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Characterization of Cr(V)-induced genotoxicity using CdTe nanocrystals as fluorescent probes

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CdTe nanocrystals capped by cysteamine were synthesized to study Cr(V)-induced genotoxicity. On the surface of TiO_2 thin films, the stepwise process of DNA breakage induced by Cr(V)-GSH complexes was vividly observed by using CdTe-DNA self-assembled fluorescent probes; In acetate buffer solution, an analytical method was developed to detect Cr(V)-induced genotoxicity with CdTe fluorescent probes.

Introduction

Chromium(VI) compounds have long been recognized as carcinogenic and mutagenic agents that pose serious risks to human health[1]. Numerous epidemiological studies indicate that high carcinogenic risks are associated with chromate exposure[2]. However, the effect of chromium on human health is perhaps one of the most controversial issues because neither trace Cr(VI) nor Cr(III) species alone contribute to DNA damage[3]. Cr(VI) can penetrate into cells rapidly through the sulfate channel, and cause metabolic reduction in the presence of glutathione, ascorbic acid, or cysteine[4]. During the reduction process of chromate in vivo, Cr(IV) and Cr(V) may be the main sources of Cr-induced genotoxicity[5]. Levina et al.[6] have proven that Cr(VI) and GSH can form unstable Cr(V)-GSH complexes, and Yang *et al.*[7] have characterized DNA conformational change and breakage induced by the interaction of Cr(VI) and GSH. However, the genotoxicity of chromium are far from fully understood because of the unstable properties of Cr(V)-GSH complexes and the complexity of Cr-induced genotoxicity.

Traditional organic small molecule DNA imaging probes, including acridine orange, fluorescein, resistance blue, rhodamine, ethidium bromide, have been widely used for DNA imaging probes. In comparison with these organic molecules, semiconductor nanocrystals (NCs) have a lot of especial advantages[8], such as symmetrical fluorescence spectra, high quantum yields, wide tunable range of excited and emission spectra, large Stokes shifts, long fluorescence lifetimes, and stronger resistance to photobleaching. Since the first report on using NCs as fluorescent labels to stain biological samples, semiconductor NCs have attracted considerable attention as novel fluorescent probes in various biochemical and bioanalytical fields[9]. In this study, CdTe NCs were exploited to investigate the genotoxicity of Cr(V)-GSH complexes. Breakage processes of DNA induced by Cr(V)-GSH complexes were observed under an inverted fluorescence microscope. In pH 5.6 acetate buffer solution, an analytical method was developed to determine Cr(V)-induced DNA damage using CdTe NCs as fluorescent probes.

Results and discussion

Synthesis of CA-CdTe NCs

CdTe NCs capped by cysteamine hydrochloride (CA, ≥98.0%, Sigma) were synthesized by a modified route[10]. Briefly, tellurium powder (Te; -30 mesh, ≥99.9%, Aldrich) was used as Te source to obtain NaHTe aqueous solution. NaHTe solution was added to a nitrogen-saturated Cd^{2+} aqueous solution in the presence of CA ligands to form CdTe NC precursor (pH 5.6). After aging for ten hours at room temperature, the precursor solution was heated at 90 ºC for different times to obtain CA-CdTe NCs. Furthermore, CdTe NCs capped by other thiol ligands were synthesized similar to the literature[11].

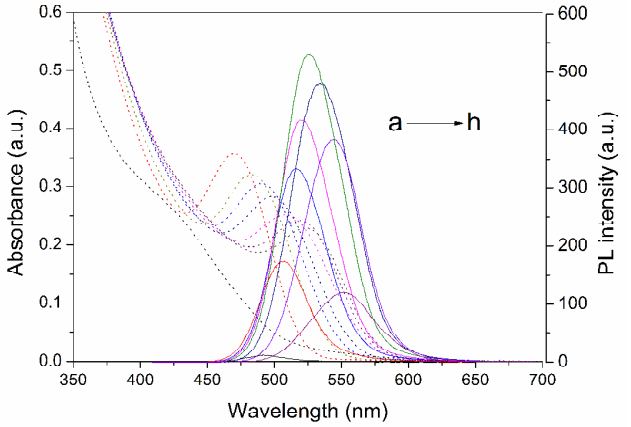

Fig. 1 Temporal evolution of absorption and PL spectra of CA-CdTe NCs, heating time (a→h): 0, 5, 10, 20, 40, 60, 90, 120 min. λ_{EX}=325 nm.

UV-Vis absorption (UV-3600 spectrometer, Shimadzu) and photoluminescence spectra (PL; RF-5301PC spectrometer, Shimadzu) of CA-CdTe NCs are displayed in Fig. 1. As the heating time increased from 0 min to 90 min at 90 ºC (Fig. 1, a→h), the PL peak shifted from 480 nm to 590 nm due to quantum confinement effect. CA-CdTe NCs emitting at 535

nm were used in succedent experiments unless otherwise specified, and the quantum yield reached up to 38%.

