Metabolic Activation of Benzo[g]chrysene in the Human Mammary Carcinoma Cell Line MCF-7

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ABSTRACT

Benzo[g]chrysene (BgC) is an environmental pollutant, and recent studies have demonstrated that anti- BgC-11,12-dihydrodiol 13,14-epoxide (anti-BgCDE) is a potent mammary carcinogen in rats. To determine whether BgC can be metabolically activated to anti-BgCDE in human cells, the human mammary carcinoma cell line MCF-7 was treated with BgC and with the racemic trans-3,4- and 11,12-dihydriodiol. The DNA adducts formed in these experiments were examined using 32P-postlabeling, and specific adducts were identified through comparisons with adducts obtained by the reaction of the racemic syn- and anti-BgCDEs with calf thymus DNA and with purine deoxyribonucleoside-3'-phosphates. BgC therefore is a potential environmental form major DNA adducts through both the syn- and anti-11,12-dihydrodiol 13,14-epoxide metabolites. BgC is therefore a potential environmental risk to humans. The major BgC-DNA adducts formed from both the dihydrodiol-epoxide diastereomers were deoxyadenosine adducts. Thus, BgC has DNA-binding properties that are very similar to those of the potent mammary carcinogens 7,12-dimethylbenz[a]anthracene and dibenzo[a,l]pyrene.

INTRODUCTION

Polycyclic aromatic hydrocarbons, many of which are carcinogenic in experimental animal systems (reviewed in Refs. 1–3), are present in airborne particulates because they arise from incomplete combustion of fossil fuels. Most of the hydrocarbons are metabolically activated by cytochrome P-450-dependent mixed-function oxidases and micromolar epoxide hydrase to dihydrodiol-epoxide metabolites that bind covalently with nucleophilic centers in cellular DNA in an early step in the carcinogenic process (1–4). The epoxide function in these reactive metabolites is usually in either a bay region or a fjord region of the molecule (5).

Dihydrodiol-epoxides from fjord region-containing hydrocarbons exhibit exceptionally high tumorigenic activities (6–10). Recently, it has been demonstrated that racemic anti-BgCDE has a tumorigenic activity comparable to that of the corresponding BcP-3,4-dihydrodiol 1,2-epoxide and that both compounds are far more active tumor initiators than anti-BaP-7,8-dihydrodiol 9,10-epoxide (7, 8). Anti-dibenzo[a,l]pyrene 11,12-dihydrodiol 13,14-epoxide, on the other hand, was significantly more active than BcPDE and BgCDE for induction of certain types of tumors (10). Given the high tumorigenicity of anti-BgCDE, the known mutagenic properties of both the anti- and syn-diastereomers in bacterial and mammalian systems (11–15), and the knowledge that the parent carcinogen, BgC (16), has been found in high-boiling petroleum distillate (17) and in a standard reference of an air particulate sample from St. Louis, Missouri, where it was present in much lower amounts (0.07 μg/g of standard reference material) than BaP (3.1 μg/g) (18), it seemed important to determine whether cells from humans were capable of generating the biologically active 11,12-dihydrodiol 13,14-epoxide from BgC.

In the present study, we examined DNA adduct formation from BgC and from the 3,4-dihydrodiol and 11,12-dihydrodiol metabolites of BgC in the human MCF-7 cell line, which is known to express several cytochrome P-450s and to metabolize hydrocarbons (19), to determine the metabolic route through which BgC forms DNA adducts in these human cells. As indicated in Fig. 1, these studies, along with in vitro reactions of synthetic dihydrodiol-epoxides with DNA and nucleotides, showed that BgC-DNA binding in human cells occurred through the intermediacy of both syn- and anti-11,12-dihydrodiol 13,14-epoxides.

