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Probing metabolic stability of CdSe nanoparticles: Alkaline extraction of free cadmium from liver and kidney samples of rats exposed to CdSe nanoparticles

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ABSTRACT

Cadmium selenide nanoparticles (CdSe NPs) exhibit novel optoelectronic properties for potential biomedical applications. However, their metabolic stability is not fully understood because of the difficulties in measurement of free Cd from biological tissues of exposed individuals. In this study, alkaline dissolution with tetramethylammonium hydroxide (TMAH) is demonstrated for selective determination of free Cd and intact NPs from liver and kidney samples of animals that were exposed to thiol-capped CdSe NPs. Aqueous suspensions of CdSe NPs (3.2 nm) were used to optimize the conditions for extracting free Cd without affecting NPs. Nanoparticles were found to aggregate when heated in TMAH without releasing any significant Cd to solution. Performance of the method in discriminating free Cd and intact NPs were verified by Dogfish Liver (DOLT-4) certified reference material. The samples from the animals were digested in 4 mL TMAH at 70 °C to extract free Cd followed by analysis of aqueous phase by ICP-MS. Both liver and kidney contained significant levels of free Cd. Total Cd was higher in the liver, while kidney accumulated mostly free Cd such that up to 47.9% of total Cd in the kidney was free Cd when NPs were exposed to UV-light before injection.

1. Introduction

Nanotechnology has been revolutionizing the human life by invention of novel nanoparticles (NPs) of intriguing physical and chemical properties for technological and medicinal applications. However, this revolution is not worry-free due to the potential health risks associated with the production and use of products containing nanoparticles [1–5]. Colloidal semiconductor nanoparticles of cadmium selenide (CdSe), are probably among the most concerned NPs because of their cytotoxicity and potential adverse effects on human and environmental health. Nanoparticles of CdSe exhibit bright, photo-stable and size-tunable emission (fluorescence) that make them optimal fluorophores and potential alternatives to traditional dyes, such as Rhodamin Green, for in vivo biomedical imaging and diagnostics [6–8]. The toxicological issues have been reviewed in several articles [1,9,10]. Nevertheless, there is still little known about the pharmacokinetics (accumulation, distribution, metabolism and elimination) of CdSe NPs and other similar nanoparticles. In vitro studies provide useful information about the toxicity issues and health risks but are not sufficient to fully address the safety issues on human and environmental health.

The major hurdle in addressing the safety issues of semiconductor nanoparticles is the lack of biosafety data mainly because of the fact that toxic effects vary substantially depending on physicochemical properties and environmental conditions, such as size, charge, outer coating, concentration, physical stability and solubility [1,5,11,12]. For instance, CdSe NPs could exhibit adverse effects even if the NPs remain stable in the body [12]. The effects become more detrimental if cadmium ions (Cd^{2+}) are released to the body as outer coating intended to enhance stability and biocompatibility undergoes metabolic degradation. Both Cd and Se are toxic to humans causing hepatic, renal, and neurologic toxicities [13–17]. Cadmium also interferes with DNA repair and metabolic proteins, and substitutes for Zn^{2+}. Initially it accumulates in the liver with a half life of 15–30 years and is gradually mobilized to kidney causing nephrotoxicity. The cadmium–metallothionein complex is also

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toxic to the kidney since most chelating agents that remove Cd^{2+} from the liver are excreted by the kidney [18–20]. Thus, accurate tracing of CdSe NPs and their species in the body is important to explore their physiological fate and safety.

Studies attempting to elucidate the effects of CdSe NPs have attributed their cytotoxicity to the formation free Cd ions from NPs [21–23]. Derfus et al. [21] for instance reported that untreated CdSe NPs were innocuous to liver hepatocyte, but cell viability increased when NPs were exposed to UV light, which was explained by the oxidation of cap and consequently liberation of free Cd ions. Similarly, the toxic effects were attributed to poisoning from Cd ions in the growth medium when a freshwater organism, *Daphnia magna*, were exposed to mercaptoundecanoic acid (MUA) coated CdSe NPs [22]. In the same study, polyetheylene oxide (PEO) coated CdSe NPs also induced acute toxicity on *D. magna*, though no significant Cd was detected in the growth medium, indicating a different mode of action from intact NPs [22]. Citrate stabilized CdSe NPs were also reported to release Cd ions to solution during the exposure of planktonic culture, *Pseudomonas aeruginosa*, and the NPs exhibited a dose dependent toxic effects [23]. At low dose, NPs induced similar effects to that of Cd ions, but were more toxic than Cd ions at higher doses [23]. These studies support the fact that the effects of CdSe NPs will not only vary among species, but also with routes of synthesis and coating materials used. Moreover, Cd ions are released from CdSe NPs if the core is not protected properly, which could infact confound the actual effects of NPs.

As occurred in vitro, CdSe NPs may degrade in vivo releasing Cd ions that accumulate through the body and mediate the effects of exposure. Thus, detection of total Cd in certain organs of exposed individual, without any knowledge of Cd ions and intact NPs, is not sufficient to fully explain their metabolic stability, toxicology and mechanism of action. To date, few in vivo studies [11,24–27] investigated the accumulation and kinetics of NPs on animal models, but were not able to provide a map of the species distribution and free Cd levels in the tissues. In most cases, localization of NPs was targeted in tissues and cells by using the fluorescence microscopy without any information about the levels of free metal ions [24–27]. This is mainly because of the lack of methodology to achieve separation of free Cd and intact NPs from soft tissues. The extraction of Cd from tissue samples requires acid treatment, but this strategy is not a viable method for selective determination of free Cd since intact CdSe NPs are also decomposed to Cd and Se [21]. Contrary to this, detection of free Cd in culture studies do not require aggressive chemical treatment, and therefore is a relatively straightforward task since free Cd ions in the growth medium can be separated from NPs by means of physical methods (e.g., dialysis or ultrafiltration). Nonetheless, the information does not reflect the free Cd levels accumulated by the cells or microorganism.

