The Potent Carcinogen Dibenzo[a,l]pyrene Is Metabolically Activated to Fjord-Region 11,12-Diol 13,14-Epoxides in Human Mammary Carcinoma MCF-7 Cell Cultures

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Abstract

Dibenzo[a,l]pyrene (DB[a,l]P), an environmental hydrocarbon and very potent carcinogen in rodent bioassays, could be activated to DNA-binding intermediates in cells through formation of three different regioisomeric bay- or fjord-region diol-epoxides or other more highly oxidized metabolites. The mechanism of metabolic activation of DB[a,l]P in the human mammary carcinoma cell line MCF-7 was elucidated by analyzing the DB[a,l]P-DNA adducts formed by [35S]phosphorothioate postlabeling, immobilized boronate chromatography, and high-performance liquid chromatography. Six DB[a,l]P-DNA adducts were detected. Comparison with those formed by DB[a,l]P-11,12-diol and by reaction of DNA with syn- and anti-(benzylidene hydroxyl and epoxide oxygen cis and trans, respectively) DB[a,l]P-11,12-diol-13,14-epoxide (DB[a,l]PDE) demonstrated that all DB[a,l]P-DNA adducts in MCF-7 cells were formed by these diol-epoxide isomers. Cellular DNA contained large amounts of two syn- and one anti-DB[a,l]PDE-DNA adducts and small amounts of one syn- and two anti-DB[a,l]PDE-DNA adducts. The ability of human cells to activate DB[a,l]P to its fjord-region 11,12-diol 13,14-epoxides suggests that environmental exposure to DB[a,l]P could pose a risk for humans.

Introduction

DB[a,l]P, an environmental PAH found in the active fraction of cigarette smoke condensate (1) and other environmental contaminants (2), exhibits both high carcinogenicity and toxicity in mouse skin and rat mammary glands (3-5). Until recently, DMBA was the most potent known carcinogenic PAH; however, on a molar basis DB[a,l]P exceeds the carcinogenic potency of DMBA (3-5). The human exposure and high carcinogenic potency of DB[a,l]P emphasized the importance of determining the mechanism of metabolic activation of this hydrocarbon and whether humans could activate it. PAHs require metabolic activation to chemically reactive intermediates in order to induce carcinogenesis and other biological effects. Sims et al. (6) first demonstrated that B(a)P was activated in cells to a diol-epoxide and that this covalently bound to the cellular DNA. Since that time, a number of other hydrocarbons have been found to undergo metabolic activation in cells to “bay-region” diol-epoxides (7). In some cases, activation to other intermediates, such as radical cations that form unstable DNA adducts, can also take place (8). Recently, Rama-Krishna et al. (9) demonstrated the formation of adducts at the 10 position of DB[a,l]P with deoxyguanine and deoxyadenine by electrochemical oxidation which are readily transformed to purine adducts. If formed in vivo, this type of reactive DB[a,l]P metabolite would be likely to form unstable DNA adducts (9). Among those PAHs activated to diol-epoxides are DMBA (10), benzo[c]phenanthrene (11), and 5-methylchrysene (12). The formation of highly reactive phenol-diol epoxides was first demonstrated for chrysene (13). However, recent studies have shown that PAH containing five or six aromatic rings are activated through more complex oxidation pathways (14-16). Metabolic activation of dibenz[a,h]anthracene was recently found to involve a 3,4,10,11-bis-diol 1,2-epoxide (14, 15). Similarly, the related symmetrical PAH dibenzo[a,l]pyrene was found to be activated through oxidation of the a and h rings (16). In the present study, we examined the metabolic activation of the potent carcinogen DB[a,l]P in a human mammary carcinoma cell line (MCF-7) to determine its mechanism of metabolic activation to DNA-binding derivatives and to determine if humans are able to activate this PAH.