MATERIALS AND METHODS

BgC and samples of racemic syn- and anti-BgCDE and BgC-trans-11,12-dihydrodiol were synthesized as described earlier (20). BgC-trans-3,4-dihydrodiol was a generous gift from Dr. Roland Lehr (Department of Chemistry, University of Oklahoma, OK; Ref. 21). Deoxyribonucleoside 3'-phosphates, calf thymus DNA, nuclease P1, micrococcal nuclease, apyrase (Sigma Chemical Co., St. Louis, MO), spleen phosphodiesterase (Boehringer Manheim), T4-polynucleotide kinase (U. S. Biochemical Corporation, Cleveland, OH), and [γ-32P]ATP with an original activity of ~3000 Ci/mmol (Amersham, Arlington Heights, IL) were all obtained commercially. Sep-Pak C18 light cartridges were purchased from Waters (Milford, MA). The PEI-cellulose plates (20 × 20 cm) were manufactured by Machery Nagel (Düren, Germany).

Cell Growth and Treatment and DNA Isolation. The human mammary carcinoma cell line (MCF-7) was grown in MEM supplemented with 10% FCS, nonessential amino acids, and sodium pyruvate. The cells were fed with fresh media when they covered more than 90% of the surface of a T175 cm2 flask. The cells were treated with 1-μM concentrations of BgC, racemic BgC-trans-3,4-dihydrodiol, or racemic BgC-trans 11,12-dihydrodiol. After 24 h, cells were harvested with trypsin-EDTA, and the cell pellet was stored at −80°C.

DNA was isolated by homogenization of the cell pellet in Tris-EDTA-SDS followed by treatment with RNase, RNase T1, and proteinase K and extraction with phenol-chloroform-isooamyl alcohol. DNA was precipitated with ethanol and dissolved in distilled water for analysis.

Preparation of the BgCDE Adduct Markers. Solutions of calf thymus DNA (1 mg in 1 ml) or of dGp, or dAp (1 mg in 100 μl) in 0.1 M Tris-HCl buffer (pH 7.0) were treated with 100 or 10 μl, respectively, of a solution of either racemic syn- or anti-BgCDE (1 mg/ml) in tetrahydrofuran. The reactions were kept at 37°C for 5 h, and then DNA reactions were extracted with water-saturated 1-butanol (3 times), ethyl acetate (3 times), and water-saturated ethyl ether (3 times). Aliquots of the resultant DNA solution were analyzed by postlabeling as described below.

To remove the hydrolysis products of the dihydrodiol-epoxides from the mononucleotide reactions, the reaction mixture was extracted with water-saturated ethyl acetate (three times), followed by three extractions with water-saturated ethyl ether. The resultant aqueous phases (100 μl) were loaded separately onto Sep-Pak C18 light cartridges that had been precondi-
tioned with methanol and distilled water. The cartridges were washed with distilled water (30 ml) to remove most of the unreacted nucleotides, and adducted nucleotides were then eluted from the cartridge with methanol (1 ml), which was concentrated to dryness under vacuum. The products were redissolved in methanol (100 μl) and stored at −20°C before 32P-postlabeling analysis.

Carcinogen-DNA Adduct Analysis. Duplicate samples of carcinogen-modified DNA (2 μg) isolated from MCF-7 cells were digested to mononucleotides at 37°C for 3.5 h with micrococcal nuclease (1 μg) and spleen phosphodiesterase (1 μg) as described earlier (23, 24). Adducted nucleotides were enriched by dephosphorylation of normal nucleotides with nuclease P1 (2 μg) treatment. The enriched adduct preparation was 32P-postlabeled by incubation with [γ-32P]ATP (100 μCi) and T4-poly nucleotide kinase (6 units). The reaction was terminated by the addition of apyrase (40 milliunits), and the total incubates were spotted onto PEI TLC plates.

