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Impeded repair of abasic site damaged lesions in DNA adsorbed over functionalized multiwalled carbon nanotube and graphene oxide

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ABSTRACT

The processing of abasic site DNA damage lesions in extracellular DNA in the presence of engineered carbon nanomaterials (CNMs) is demonstrated. The efficacy of the apurinic-apyrimidinic endonuclease 1 (APE1) in the cleavage of abasic site lesions in the presence of carboxylated multi-walled carbon nanotubes (MWCNT-COOH) and graphene oxide (GO) are compared. The CNMs were found to perturb the incision activity of APE1. The reason for such perturbation process was anticipated to take place either by the non-specific adsorption of APE1 over the free surface of the CNMs or steric hindrance offered by the CNM-DNA complex. Accordingly, bovine serum albumin (BSA) was selectively utilized to block the free surface of the CNM-DNA hybrid material. Further treatment of the CNM-DNA-BSA complex with APE1 resulted in a marginal increase in APE1 efficiency. This indicates that APE1 in solution is unable to process the abasic sites on DNA adsorbed over the CNMs. However, the cleavage activity of APE1 was restored in the presence of non-ionic surfactant (Tween 20) that inhibits adsorption of the DNA on the surface of the CNMs. The conformational deformation of the DNA, along with steric hindrance induced by the CNMs resulted in the inhibition of abasic site DNA repair by APE1. Moreover, appreciable changes in the secondary structure of APE1 adsorbed over the CNMs were observed that contribute further to the repair refractivity of the abasic sites. From a toxicological viewpoint, these findings can be extended to the study of the effect of engineered nanoparticles in the intracellular DNA repair process.

1. Introduction

Engineered carbon nanomaterials find an important position in the field of materials science and technology due to their unique structure and properties [1]. The ability to tune the different properties of these nanomaterials by modifying them structurally for multifaceted applications has resulted in their widespread usage [2,3]. Carbon nanomaterials (CNMs) like graphene and carbon nanotubes (CNT) are being constantly researched to explore their applications [4–6]. They have a broad range of applications in structural nanocomposites, battery electrodes, supercapacitors, biomedicine, biomedical imaging, drug delivery, water treatment, even agricultural fertilizers and others [7–9]. Despite the presence of such advantageous properties associated with these CNMs, there have been lot of debates over the risk assessment and safety issues of CNMs [10]. This makes it important to understand the toxicological aspects of CNMs in the environment and towards the human and other organisms [11,12]. Herein, we demonstrate the effect of graphene oxide (GO) and carboxylated multi walled carbon nanotube (MWCNT-COOH) on the processing of solitary and clustered abasic site lesions in DNA, which is considered as one of the most common type of DNA damage lesions.

Abasic sites are formed by the stripping of the purine or pyrimidine bases of the DNA strands. This occurs due to the spontaneous hydrolysis, oxidative processes, and exposure to radiation and exogenous toxins [13]. Around 10000 abasic sites are generated naturally per cell/day in humans, which makes them the most frequently encountered DNA damage lesions [13]. Abasic sites are highly mutagenic if not repaired. Moreover, multiple abasic sites present in close proximity (within 1–2 helical turns of DNA) give rise to clustered DNA damage that are repair refractive. Bistranded abasic clusters have been found to produce Double strand Breaks (DSBs) during their attempted repair that are deleterious to the cell [14]. The abasic site lesions are repaired in a series of steps. Apurinic/apyrimidinic endonuclease, commonly known as APE1, plays a vital role in the cleavage of the abasic site to initiate the repair process by Base Excision Repair (BER) mechanism [15]. The

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repair of abasic sites in extracellular DNA is important because it makes the DNA less susceptible towards mutation while recombining during the natural transformation process [16,17]. The effect of CNMs on the repair process of these mutagenic lesions present in DNA is largely unknown.

The in-vitro as well as in-vivo studies of the interactions of cells with CNMs have demonstrated various toxic routes for cell death [18–20]. The significant increase in the intracellular reactive oxygen species (ROS) by CNMs cause oxidative stress mediated cellular toxicity [21–24]. CNMs inhibit cell proliferation, decrease the cell adhesive ability, induce the expression of a number of apoptotic genes, induce membrane perturbation, and inhibit the expression of genes connected with the cell cycle and signal transduction [25–29].

Recently, functionalized CNMs have been used in many biomedical applications including enzyme immobilizations and gene delivery [30–33]. These have demonstrated several unique modes of interactions of CNMs with extracellular DNA/RNA regarding the action of various nuclease enzymes [34–38]. CNMs also enhance the natural transformation capacity between two competent bacteria by interacting with DNA [39,40]. Thus, there is no doubt that CNMs can exclusively interact with DNA and enzymes and may have specific impact on the DNA repair machinery of the cell.

Even though there are several reports dealing with the impact of CNMs on the environment [41–43] and their effect on growth and various metabolic processes, very few works have been persuaded to understand the effect of CNMs on the repair process of DNA [44–49]. The fact that the cell spends a substantial amount of energy in producing various DNA repair enzymes, the DNA repair process is considered as the most important phenomenon that maintains the integrity of the genome and prevents mutation. Thus, it is foremost important to understand and evaluate the effect of CNMs on the process of DNA repair at the molecular level. Apart from the toxicological aspect of CNMs intervening in the normal DNA repair process; this kind of studies can help to understand the effect of CNMs on evolution concerning the natural transformation process.