Peparation of Cr(V)-GSH complexes

The formation process of Cr(V)-GSH complexes is similar to the reference[7], and the ratio of GSH to Cr(VI) was set to 7.5/1. In brief, $K_2Cr_2O_7$ (5.0 μM, 10 mL; ≥99.5%, BioXtra) and L-Glutathione reduced (37.5 μM, 10 mL; GSH, ≥98.0%, Sigma) was mixed with a magnetic stirrer in pH 5.6 acetate buffer solution and then incubated at 37 ºC for 20 min. Freshly prepared Cr(V)-GSH complexes were used for subsequent experiments.

Visualization of the Cr(V)-induced DNA breakage

Formation of TiO_2 thin films on silanized slides is similar to the literature that used titanium dichloride diethoxide (TDD, Tetrahedron) as a starting material[12]. Silanized glass slides were immersed into an anhydrous toluene (99.8%, Aldrich) solution containing 0.1 M TDD for 30 min at room temperature under a N_2 atmosphere (≥99.99%). Bright-field microscope images of TiO_2 thin films on silanized slides were characterized (section I in ESI†).

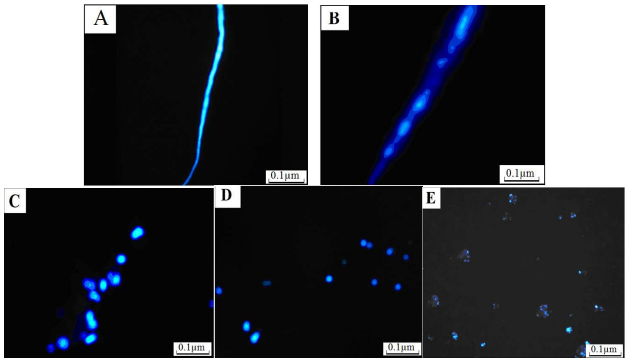

Fig. 2 Stepwise process of Cr(V)-induced DNA breakage captured with an inverted fluorescence microscope. Images (A-E) incubated for 0, 10, 20, 40, 60 min.

Deoxyribonucleic acid (DNA; calf thymus, Sigma; C_{DNA} = 0.05 *m*M, 10 mL) and CdTe (5.0 *m*M, 0.1 mL) solution were mixed using a magnetic stirrer, spread evenly on the surface of TiO_2 thin films, and observed with an inverted fluorescence microscope (TE2000, Nikon). Fig. 2 (A) is the entire image of DNA-CdTe, which appears as a long luminescent chain. Further experimental results showed that CdTe NCs with positive surface charge cannot form DNA-CdTe fluorescent probes on TiO_2 thin films, whereas CdTe NCs with negative surface charge can form fluorescent probes (Section I in ESI†). The interaction between positive charges of NCs and negative charges of DNA's phosphate backbone may be playing a main role in the formation of CdTe-DNA fluorescent chains.

Cr(V)-GSH (37.5 μM, refer to the concentration of Cr; 2.0 mL) and DNA-CdTe solution (50.0 μM, 10.0 mL) were mixed thoroughly and spread evenly on the surface of TIO_2 thin films. Images were captured with an inverted fluorescence microscope incubated for 0, 10, 20, 40, 60 min at room temperature. Fig. 2(B) shows the image of DNA-CdTe solution incubated with Cr(V)-GSH complexes for 10 min, which an apparent change of DNA conformation appears. For 30 min of reaction, DNA fragments with different lengths can be observed from (C), showing a clear chain scission. Seen from images (D) and (E), the DNA strand can be observed and the majority of DNA strand breaks into pieces with the gradual drop of PL intensity. The results from Fig. 2 reveal that the Cr(V)-induced DNA conformational changes are subsequently accompanied by constant DNA strand breakage. To our knowledge, it's the first time that the stepwise process of Cr(V)-induced DNA damage is vividly observed using semiconductor NCs as fluorescent probes, and a possible reaction mechanism is proposed (section II in ESI†).

A similar phenomenon of Cr(V)-induced DNA breakage has been observed with an atomic force microscopy (AFM; Nanoscopy IIIa, Digital). AFM images display the change of DNA conformation and DNA strand breakage (section III in ESI†), which are consistent with the observation under the inverted fluorescence microscope.

Determination of DNA damage with CdTe NCs probe

Cr(V)-induced DNA damage in human B lymphoblastoid cells was detected by a single-cell gel electrophoresis assay (comet assay, section IV in ESI) as described previously[13]. The degree of Cr(V)-GSH complexes induced DNA damage was detected and the corresponding curve between DNA and Cr(V)-GSH complexes was plotted. As seen from Fig. 3 (solid line), when Cr(V)-GSH complexes were successively added from 0, 0.5, 1.0, 2.0, 5.0 to 10.0 μM, the degree of Cr(V)-induced DNA damage (Tail DNA%) gradually increased from 3.2%, 7.3%, 11.6%, 20.4%, 37.5% to 48.2%.