Materials and Methods

Cell Culture and Treatment. The human mammary carcinoma cell line (MCF-7) was grown in minimum essential medium supplemented with 10% fetal calf serum, pyruvate, and nonessential amino acids. The MCF-7 cells were refed with the complete medium 24 h prior to treatment with the compounds described below. Dibenzo[a,l]pyrene was purchased from Chemsyn Science Laboratories, Lenexa, Kansas. DB[a,l]P-11,12-diol was produced enzymatically by incubation of DB[a,l]P with Aroclor 1254-induced rat liver S9 as described by Devanesan et al. (8). Chemically synthesized DB[a,l]P-11,12-diol and syn- and anti-DB[a,l]PDE were characterized by mass spectrometry and NMR spectroscopy; the synthetic methods and spectral data are being prepared for publication elsewhere. Calf thymus DNA was treated with 50 μg of either the syn- or anti-diol epoxide in a solution of 0.1 μ Tris (pH 7.4) at 37°C. After incubation for 4 h, the mixture was extracted with ethyl acetate (three times) and diethyl ether (three times), and the modified DNA was precipitated by addition of ethanol (2 vol.) and sodium chloride (5 μ, 0.1 vol.), washed with ethanol (70%), and redissolved in water. Cultures were treated with DB[a,l]P (1 mg/ml acetone; final concentration, 8 μM) and DB[a,l]P-11,12-diol (1 mg/ml dimethyl sulfoxide; final concentration, 0.3 μM). Twenty-four h later, the MCF-7 cells were harvested, and the DNA was isolated by phenol-chloroform extraction and ethanol precipitation (17).

35S-Postlabeling Analysis. DNA samples (30 μg) were digested with nuclease P1 (1.2 units) and prostatic acid phosphatase (0.6 units). Samples were then 35S-postlabeled by incubation with [γ-35S]ATP (50 μCi) and T4 polynucleotide kinase (60 units) at room temperature overnight, followed by digestion with snake venom phosphodiesterase (17, 18). The DB[a,l]P-deoxyribo- nucleotide adducts were isolated by chromatography on a Sep-Pak C-18 cartridge eluted with ammonia/methanol (5%/95%). Immobilized boronate chromatography was carried out by loading the 35S-labeled DB[a,l]P- and DB[a,l]P-11,12-diol-DNA adducts onto an immobilized

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cells treated with 0.3 \mu M DB[a,I]P for 24 h. The DB[a,I]P-DNA adducts were postlabeled with [\textsuperscript{35}S]phosphorothioate, and the mononucleotide adducts were analyzed as described in "Materials and Methods." The total level of DB[a,I]P-DNA adducts was 25 pmol/mg DNA. To further characterize the DB[a,I]P-deoxyribonucleotide adducts, another portion of the above DNA samples was applied to an immobilized boronate column which retains compounds containing cis-vicinal hydroxyl groups. Unretained material was eluted with a HEPES/methanol buffer, isolated by chromatography on a Sep-Pak, and analyzed by HPLC (Fig. 1A). Adducts retained by the immobilized boronate column were eluted with a Tris/sorbitol buffer, concentrated on a Sep-Pak, and analyzed by HPLC (Fig. 1C). The unretained material contained two of the larger adduct peaks (peaks 3 and 6) and one of the smaller adduct peaks (peak 5) (Fig. 1B). Three of the DB[a,I]P-DNA adducts were retained by the column, one of the large peaks (peak 4) and two of the smaller peaks (peaks 1 and 2; Fig. 1C), indicating that they contained cis-vicinal hydroxyl groups. Cis-vicinal hydroxyl groups are formed upon reaction of DNA bases with the benzylic carbon of the anti-isomers of PAH diol epoxides, suggesting that DB[a,I]P was metabolized to an anti-diol epoxide in MCF-7 cells.