Calf thymus DNA (2 μg) that had been separately treated in vitro with syn-and anti-BgCDEs was digested as above but using micrococcal nuclease (2 μg) and spleen phosphodiesterase (2 μg). After enrichment with nuclease P1 (2 μg), the sample was diluted to 2 ml with distilled water. A 5-μl portion of the diluted solution was postlabeled using 100 μCi of [γ-32P]ATP and T4-poly nucleotide kinase (3 units) at 37°C for 30 min. The mixture of modified nucleoside 3'-phosphate markers (2 μl) was also postlabeled as described earlier (25) using 50 μCi of [γ-32P]ATP for each sample. The reactions were terminated by the addition of apyrase.

Adducts were resolved by multidirectional PEI-cellulose TLC (20 × 20 cm) with the 32P-postlabeling mixture spotted 1.5 cm from the left corner of the plates. The following conditions were employed: D1 and D5, 1.7 M sodium phosphate (pH 6.0), 18 h; D3, 3.5 M lithium formate and 8.5 M urea (pH 3.4), 24 h; and D4, 0.8 M sodium phosphate, 0.5 M Tris-HCl, and 8.5 M urea (pH 7.8), 24 h. The corners of the air-dried TLC plates (after two-dimensional chromatography) were marked with a nonradioactive marker pen (Scienceware, Pequannock, NJ) for alignment after exposure. The postlabeled adducts were located on the TLC plates with autoradiography using Kodak XAR-5 films. Autoradiographic exposures were at room temperature for 5–8 h. The adduct spots were excised from the plates, and the radioactivity on each spot was determined using Cerenkov counting.

RESULTS AND DISCUSSION

To investigate the metabolic route to DNA adduct formation for BgC in MCF-7 cells, adducts formed from BgC were compared with those formed in these same cells from the racemates of BgC-trans-3,4-dihydrodiol, BgC-trans-11,12-dihydrodiol, and anti-BgCDE using 32P-postlabeling analysis (Fig. 2). It was immediately clear that the racemic 3,4-diol did not yield any detectable adducts (Fig. 2c), indicating that this metabolite was not an intermediate in the formation of a DNA-binding metabolite, i.e. BgC-DNA adducts were not derived from a 3,4-dihydrodiol 1,2-epoxide.

In contrast, all the other treatments led to adduct formation, and the major adduct spots formed from the anti-11,12-dihydrodiol 13,14-epoxide (1 and 2, Fig. 2d) were the major 11,12-dihydrodiol adducts (Fig. 2b) and were also two of the major adducts formed by the parent BgC itself (Fig. 2a). Thus, conversion of BgC to the 11,12-dihydrodiol followed by further metabolism to anti-11,12-dihydrodiol 13,14-epoxide is the route through which the major adducts, 1 and 2, are formed from BgC. Another major adduct (3 in Fig. 2a) was in the BgC pattern, but this was only a minor adduct in the 11,12-dihydrodiol exposure and was not detected in the anti-dihydrodiol-epoxide-treated cells. Thus, the reactive metabolite responsible for this adduct is a minor product of metabolism of the racemic 11,12-dihydrodiol and is not the anti-dihydrodiol-epoxide. A logical supposition based on previous work (22, 26) would be that this reactive metabolite was the syn-dihydrodiol-epoxide and, as shown later, this seems to be the case.

All adducts were numbered in Fig. 2, and those that had similar chromatographic mobilities were given the same number in each panel of each figure. Eleven separate adduct spots were detected in the DNA from cells exposed to BgC (Fig. 2a), and tentative identifications of these were made from comparisons of their chromatographic properties with those of adducts derived from reactions of DNA or purine deoxyribonucleoside-3'-phosphates with the racemic syn- and anti-11,12-dihydrodiol-epoxides.