Detection of free metal ions from the tissues of individuals that are exposed to NPs is a challenging task without altering the composition of intact NPs. Yet, the development of robust and reliable methods will provide indispensable tools to address the concerns associated with environmental and human health effects of nanoparticles. For this purpose, we have investigated the experimental conditions for selective determination of Cd ions and CdSe NPs from biological samples. The specific objectives of this study are (1) to develop a method for quantitative separation of Cd ions and intact NPs originating from degradation of thiol-capped CdSe NPs, (2) to apply the procedure to samples collected from rats exposed to CdSe NPs to determine the distribution of the Cd ions and intact CdSe NPs. Thiol-capped CdSe NPs were synthesized, purified and used without further modification or additional ZnS coating. We opted for bare thiol-cap as it provides minimal surface protection for NP core, and therefore, is ideal to delineate the metabolic stability of the NPs and the fates of degradation products in vivo.

2. Experimental

2.1. Materials and solutions

Cadmium chloride ($CdCl_2{\cdot}2H_2O$ 98%, Sigma–Aldrich), selenium powder (99%, Acros Chemicals), thioglycolic acid (98%, $d = 1.325\ g\ mL^{-1}$, Sigma–Aldrich) and $NaBH_4$ (98% Sigma–Aldrich) were used for synthesis of CdSe QDs. Tetramethyl ammonium hydroxide (TMAH) solution (99.99%, $d = 1.016\ g\ mL^{-1}$) was purchased from Alfa Aesar. Trace metal grade nitric acid (HNO_3) and hydrochloric acid (HCl) were used. Teflon tubes (Savillex, Minnetonka, MN) were used for heat-assisted extraction and dissolutions. Stock Cd(II) solution (1.0 M) was prepared by dissolving 9.15 g of $CdCl_2$ in 50 mL water. Selenium stock solution (0.5 M) was made by dissolving 3.95 g Se powder in sufficient volume (ca. 4 mL) of concentrated HNO_3. Upon dissolution, the solution was heated to get rid of excess HNO_3 and then diluted to 100 mL with 10% HCl to reduce from Se(VI) to Se(IV). Thioglycolic acid was used directly from the bottle. $NaBH_4$ solution (10% w/v) was prepared freshly in 0.1% NaOH solution. Multi-element solutions and calibration standards were prepared from 10 $\mu g\ mL^{-1}$ multi-element solution (Spex Certiprep).

2.2. Apparatus

UV–vis absorption spectra were recorded by using a HP 8453 UV-visible spectrometer. Fluorescence spectra were taken by Horiba Jobin Yvon benchtop FluoroMax-2 spectrofluorometer. A Fisher Scientific Model 100 Sonic Dismembrator equipped with titanium alloy probe (12.5 cm long, 3.1 mm wide tip) was used for ultrasonic agitation of samples. The transmission electron microscopy (TEM) images of the NPs were acquired by JEOL-1011 TEM instrument. The resolution of JEM-1011 is 0.2 nm lattice with magnification of 50 to 1×10^6 under the accelerating voltage of 40–100 kV. The colloidal solution in water was dropped onto 50 Å thick carbon-coated copper grids and allowed to dry. A 48-well digestion block (Digiprep MS, SCP Science, Champlain, NY) was used for extractions and dissolutions. Elemental measurements were performed by Varian 820-MS ICP-MS instrument (Varian, Australia), which was equipped with a peltier-cooled glass concentric spray chamber, micromist nebulizer (400 $\mu L\ min^{-1}$), standard one-piece, low flow, ball-and-socket connection torch quartz, standard Ni sampler and skimmer cones, patented Collision Reaction Interface (CRI), a unique 90 degree ion mirror delivering exceptional sensitivity, all-digital detector; Discrete Dynode Electron Multiplier (DDEM, Model AF250, ETP Australia) providing nine decades of linear dynamic range. Samples were introduced via Varian autosampler (Model SPS3). The ICP-MS instrument was optimized by using 5 ppb Ba, Be, Ce, In, Mg, Th, for sensitivity, doubly charged ions and oxides. Data were collected using ^{111}Cd and ^{114}Cd isotopes at peak hopping mode using manufacturers recommended settings. The results for Cd are reported as average of ^{111}Cd and ^{114}Cd signals. Internal standard correction was performed with ^{103}Rh that was added to the sample stream on-line.

2.3. Preparation of CdSe NPs

Procedure for synthesis of water soluble CdSe NPs was adapted from the protocols described by Gaponik et al. [28] and Chen et al. [29]. In a typical synthesis 0.5 mL of 1.0 M $CdCl_2$ solution and 0.5 mL of thioglycolic acid ($HSCH_2COOH$) as capping agent were added to 250 mL deionized water in a three-neck flask under stirring. The solution was initially turbid because of the incomplete solubility of Cd–thiolate complex, but clear solution formed at around pH 9–9.2 by adding appropriate volume of 1.0 M NaOH. Selenium solution (10 ml of 0.5 M Se(IV) in 10% HCl) was placed in another three neck