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Visual detection of cyclobutane pyrimidine dimer DNA damage lesions by Hg^{2+} and carbon dots

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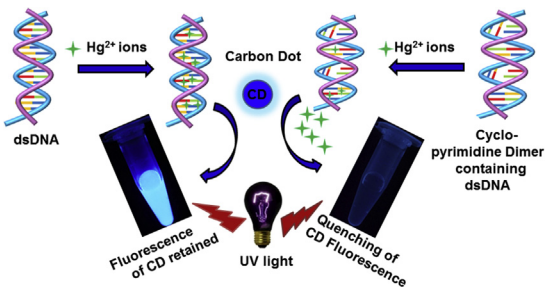
HIGHLIGHTS

- Biosensing of CPD lesion in DNA using Hg^{2+} ions and carbon dots (CDs) nanoparticle.
- Binding of Hg^{2+} is disrupted in the presence of CPD in DNA.
- Free Hg^{2+} ions in solution get adsorbed over CDs and quench the fluorescence of CDs.
- Thermodynamic parameter of Hg^{2+} binding with DNA in correlation with optical studies.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyclobutane pyrimidine dimmers (CPDs) and 6–4-[pyrimidine-2′-one] pyrimidine (6-4 PP) are major UV induced DNA damage lesions formed from solar radiation and other sources. CPD lesions are presumably mutagenic and carcinogenic that inhibit polymerases and interfere in DNA replication. An easy and cost effective way for visual detection of these lesions by using fluorescence based method is shown here. Artificial UVA and UVB lights were used for the generation of CPD and 6-4 PPs in selected DNA samples. Binding of Hg^{2+} ions with DNA before and after induction of CPD and 6-4 PP lesions was evaluated in the presence of highly fluorescent blue emitting carbon dots (CDs). Induction of CPD and 6-4 PPs in DNA causes distortion of DNA structure which hinders the binding of Hg^{2+} ions to DNA nucleobases. Quenching of fluorescence intensity of CDs by unbound Hg^{2+} ions was found to be proportional to the amount of CPD and 6-4 PP lesions induced by UV irradiation of DNA samples that offer a biosensing platform for the sensitive detection of CPD lesions in DNA. The fluorescent quenching was visually detectable using hand held UV light without the intervention of any equipment.

1. Introduction

Cyclobutane Pyrimidine Dimer (CPD) and 6–4-[pyrimidine-2′-one] pyrimidine photoproducts (6-4 PPs) in DNA are UV light induced DNA damage lesions formed by covalent linking of adjacent pyrimidine bases [1–3]. Exposure to UV-A & B light reaches the surface of earth from Sun and could effectively generate CPDs and 6-4 PPs in our DNA [4–6]. The comparative rate for CPD production is ~90% while it is ~10% for 6-4 PP [7,8]. Pyrimidine dimers are the major cause of mutation of cytosine (C) to thymine (T), cell killing, immunosuppression and carcinogenesis [9–11]. Enhanced exposure to UV radiation results in the profound rise in CPD production with slow repair that are related

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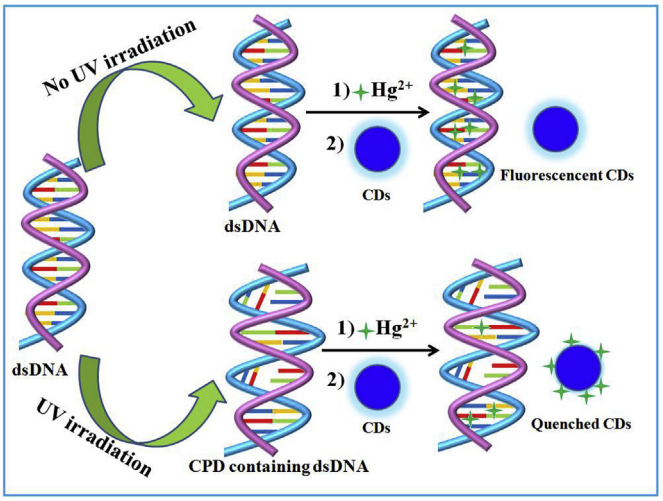
to skin related diseases including induction of melanoma and skin cancer in humans [12–14]. Reportedly, p53 mutations (C to T conversion) in TP53 gene of Xeroderma Pigmentosum patients have decreased repair efficiency of these lesions underlining the important role of CPD in melanoma [15,16]. Thus, detecting these mutagenic pyrimidine dimers in DNA is essential to diagnose premature aging, skin cancer and maintenance of genome integrity [17–19]. Consequently, development of tools to identify these DNA lesions is of broad scientific interest. Herein, we report an easy, economic and sensitive method for visual detection of the presence of pyrimidine dimer lesions in DNA by using mercury (Hg^{2+}) and carbon dots (CDs) as an effective solution phase biosensing platform.