32P-Postlabeling adduct maps resulting from reactions of anti- and syn-BgCDEs with calf thymus DNA in vitro are shown in Fig. 3. The racemic anti-BgCDE yielded six spots (Fig. 3a). Three adducts (1, 2, and 12) were present in large amounts, and the remaining three adduct spots (4, 5, and 13) were formed in small quantities. Chromatographic comparison of the 32P-postlabeled maps of this anti-BgCDE-DNA sample (Fig. 3a) with the map of adducts from BgC-exposed MCF-7 cells (Fig. 2a) confirmed that only 2 major (1 and 2) and 2 minor (4 and 5) DNA adducts of the 11 BgC adduct spots were present in both of these samples. These same four adduct spots were also found in DNA from BgC-11,12-dihydrodiol-exposed MCF-7 cells (Fig. 2b). The comparison with the anti-dihydrodiol-epoxide reaction with DNA in vitro confirms, therefore, that adduct spots 1, 2, 4, and 5 were all derived from reaction of the anti-11,12-dihydrodiol 13,14-epoxide with DNA. Moreover, because cellular metabolism usually leads to the preferential or exclusive formation of one enantiomer, i.e. the R,S-dihydrodiol S,R-epoxide (3, 22, 26, 27), these adducts were presumably formed from this particular stereoisomer. Adduct spots 12 and 13, present in the 32P-postlabeled sample of anti-BgCDE-DNA, did not comigrate with any spots from BgC or the 11,12-dihydrodiol activated in MCF-7 cells and, therefore, probably arose from the
S,R-dihydrodiol R,S-epoxide present in the synthetic racemic preparation. Adduct spot 12 was present in the adduct map derived from the racemic anti-BgCDE in MCF-7 cells. Some of the adducts formed from the racemic syn-BgCDE-DNA reaction (Fig. 3b) migrate at the same positions as some remaining adducts in the DNA from BgC- and the 11,12-diol-treated MCF-7 cells. As is evident from the data shown in Fig. 3b, the two major DNA adducts formed from syn-BgCDE-DNA were chromatographically identical to adducts 3 and 11 in Fig. 2a, and four of the minor adducts (6, 7, 8, and 9) also seemed to be common to both BgC in MCF-7 cells (Fig. 2a) and the syn-dihydrodiol-epoxide-DNA reactions (Fig. 3b). The 11,12-dihydrodiol-treated MCF-7 cell DNA contained a very small amount (~3%) of syn adduct spot 3 (Fig. 2b). Thus, in MCF-7 cells, BgC is converted to a syn-dihydrodiol-epoxide as well as to an anti-dihydrodiol-epoxide at the 11,12,13,14-ring, and both of these metabolites react with cellular DNA to form DNA adducts.

The adduct spots generated from anti- and syn-isomers of the 11,12-dihydrodiol 13,14-epoxide derivatives of BgC were further characterized by separately reacting these dihydrodiol-epoxides with dAp and dGp (Fig. 4). Of the 32P-postlabeled racemic anti-BgCDE-dAp reaction products (Fig. 4a), adduct spot 1 was a major component in the labeled DNA adducts from the BgC-exposed MCF-7 cell DNA, and adduct spot 4 was a minor component. Thus, it is established that adduct spots 1 and 4 in Fig. 2a arose from reaction of the anti-11,12-...
dihydrodiol 13,14-epoxide with deoxyadenosine residues in DNA. Although we have no structural data on these adducts, it would be anticipated from comparisons with findings for BcP dihydrodiol-epoxide reactions with DNA (28-30) that the major adduct, 1, arose from trans opening of the epoxide, whereas the minor adduct arose from cis opening.

In the same fashion, comparisons between Fig. 2a and Fig. 4, b-d allowed the origin of the remaining adduct spots in Fig. 1a to be assigned. Thus, adduct spots 3, 6, 7, and 9 were shown to be derived from the racemic syn-BgCDE-dAp reaction products (Fig. 4b). The formation of all four of the dihydrodiol-epoxide-dAp products in MCF-7 cells suggests that both syn-enantiomers might be formed therein because these four adducts presumably result from cis and trans opening of the epoxide ring in each enantiomer. However, the findings for the reactions with dGp described below require that one of these enantiomers be formed in considerable excess over the other.

