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Benzene-derived N^2-(4-hydroxyphenyl)-deoxyguanosine adduct: UvrABC incision and its conformation in DNA

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ABSTRACT

Benzene, a ubiquitous human carcinogen, forms DNA adducts through its metabolites such as *p*-benzoquinone (*p*-BQ) and hydroquinone (HQ). N^2-(4-Hydroxyphenyl)-2′-deoxyguanosine (N^2-4-HOPh-dG) is the principal adduct identified *in vivo* by ^{32}P-postlabeling in cells or animals treated with *p*-BQ or HQ. To study its effect on repair specificity and replication fidelity, we recently synthesized defined oligonucleotides containing a site-specific adduct using phosphoramidite chemistry. We here report the repair of this adduct by *Escherichia coli* UvrABC complex, which performs the initial damage recognition and incision steps in the nucleotide excision repair (NER) pathway. We first showed that the *p*-BQ-treated plasmid was efficiently cleaved by the complex, indicating the formation of DNA lesions that are substrates for NER. Using a 40-mer substrate, we found that UvrABC incises the DNA strand containing N^2-4-HOPh-dG in a dose- and time-dependent manner. The specificity of such repair was also compared with that of DNA glycosylases and damage-specific endonucleases of *E. coli*, both of which were found to have no detectable activity toward N^2-4-HOPh-dG. To understand why this adduct is specifically recognized and processed by UvrABC, molecular modeling studies were performed. Analysis of molecular dynamics trajectories showed that stable G:C-like hydrogen bonding patterns of all three Watson–Crick hydrogen bonds are present within the N^2-4-HOPh-G:C base pair, with the hydroxyphenyl ring at an almost planar position. In addition, N^2-4-HOPh-dG has a tendency to form more stable stacking interactions than a normal G in B-type DNA. These conformational properties may be critical in differential recognition of this adduct by specific repair enzymes.

1. Introduction

Benzene is a common environmental pollutant with wide usage in industry (Wallace, 1996). Human exposure to benzene comes from gasoline and automobile combustion products, and from certain industries such as oil refineries and petrochemical/rubber manufacturing (IARC, 1982; NTP, 2005). Cigarette smoking is another major source as it is estimated to account for about half of the entire nationwide exposure to benzene (Wallace, 1996). Benzene is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) and National Toxicology Program (NTP) (IARC, 1982; NTP, 2005). In rodents, benzene exposure has been demonstrated to cause tumors at multiple organ sites (Huff et al., 1989). Several human epidemiological studies (e.g., Sorahan et al., 2005; Yin et al., 1996) have revealed a relationship between occupational benzene exposure and an increased incidence of leukemia, primarily acute myeloid leukemia. An increased risk of breast and lung cancer among benzene exposed populations has also been suggested from limited epidemiological or population-based studies (Costantini et al., 2009; Hansen, 2000; Petralia et al., 1999; Yin et al., 1996).

Benzene must be metabolized to more reactive compounds to exert its effects. Despite extensive investigation of the biological effects of benzene and its major metabolites for many years, the precise mechanisms underlying benzene mutagenicity and carcinogenicity remain unclear. Nevertheless, several possible mechanisms have been proposed with focuses on interactions of benzene with different cellular components as well as resulting structural and biological impacts. One of the mechanisms concerns the formation of mutagenic DNA damage by benzene metabolites in certain types of target cells/tissues, particularly the hematopoietic progenitor cells in the bone marrow (McDonald et al., 2001;

Abbreviations: HQ, hydroquinone; *p*-BQ, *p*-benzoquinone; N^2-4-HOPh-dG, N^2-(4-hydroxyphenyl)-2′-deoxyguanosine; AP, apurinic/apyrimidinic; UvrABC, *E. coli* UvrABC complex; NER, nucleotide excision repair; BER, base excision repair; Exo III, exonuclease III; Endo IV, endonuclease IV; APE1, human AP endonuclease 1; MD, molecular dynamics.

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Whysner et al., 2004). DNA adducts have been detected in these cells or tissues following exposure to benzene or its metabolites (Whysner et al., 2004). The role of DNA adduct formation is also supported by the evidence from induced mutagenesis following transfection of *p*-benzoquinone (*p*-BQ)- or hydroquinone (HQ)-damaged shuttle vectors into cultured mammalian cells (Gaskell et al., 2004, 2005; Nakayama et al., 2004).