Numerous methods have been adopted for detection of CPD such as flow cytometry, radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), alkaline gel electrophoresis by T4 endonuclease V, mass spectrometry, Atomic Force Microscopy (AFM) and near-infrared spectroscopy [20–24]. However, multistep tedious process, time consumption and costly devices may limit their applications and practical biomedical utility. This essentially necessitates the development of a simple yet reproducible strategy for the detection of these DNA damage lesions. Recently, graphene oxide has been utilised for detection of UV induced mutagenic thymine dimers [25]. The detection strategy requires conjugation of DNA with fluorophores that are subsequently quenched to give a signal. In the current study, we have used CDs as a fluorescent probe to track the differential binding of Hg^{2+} ions with normal and CPD lesion containing DNA.

The interaction of DNA nucleobases and metal ions has emerged as an area of focus for quite some time [26–28]. The transition metal ions preferably bind with the donor atoms of heterocyclic nucleobases unlike the counter ions (Mg^{2+} and Na^{+}) of DNA that interact with oxygen atoms of phosphate backbone of DNA [29]. Among the transition metal ions, binding of Hg^{2+} to DNA's nucleobases particularly with thymine (T) and then cytosine (C) is remarkable [30]. Specific interaction of Hg^{2+} ions with pyrimidine nucleobases have shown promising applications in DNA nanotechnology such as single nucleotide polymorphism detection, ultrasensitive detection of Hg^{2+} in drinking water and DNA assembly formation [31–33]. The source of Hg^{2+} being easily available low cost mercury salts, it instigates special interest to explore this metal ion for broad biomedical applications.

Carbon derived nanoparticles like CDs, carbon nanotubes, graphene etc. have shown diverse physical, chemical and optical properties. In particular, CDs are considered as an effective alternative to semiconductor quantum dots and other metal based nanoparticles [34]. Low cost convenient route of synthesis, biocompatibility and high fluorescent properties of CDs pave the way for its use in biosensing, drug delivery, catalysis, electronic applications etc [35–37]. The intense fluorescence of CDs are quenched by Hg^{2+} -a well-known electron acceptor which can interact with CDs through electrostatic interaction that prompt the ultrasensitive detection of the latter [38]. In the present study, the interaction of Hg^{2+} ions with DNA and CDs were put together to develop a biosensing method for the detection of UV induced damage in DNA, in particular CPD and 6-4 PP lesions. The CPD and 6-4 PP lesions in T rich oligonucleotide and pUC19 were created on irradiation by using UVA and UVB lamps. The presence of CPD in oligonucleotide and pUC19 plasmid DNA introduces conformational distortion of the DNA that results in poor interaction of Hg^{2+} with these UV irradiated dsDNA. The exclusion of Hg^{2+} from DNA and their subsequent presence in solution quantitatively quench the fluorescence of CDs (Scheme 1) that is visually detectable following irradiation with a handheld UV lamp only.



Scheme 1. UV induced CPD detection in DNA using Hg^{2+} and CDs.

2. Materials and methods

2.1. Material and reagents

Citric acid monohydrate, glycine, mercury (II) acetate $Hg(OAc)_2$ were purchased from Sigma. pUC19 plasmid, *Hin*dIII restriction endonuclease and T4 PDG (T4 endonuclease V) were purchased from New England Biolabs, USA. Nanopure water from Millipore was used in all experiments. The oligomeric DNA sequences were obtained from Sigma custom oligo service (Table 1). For all measurements, DNA concentration is given in terms of concentration of nucleobases.

2.2. Experimental measurements

Irradiation of DNA samples with UV lamps was done in photoreactor (Luzchem, LZC-4X, Canada). Formation of CPD in oligomer DNA on UV irradiation was observed by 20% polyacrylamide gel electrophoresis (PAGE) in Tris Taurine EDTA (TTE) buffer. Similarly, for pUC19 the lesion was analyzed by 1.5% agarose gel electrophoresis in tris acetate EDTA (TAE) buffer at 100 V and images were captured using a CCD camera fitted with a gel documentation system (UVP, UK). Absorption spectra of samples in UV–visible range were measured on an UV-2550 spectrophotometer (Shimadzu, Japan). Steady-state fluorescence spectra were obtained on Fluoromax-4 spectrofluorometer (Horiba, Japan). For transmission electron microscopy (TEM), a dilute solution of CDs (0.5 mg/mL) were dispersed on a carbon grid and captured through Hitachi (H-7500) electron microscope at 100 kV accelerating voltage. The lattice spacing and crystalline phase of CDs were analyzed through X-ray diffractometer (XRD). Hydrodynamic radii and zeta potential of samples were measured by dynamic light scattering (DLS) on a Delsa™ Nano particle analyzer (Beckman Coulter). Surface functionality of CDs were measured by Fourier transform infrared spectroscopy (FTIR, Shimadzu, Japan). CDs were analyzed with X-ray photoelectron spectrum (XPS) (Oxford Instrument Germany)

Table 1
Oligomeric DNA sequences used for template formation.

Oligo	Sequence
A1	5′- AGCT GCG CGA AAA CAC GTG CAC ATCTTTTCA GAT ACGCG -3′
A2	3′- CGC GCT TTT GTG CAC GTG TAG AAA AGT CTA TGC GC-5′

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