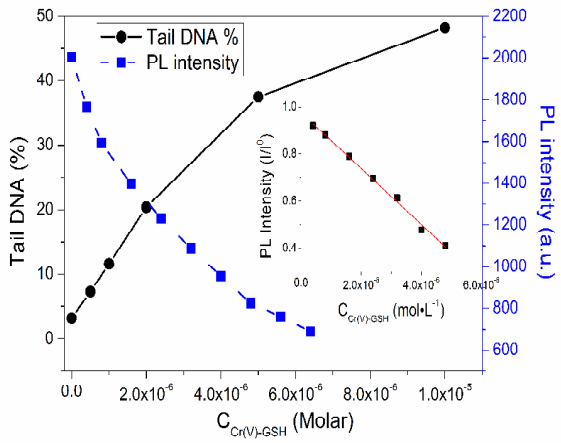

Fig. 3Percent of tail DNA and CdTe NCs' PL intensity change induced by Cr(V)-GSH complexes. Insert: linear relation between PL intensity of CdTe-DNA solution and Cr(V)-GSH complexes.

In pH 5.6 acetate buffer solution, CA-CdTe NCs and DNA were mixed to form a clear, transparent solution (C_{CdTe} = 1.0 *m*M and C_{DNA} = 10.0 μM). When Cr(V)-GSH complexes were successively added from 0, 0.4, 0.8, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6 to 6.4 μM, the PL intensity of the mixed solution gradually decreased from 2003 to 690 (Fig. 3, dashed line). Moreover, the PL intensities of CdTe NCs are quenched by Cr(V)-GSH complexes in a concentration-dependent manner, from 0.4 μM to 4.8 μM (R^2 = 0.995, insert in Fig. 3), which could be best

expressed by Stern-Volmer equation[14]: $I_0/I = 1+ K_{sv}C$. Where I_0 and I are the intensities in the absence and presence of Cr(V)-GSH complexes, respectively. K_{sv} is the Stern-Volmer quenching constant, and C is the concentration of the quencher.

in vivo, Cr(V)-GSH complexes have a vital role in Cr-induced genotoxicity [15]. In this study, CdTe NCs can be developed as a sensitive fluorescent probe to detect the concentration of Cr(V)-GSH complexes, which can be an indicator for estimating Cr(V)-induced DNA damage.

Possible mechanism of Cr(V)-induced DNA damage

Cular dichroism (CD; Chirascan, Applied Photophysics), Zeta potential (ζ; Zetasizer Nano ZS, Malvern) and time-resolved luminescence spectra (FLS920 spectrometer, Edinburgh) were conducted to reveal a possible mechanism of Cr(V)-induced DNA breakage.

Zeta potential was used to determine the surface charge density of CA-CdTe NCs. The ζ of CdTe-DNA solution reaches 20.2 mV (Fig. S3, section V in ESI). When Cr(V)-GSH complexes (5.0 μM) are added, the ζ of mixed solution decreases to 4.6 mV. The changes in ζ may be attributed to the electronic interaction of DNA-CdTe (positive charges) and Cr(V)-GSH complexes (negative charges).

The absorption bands of CD spectra at 278 and 246 nm (section VI in ESI) confirmed that calf thymus DNA has a double-helix conformation[16]. The CD spectra are altered with the constant blue-shifting of bands at 246 nm and red-shifting at 278 nm, indicating that the DNA double-helix conformation is substantially changed.

PL intensity of NCs can be quenched by inorganic ions or biological molecules, and two main types of reaction mechanisms have been proposed[17]: one is the formation of new complexes between quenchers and NCs, resulting in a red-shift of the PL spectra; and the other is the electron transfer process, leading to the change of PL intensity and fluorescence lifetime. As seen from Fig. S5 (section VII in ESI†), the gradual red-shifting of PL spectra is observed when Cr(V)-GSH complexes are constantly added; at the same time, the PL intensity and PL decay constantly decrease, indicating that electron transfer process and new complexes may be produced of NCs, Cr(V)-GSH, and DNA. New complexes may be formed between the reduction product of Cr(V)-GSH complexes and DNA fragments.

Conclusion

The stepwise process of DNA breakage induced by Cr(V)-GSH complexes were visually characterized with an inverted fluorescence microscope using CdTe-DNA self-assembled fluorescent probes. In acetate buffer solution, Cr(V)-induced DNA damage could be determined with CdTe NCs fluorescent probes. Zeta potential, CD spectra, and time-resolved fluorescence spectra experiments revealed that electronic interaction and new complexes may be produced during the Cr(V)-induced DNA conformational change, denaturation, and DNA strand breakage. The current data expand the application fields of nanomaterial and add new information for understanding of Cr(V)-induced genotoxicity.

Notes and References

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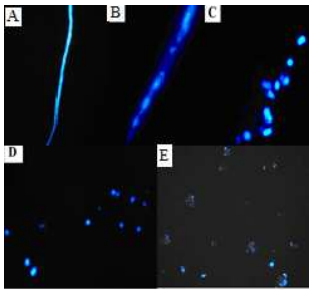
Graphical Abstract



Stepwise process of Cr(V)-induced DNA breakage incubated for different time under an inverted fluorescence microscope.