A major class of DNA adducts found to be formed *in vitro* by *p*-BQ or HQ are the exocyclic benzetheno adducts of dG, dA and dC (Gaskell et al., 2002; Jowa et al., 1986; Pongracz and Bodell, 1991; Pongracz et al., 1990). To study their biochemical and biological properties, we previously synthesized oligonucleotides containing these adducts at a single location (Chenna and Singer, 1995, 1997). Our site-directed mutagenesis experiments showed that these adducts are highly mutagenic in the cell (Xie et al., 2005). We have also demonstrated that all three benzetheno adducts are substrates for exonuclease III (Exo III) and endonuclease IV (Endo IV) of *Escherichia coli* and the major apurinic/apyrimidinic (AP) endonuclease (APE1) of humans (Chenna et al., 1995; Guliaev et al., 2004; Hang et al., 1996; Hang et al., 1998). These enzymes hydrolyze the phosphodiester bond immediate 5′ to the adduct, leaving the benzetheno derivative on the 5′-terminal of the 3′ fragment as a "dangling base" (Hang et al., 1996), a mechanism which was later referred as nucleotide incision repair (NIR) (Ischenko and Saparbaev, 2002).

Benzene-derived DNA adducts have also been reported in certain tissues or cultured cells after exposure. By ^{32}P-postlabeling, Bauer et al. (1989) showed the formation of DNA adducts in liver of rabbits treated with benzene. Bodell and co-workers later detected several adducts in human promyelocytic leukemia HL60 cells (Levay et al., 1991; Pongracz and Bodell, 1996) and in the bone marrow cells of mice (Bodell et al., 1996) treated with benzene, *p*-BQ or HQ. The principal adduct detected in these cells/tissues by ^{32}P-postlabeling corresponded to N^2-4-HOPh-dG-3′-phosphate (Levay et al., 1991; Pongracz and Bodell, 1996) (see Fig. 1 for adduct structure). The same adduct could be identified *in vitro* by allowing guanosine 3′-phosphate to react with *p*-BQ (Levay et al., 1991; Pongracz and Bodell, 1996). To date, to the best of our knowledge, both biochemical and biological properties of this adduct have not been studied. In light of the observation that induction of benzene toxicity in the bone marrow correlates with the adduct formation (Bodell et al., 1996) and that induced mutagenesis studies suggest a major role of the *p*-BQ- or HQ-induced dG adducts in benzene mutagenesis (Gaskell et al., 2004, 2005; Nakayama et al., 2004), it was of great interest for us to investigate the removal of N^2-4-HOPh-dG by cellular repair mechanisms.

Based on the chemical structure of N^2-4-HOPh-dG and known substrate specificity of respective repair pathways, we examined the specificity toward this adduct of enzymes from three different *E. coli* repair mechanisms, all of which have been well characterized and are highly conserved from bacteria to mammalian cells. Such work was greatly facilitated by our ability to synthesize the defined DNA oligonucleotides containing N^2-4-HOPh-dG at a specific position (Chenna et al., 2008). As stated above, AP endonucleases Exo III and Endo IV are able to recognize and repair benzene-derived benzetheno adducts which have gained two extra rings on the nucleotide base. While the BER pathway primarily targets relatively small base modifications such as alkylated and oxidized bases, it is able to repair certain 5- or 6-membered exocyclic ring adducts, as shown by us and others (Hang, 2004). The NER pathway is the major mechanism for repair of bulky DNA adducts of various chemical structures (Friedberg et al., 2005; Reardon and Sancar, 2005), which was expected by us to be the primary mechanism for repair of N^2-4-HOPh-dG adduct. In *E. coli*, the initial steps of NER are carried out by the UvrABC proteins, which perform at least two sequential reactions. First, UvrA and UvrB are involved in recognition of chemical modification and structural distortion in duplex DNA, leading to the formation of the UvrB-DNA complex; Second, UvrC is recruited to the damaged site and makes incisions on the sides of the damage.