Fig. 4, c and d, indicates the location on the postlabeling maps of BgCDE-dGp adducts. Major adduct spot 2 and minor adduct spot 5 (Fig. 2a) were both derived from reaction of the anti-BgCDE with dGp (Fig. 4c) and, as noted earlier, it is likely that these result from trans and cis opening of the R,S-dihydrodiol S,R-epoxide, respectively. The reaction of syn-BgCDE with dGp gave rise to two major spots, 8A and 11, and one minor spot, 8 (Fig. 4d). Only two of these spots, 8 and 11, comigrated with the adduct spots in Fig. 2a from the treatment of MCF-7 cells with BgC, suggesting that the dihydrodiol-epoxide enantiomer responsible for formation of 8A is not extensively made in the human cells.

Quantitative analysis of the adduct spots from the BgC MCF-7 cell study indicated that three adducts (1, 2, and 3 in Fig. 2a) accounted for 75% of total adducts. These adducts are shown to have arisen from the anti-dihydrodiol-epoxide reaction with deoxyadenosine residues (adduct 1, 36%), from the same dihydrodiol-epoxide reacting with deoxyguanosine residues (adduct 2, 11%) and from the reaction of the syn-dihydrodiol-epoxide with deoxyadenosine residues in DNA (adduct 3, 28%). The formation of these three major adducts is reminiscent of findings reported previously for DMBA in mouse cells (26, 27, 31) and for BcP in mouse, hamster, and rat cells (32), and both of these hydrocarbons, like BgC, have a sterically hindered bay region or a fjord region.

The finding that the parent BgC bound to DNA through an 11,12-dihydrodiol 13,14-epoxide was anticipated because the active dihydrodiol-epoxide was expected to have the epoxide in the fjord region (5), and the hydroxyl groups of a 3,4-dihydrodiol 1,2-epoxide would be in a bay region and, therefore, would be diaxial, a conformation not usually associated with reactivity toward DNA or carcinogenic potential (33, 34). This mode of activation is also consistent with that reported for related hydrocarbons such as BcP (32), DMBA (26), and dibenzof[a]anthracene (22, 35). Additionally, previous work has established the mutagenicity of racemic anti- and syn-BgCDEs (11-15), and racemic anti-BgCDE has been found to be a potent carcinogen (8, 10).

The present investigations have shown that BgC is metabolically activated to DNA-binding metabolites in human MCF-7 cells and that the reactive metabolites that mediate this binding are syn- and anti-11,12-dihydrodiol 13,14-epoxides, as reported for activation in mouse skin (36). In addition to a number of minor adducts, the former epoxide primarily reacted with deoxyadenosine residues in DNA, and the latter one formed two major adducts, one with deoxyguanosine and one with deoxyadenosine residues (37). Analogous extensive reaction with deoxyadenosine residues in DNA was first described for the very potent hydrocarbon carcinogen DMBA (27, 31) and has since been attributed to all dihydrodiol-epoxides derived from nonplanar hydrocarbons (38). Moreover, recent studies with the configurationally isomeric BcP dihydrodiol-epoxides in vivo have suggested that the deoxyadenosine adducts may be more potent in tumor initiation than the corresponding deoxyguanosine adducts (30). In contrast,
dihydrodiol-epoxides derived from planar hydrocarbons react mostly with deoxyguanosine residues of DNA (39–41). It has already been shown that racemic anti-BgC is a potent carcinogen because its activity is comparable to that of anti-BcP (23, 25), which in turn is much more potent than the planar anti-BaP dihydrodiol-epoxide (8). Additionally, it is known that BgC is present in the environment (17, 18). Thus, the present demonstration that the carcinogenic anti-dihydrodiol-epoxide is formed from the parent hydrocarbon in a human mammary carcinoma cell line suggests that BgC, even in a small amounts, could pose a risk for cancer in humans.

REFERENCES


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