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Mutagenesis by (+)-*anti*-B[*a*]P-N^2-Gua, the major adduct of activated benzo[*a*]pyrene, when studied in an *Escherichia coli* plasmid using site-directed methods

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The suspected major mutagenic adduct of benzo[*a*]pyrene, (+)-*anti*-B[*a*]P-N^2-Gua, is built into the unique *Pst*I recognition site of the *Escherichia coli* plasmid, pUC19, in order to study its mutagenic potential. The adduct can either be at G_{437}, which is replicated during leading strand DNA synthesis, or at G_{438}, which is replicated during lagging strand DNA synthesis. The DNA strand complementary to the strand containing the (+)-*anti*-B[*a*]P-N^2-Gua adduct is saturated with UV lesions to minimize its potential to generate progeny. Although all in-frame mutations could have been detected, a $G_{437} \rightarrow T$ transversion mutation is virtually exclusively obtained at a frequency of ~0.04% per adduct following transformation into Uvr$^+$ *E.coli* when SOS is not induced, and ~0.18% when SOS is induced. The mutation frequency of the adduct in a Uvr$^-$ background is estimated to be ~0.2% when SOS is not induced, and ~0.9% when SOS is induced. The absence of $G_{438} \rightarrow T$ mutations is rationalized. G → T mutations from (+)-*anti*-B[*a*]P-N^2-Gua are compared to the mutational specificity of the ultimate mutagenic form of activated benzo[*a*]pyrene.

Introduction

Mutagenic processes are fundamental to cancer causation as evidenced by the observation that oncogenes are derived from their normal cellular counterparts (protooncogenes) via mutations (1). Mutagenic events can occur spontaneously or can be induced by exogenous agents (2). Many potent mutagens/carcinogens are bulky and three-dimensionally complex, such as benzo[*a*]pyrene (B[*a*]P*), aflatoxin B_1 (AFB_1), 2-aminofluorene (2-AF), 2-acetylaminofluorene (2-AAF) and *cis*-diammindichloroplatinum(II) (*cis*-DDP), and understanding the mechanisms by which they induce mutations is only now becoming possible (3–7).

B[*a*]P, a polycyclic aromatic hydrocarbon, reacts with DNA following activation to its corresponding 7,8-diol-9,10-epoxide (BPDE) (reviewed in refs 8 and 9). The majority of adducts *in vivo* are thought to be derived from the (+)-*anti*-isomer of BPDE (Figure 1) (8,9), though this may not always be the case (10–12). The most prevalent adducts derived from the racemic mixture of (±)-*anti*-BPDE are B[*a*]P-N^2-Gua (Figure 1) (8,9,13–15) and B[*a*]P-N^6-Ade (16), where *trans* addition to the epoxide prevails, as well as B[*a*]P-*N*(7)-Gua (17,18), which has not been carefully characterized. In addition, evidence exists for the formation of cytosine adducts (19,20).

The adducts of (±)-*anti*-BPDE are reported to block DNA replication (21), and induce homologous (22) and illegitimate recombination (23). In addition (±)-*anti*-BPDE is mutagenic and induces both base pairing and insertion/deletion mutations (24–35). The mutational specificity of the racemic mixture of (±)-*anti*-BPDE in bacteria has been determined in several systems; the most prevalent mutations are GC → TA and AT → TA (27–29). Mutational spectra for (±)-*anti*-BPDE have been determined in human cells using a shuttle vector system, (30), and in CHO cells (31,32) and human cells (33–35) using endogenous target genes, where the specificity is reasonably similar to that determined in bacteria, though GC → CG mutations appear to become more prevalent. Mutations induced in the Ha-*ras* oncogene at the 61st codon have been determined with cells in culture (36). Finally, GC → TA and AT → TA mutations in Ha-*ras* have been identified in tumors derived from animals treated with B[*a*]P (1, 37–39).

Although much information is known about both the adducts and the mutations arising from BPDE, the relationship between these two is poorly understood. During the last several years it has become possible to study the biological consequences of individual DNA adducts because of the development of techniques

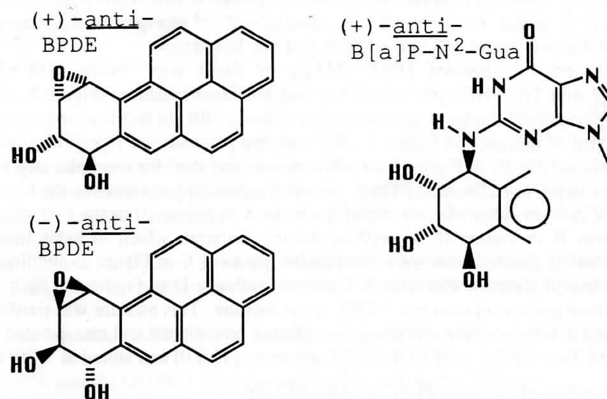

Fig. 1. Structures of (+)-*anti*-BPDE, (−)-*anti*-BPDE, and the adduct, (+)-*anti*-B[*a*]P-N^2-Gua (*trans* addition).

*__Abbreviations:__ B[*a*]P, benzo[*a*]pyrene; (+)-*anti*-BPDE, (+)-*r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*) (Figure 1); (+)-*anti*-B[*a*]P-N^2-Gua, the *trans* additional product between the *C*10 position of (+)-*anti*-BPDE and the N^2 position of guanine (Figure 1); *cis*-DDP(GpG), the *N*(7)Gua-*N*(7)-Gua diadduct of *cis*-diammindichloroplatinum(II); *cis*-DDP(ApG), the *N*(7)Ade-*N*(7)-Gua diadduct of *cis*-DDP; AF-C8-Gua, the *C*(8)-Gua adduct of 2-aminofluorene; AAF-C8-Gua, the *C*(8)-Gua adduct of 2-acetylaminofluorene; AFB_1-N7-Gua, the *N*(7)-Gua adduct of aflatoxin B_1; 5′-pT(B[*a*]P-N^2)GCA-3′, an oligonucleotide containing (+)-*anti*-B[*a*]P-N^2-Gua; (+)-*anti*-B[*a*]P-N^2-Gua-pUC19, the plasmid pUC19 with 5′-pT(B[*a*]P-N^2)GCA-3′ ligated into the *Pst*I recognition site; C-pUC19, the plasmid pUC19 with 5′-TGCA-3′ ligated into the *Pst*I recognition site; IPTG, isopropyl β-D-thiogalactopyranoside; X-gal, 5-bromo-4-chloro-3-indoyl β-D-galactopyranoside; WT^b, *Pst*I$^+$ (blue); M^b, *Pst*I$^-$ (blue); M^w, *Pst*I$^-$ (white); M^{lb}, *Pst*I$^-$ (light blue); M^{fb}, *Pst*I$^-$ (faintly blue)—the latter five abbreviations are presented more thoroughly in Table II; R_i is defined in equation (1) (Materials and methods); $R_{G\rightarrow T}$ is defined in equation (2a) (Materials and methods); $MF^{+ER}_{G\rightarrow T}$ is defined in equations (3) and (4) (Materials and methods) and $MF^{-ER}_{G\rightarrow T}$ is defined in equation (5) (Materials and methods); *MF*, mutation frequency; +ER, excision repair proficient cells (AB1157); −ER, excision repair deficient cells (AB1885); ds, double strand.

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