In this study, the results showed that N^2-4-HOPh-dG is specifically recognized and processed by the UvrABC complex, but not by the glycosylases and AP endonucleases tested. To gain a structural rationale for this novel substrate specificity, molecular dynamics (MD) simulations were performed to analyze the effect of N^2-4-HOPh-dG on local conformation of the DNA duplex. The significance and implications of these findings were discussed.

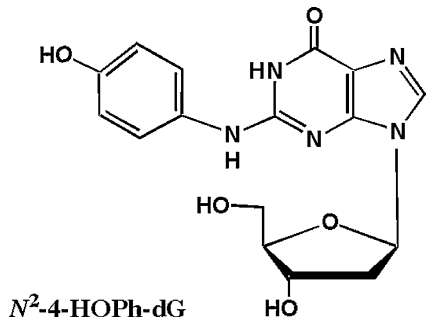

Fig. 1. Chemical structure of N^2-(4-hydroxyphenyl)-2′-deoxyguanosine (N^2-4-HOPh-dG).

2. Materials and methods

2.1. DNA substrates and repair proteins

The synthesis of the 5′-DMT-3′-phosphoramidite of N^2-4-HOPh-dG and its site-specific incorporation into defined oligonucleotides were previously described by Chenna et al. (2008). The unmodified controls, complementary strands and DNA size markers were purchased from Operon Inc. (Alameda, CA). All oligomers were HPLC- and/or PAGE-purified before assayed (see Figs. 3 and 5 for sequences used in this study).

To generate plasmid globally modified by *p*-BQ, pCMV vector (Invitrogen) (15 μg) was treated with several concentrations of *p*-BQ (Sigma, St. Louis, MO, stock: 500 mg/ml in methanol) or methanol only in 80 μl at 37 °C overnight. Both *p*-BQ-treated and untreated plasmids were purified using the QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA) and dissolved in TE buffer (pH 8.0).

Purified recombinant UvrA, UvrB and UvrC proteins were purchased from BD Biosciences (San Jose, CA). These subunits were purified from *E. coli* DR153 overexpressing the uvrA, uvrB and uvrC genes, respectively. Exo III was purchased from Gibco BRL (Carlsbad, CA). Endo IV was from R&D Systems (Minneapolis, MN). Mismatch uracil-DNA glycosylase (Mug), endonuclease III (Nfo), endonucleae VIII (Endo VIII), formamidopyrimidine-DNA glycosylase (Fpg), and MutY were all purchased from Trevigen (Gaithersburg, MD).

2.2. Incision assays

UvrABC incision of *p*-BQ-modified plasmid DNA was measured at 30 °C with varying UvrABC concentrations or times in a total volume of 50 μl. The UvrABC subunits were diluted with a dilution buffer and premixed prior to the reaction. The reactions were carried out using 0.75 μg plasmid DNA in a UvrABC buffer containing 50 mM Tris–HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 0.1 mg/ml BSA, 5 mM DTT and 2 mM ATP. All reactions, after terminated with 25 mM EDTA, were resolved on a 1% agarose gel with 0.3 μg/ml ethidium bromide and visualized with an IS-500 Digital Imaging System (Alpha Innotech). DNA nicking was determined by the conversion of supercoiled plasmid into the linear form.

To test for UvrABC activity toward N^2-4-HOPh-dG, both adduct-containing and unmodified 40-mers were 5′-terminally labeled with [γ-^{32}P] ATP (specific activity 6000 Ci/mmol; 1 Ci = 37 GBq, PerkinElmer, Boston, MA) and annealed to a complementary strand in a buffer containing 10 mM HEPES-KOH (pH 7.5) and 100 mM NaCl. The reaction mixtures contained 2 nM radiolabeled DNA substrate in a UvrABC buffer containing 50 mM Tris–HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 0.1 mg/ml BSA, 5 mM DTT, 2 mM ATP, and varying amounts of UvrABC subunits as indicated in the figures. The reactions were terminated by adding an equal volume of F/E solution (90% formamide, 50 mM EDTA, and 0.2% bromophenol blue) followed by heating at 95–100 °C for 5 min. The reaction products were resolved on a 12% denaturing PAGE and the radioactivity was scanned with a Molecular Imager FX (Bio-Rad, Hercules, CA). For quantification of radioactivity of the bands, Quantity One software (version 4.0.1) was used.