercaptoacetic acid (MA)-capped CdTe QDs, neither of which exhibited significant copper quenching. All of these

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[1] *Abbreviations used:* NP, nanoparticle; QD, quantum dot; GSH, glutathione; MA, mercaptoacetic acid; LB, Luria–Bertani; AAS, atomic absorption spectroscopy; RCF, relative centrifugal force; EDTA, ethylenediaminetetraacetic acid; MIC, minimal inhibitory concentration.

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antecedents indicate that QDs are a suitable, sensitive, and specific tool for detection of different metal ions. However, no QD-based detection assay has been developed for copper metal detection in biological culture media. A variety of metal ions are relevant to be studied and detected on cell growth cultures, especially when studying microorganisms that display an intricate relationship between metals and metabolism. One of these biologically relevant metals is copper, specifically the copper cation Cu^{2+}, which is essential for all living organisms and is required for redox reactions catalyzed by cellular enzymes, among other functions [16]. In addition, Cu^{2+} displays many industrial and technological applications because of its semiconductor properties [17,18].

To date, several analytical methods, such as colorimetry, atomic absorption spectrometry, and inductively coupled plasma atomic emission spectrometry, have been used for copper determination [19,20]. However, simple and inexpensive Cu^{2+} quantification methods in liquid media are required in studies of copper interaction with microorganisms, particularly in applications related to biomining and bioremediation and, most recently, with the capacity of some bacteria to biosynthesize copper NPs [18]. Such a method will contribute to the study of bacterial/Cu^{2+} interaction by allowing Cu^{2+} determination (i) when investigating whether metal resistance of bacterial strains is related to the ability to avoid copper uptake and decrease toxicity (a particularly relevant issue when examining new copper-resistant environmental isolates), (ii) when studying the ability of microorganisms to obtain Cu^{2+} from the environment for bioremediation strategies, (iii) when determining the effectiveness of bacterial bioleaching in the mining industry by determining Cu^{2+} solubilization from ores, and (iv) when investigating the production of copper sequestration by molecules produced by bacterial cells (phosphates, peptides, and polymers), among others.

In this context, determination of copper ion consumption by microorganisms in culture media using QDs will allow a fast and easy determination of metal absorption kinetics.

Based on this information and using biomimetically synthesized QDs, we have developed a rapid and inexpensive copper quantification assay using CdTe–GSH QDs. The method was validated in different reaction matrices and used to study bacterial copper consumption in both complex and well-defined growth media.

Materials and methods

Reagents

$MnCl_2$, $CaCl_2{\cdot}2H_2O$, KCl, NaCl, $MgCl_2{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, CsCl, $HgCl_2$, $AgNO_3$, and $NiSO_4{\cdot}6H_2O$ were obtained from Merck.

$Co(NO_3)_2{\cdot}6H_2O$, $CdCl_2$, $NaAsO_2$, $Na_2AsO_4{\cdot}7H_2O$, $InCl_3$, $CuSO_4{\cdot}5H_2O$, Na_2SeO_3, K_2TeO_3, Li-acetate, TiO_2, GSH, and $NaBH_4$ were obtained from Sigma–Aldrich and used as received. Bacterial growth medium ingredients were purchased from Difco.

Cu^+ as copper(I) tris–thiourea sulfate complex was obtained by reducing 680 mM $CuSO_4{\cdot}5H_2O$ in the presence of thiourea (2.2 M) under aerobic atmosphere and heating at 55 °C for 10 min. The dissolved mixture was cooled, and a white precipitate indicative of Cu^+ formation was observed. The solution was filtered and recrystallized in 1.3 M thiourea solution.

Sea water samples were collected from Ventanas, Chile, and stored at room temperature. Tap water samples were taken directly from the Santiago water system and stored at room temperature.

QD synthesis

QD synthesis was carried out following a biomimetic protocol described previously by our group that produces highly fluorescent QDs that have already been characterized [7,8,10]. Two different sizes of QDs—with emission peaks at 510 (green) and 610 nm (red) when excited at 350 nm—were synthesized. The synthetic yield was determined by precipitating QDs with two volumes of isopropyl alcohol and drying and weighing the precipitate as described previously [7].

Quenching experiments

Green and red CdTe–GSH QDs (1160 μg/ml) were exposed to 0.1, 7.9, 15.7, and 157 nM Cu^{2+} solutions and fluorescence spectra after excitation at 350 nm was determined using a Synergy H1 M multiple-well plate reader (BioTek). Optimal concentration of QDs for Cu^{2+} quenching experiments was evaluated at 580, 1160, and 2380 μg/ml green CdTe–GSH QDs. To determine optimal copper incubation times, QD fluorescence decay was evaluated at different times after copper exposure using the conditions mentioned above.

Quenching effect of ions

Fluorescence of CdTe–GSH QDs (1160 μg/ml) was assayed in solutions amended with cadmium (Cd^{2+}), copper (Cu^+/Cu^{2+}), calcium (Ca^{2+}), sodium (Na^+), potassium (K^+), cobalt (Co^{2+}), zinc (Zn^{2+}), manganese (Mn^{2+}), magnesium (Mg^{2+}), indium (In^{3+}), arsenic (As^{3+} and As^{5+} oxyanions), selenium (Se^{4+} as the oxyanion selenite), tellurium (Te^{4+} as the oxyanion tellurite), lithium (Li^+), titanium (Ti^{4+}), cesium (Cs^+), mercury (Hg^{2+}), silver (Ag^+), and nickel (Ni^{2+}) at 1 μg/ml final concentrations. QD fluorescence in the presence of ions was determined as described above.

Effect of different aqueous culture media and water

Copper quenching on green CdTe–GSH QDs (1160 μg/ml) prepared in distilled water and Luria–Bertani (LB), R2A, and M9 media was determined. LB, R2A, and M9 media were prepared as described by Baev and coworkers [21], Massa and coworkers [22], and De Kievit and coworkers [23], respectively. LB and R2A are complex media using yeast extract, and M9 is a well-defined medium using glucose as carbon source. QDs were incubated with different Cu^{2+} concentrations ranging from 1 to 188 nM. Standard curves were constructed in distilled water and bacterial growth media by using 1, 1.97, 3.95, 7.9, and 15.7 nM Cu^{2+} and 19.7, 39.5, and 79 nM Cu^{2+}, respectively.

Cu^{2+} quantification assays

Cu^{2+} solutions (94 nM) were prepared in distilled, tap, and sea water. Copper content was determined with green CdTe–GSH QDs (1160 μg/ml) using conditions described above (see quenching experiments). The same experiment was performed in bacterial growth media (LB, R2A, and M9) but using 7.9- and 790-nM Cu^{2+} solutions. In all experiments, Cu^{2+} was also quantified by flame atomic absorption spectroscopy (AAS). All solutions for analysis by fluorescence quenching and by AAS were diluted before analysis to accommodate the linear range.

Copper and cadmium AAS detection

Copper and cadmium were detected employing an AA-6200 flame atomic absorption spectrometer (Shimadzu) at 324.7 and 228.8 nm, respectively. In cadmium AAS experiments, background correction was achieved using a deuterium lamp. Samples were diluted in distilled water for their determination.