2. Experimental section

2.1. Materials

Expanded Graphite (Grade 3777) was procured from Asbury Carbon, NJ, USA. MWCNT was obtained from Helix Materials, TX, USA. Potassium permanganate, Concentrated Sulphuric and Nitric acid, 30% H_2O_2 was obtained from CDH Chemicals, India and used without further purification. Uracil DNA Glycosylase (UDG), Mung bean Nuclease (MBN) and APE1 enzyme were purchased from New England Biolabs (USA). Sybr Gold® was purchased from Thermo Fischer Scientific. HPLC purified mono uracil containing synthetic deoxyoligomers were purchased from Sigma and concentrations adjusted as per the manufacturer's yield report. Chemicals for gel electrophoresis and buffer preparation and synthesis were purchased from Sigma Aldrich or Alfa Aesar and were used without further purification. Gel images were captured by UVP GelDoc-It 310 gel documentation system after staining with Sybr Gold®. All experiments of CNMs-DNA interactions were done in triplicate and error bars were generated after calculating the mean SD from the average value wherever applicable.

2.2. Synthesis of carbon nano materials (CNMs)

2.2.1. Synthesis and characterization of GO

GO was synthesized by following the Hummers method [50]. Briefly, 1 g of expanded graphite was added to 40 ml of concentrated sulfuric acid in ice bath condition. Further, 3 g of potassium permanganate was added slowly to the solution with vigorous stirring (caution: explosive mixture, the temperature should be maintained low while adding potassium permanganate) at 35 °C for 30 min. 240 ml of water was added to the solution and heated to 90 °C for 15 min. After vigorous stirring, 700 ml of de-ionized water was introduced to the mixture along with drop wise addition of 30 ml of H_2O_2 (30%). The product was isolated by centrifugation and washed with 10% HCl followed by water. The isolated product was characterized by Raman spectroscopy using an argon ion laser as the source. The extent of covalent modification of the graphitic material was evaluated by comparing the ratio of the intensity of the D-band to the G-band for the pristine material with that of the functionalized derivative.

2.2.2. Synthesis of MWCNT-COOH

50 mg of MWCNT was dispersed in a 3:1 mixture of sulfuric acid and nitric acid (200 ml) by methodology as described elsewhere [51]. This mixture was sonicated for 5 h at 60 °C and the product was isolated from the reaction mixture by centrifugation. The isolated product was further washed with deionized water to remove the acid completely. The isolated product was characterized by Raman spectroscopy. The Raman spectra of the pristine material was compared and contrasted against the functionalized material.

2.3. Preparation of oligomer duplexes with abasic sites

The custom synthesized oligonucleotides (R1 and R2) used in the study is shown in Table 1. The annealing of the oligonucleotides was performed in 10 mM sodium phosphate buffer at pH 7.0. The abasic site in the DNA duplex the oligomer duplex (2 μg) was generated by treating the sample with 5 units of UDG enzyme at 37 °C for 1 h. The UDG enzyme in the sample was heat-inactivated at 65 °C for 20 min and oligomer duplex was purified by ethanol precipitation. The UDG enzyme treated samples was incubated with 0.5 M NaOH (PAGE not shown) and putrescine separately for 1 h and further visualized on denaturing PAGE gel to check the complete conversion of the abasic sites into strand breaks [52,53].

2.4. Adsorption and desorption of dsDNA on GO and MWCNT-COOH surface

The stock solutions (0.5 mg/ml) of the GO and MWCNT-COOH were prepared in nuclease free nanopure water. Abasic site containing DNA duplex (R1–R2, 2.25 μg) was adsorbed on 250 μg of both GO and MWCNT-COOH in 1000 μl of 25 mM HEPES buffer, containing 1 mM $MgCl_2$ and 75 mM NaCl at pH 6.0. The CNMs-DNA mixtures were gently shaken for 12 h at room temperature followed by centrifugation at 20,000*g* for 20 min. The supernatants were carefully removed and the corresponding pellets containing DNA adsorbed over CNMs were washed with the adsorption buffer. The absorbance (A_{260}) of the supernatants was recorded at 260 nm to calculate the amount of unbound DNA present in the solution after centrifugation following Eq. (1).

$$\text{The amount of DNA } \left(\frac{\mu g}{\text{mL}}\right) \text{ adsorbed over CNMs DNAmixture pellets} = (\text{A 260of DNA i nwater} - \text{A 260 of supernatant}) \times 50 \frac{\mu g}{\text{mL}} \quad (1)$$

We used two types of buffers for the desorption of DNA from the CNMs. Buffer 1 (4 mM EDTA, 25 mM HEPES, pH 8.5) and buffer 2 (50 mM NaCl, 25 mM HEPES) were used successively. The desorption of R1–R2 duplex as well as APE1 treated R1–R2 from the GO and MWCNT-COOH was achieved by incubating pellets with the buffers at 50 °C for 3 h separately.