The positions of DB[a,I]P involved in metabolic activation of these DNA binding derivatives were investigated by treating MCF-7 cultures with 0.3 \mu M DB[a,I]-11,12-diol that was enzymatically synthesized with rat liver S-9 as described by Devanesan \textit{et al.} (8) for 24 h. The DNA was isolated, and the adducts were [\textsuperscript{35}S]-postlabeled and analyzed by HPLC (Fig. 2A). Three DNA adducts were present in the DB[a,I]-11,12-diol-treated MCF-7 cell DNA, one large DNA adduct peak (peak 4) and two smaller adduct peaks (peaks 1 and 2). The [\textsuperscript{35}S]-labeled DB[a,I]-11,12-diol-DNA adducts were also analyzed by immobilized boronate chromatography followed by HPLC. No adducts were eluted with the HEPES/methanol buffer (Fig. 2B). All of the DB[a,I]-11,12-diol-DNA adduct peaks were retained by the boronate column (Affigel 601) and incubating the adducts on ice for 1 h. The first fifty 1.0-ml fractions were eluted with a HEPES/methanol buffer. C. HPLC profile of the DB[a,I]-DNA adducts retained by the immobilized boronate column (peaks 1, 2, and 4) after elution with a Tris/sorbitol buffer.

Results

The human mammary carcinoma cell line MCF-7 has been shown to metabolically activate PAH such as B[a,I]P to DNA-binding metabolites.\textsuperscript{6} To determine whether DB[a,I]P is activated to DNA binding intermediates in this cell line, MCF-7 cells were treated with 8 \mu M DB[a,I]P and harvested 24 h later. The DNA was isolated and degraded with the nuclease P\textsubscript{1}-prostatic acid phosphatase procedure, the DB[a,I]-DNA adducts were postlabeled with [\textsuperscript{35}S] phosphorothioate, cleaved with snake venom diesterase to give [\textsuperscript{35}S]-DB[a,I]-5'-mononucleotide adducts, and the adducts were analyzed by reverse-phase HPLC (Fig. 1A). Three major DB[a,I]-DNA adduct peaks (peaks 3, 4, and 6) and three smaller DNA adduct peaks (peaks 1, 2, and 5) were detected. At this dose and treatment time, the total level of DB[a,I]-DNA binding was 25 pmol/mg DNA. To further characterize the DB[a,I]-deoxyribonucleotide adducts, another portion of the above DNA samples was applied to an immobilized boronate column which retains compounds containing cis-vicinal hydroxyl groups. Unretained material was eluted with a HEPES/methanol buffer, isolated by chromatography on a Sep-Pak, and analyzed by HPLC (Fig. 1B). Adducts retained by the immobilized boronate column were eluted with a Tris/sorbitol buffer, concentrated on a Sep-Pak, and analyzed by HPLC (Fig. 1C). The unretained material contained two of the larger adduct peaks (peaks 3 and 6) and one of the smaller adduct peaks (peak 5) (Fig. 1B). Three of the DB[a,I]P-DNA adducts were retained by the column, one of the large peaks (peak 4) and two of the smaller peaks (peaks 1 and 2; Fig. 1C), indicating that they contained cis-vicinal hydroxyl groups. Cis-vicinal hydroxyl groups are formed upon reaction of DNA bases with the benzylic carbon of the anti-isomers of PAH diol epoxides, suggesting that DB[a,I]P was metabolized to an anti-diol epoxide in MCF-7 cells.

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\textsuperscript{6} S. Ralston, H. S. L. Lau, and W. M. Baird, unpublished results.
ronate column (Fig. 2C; peaks 1, 2, and 4). A similar adduct profile was also obtained after treating the MCF-7 cells with synthetic racemic DB[a,I]P-11,12-diol (data not shown). The total level of DB[a/I]P-11,12-diol-DNA binding for the enzymatically synthesized diol and the chemically synthesized diol in MCF-7 cells were 82 and 70 pmol/mg DNA, respectively. Comparison of elution profiles with those of DB[a/I]P-treated cells (Fig. 1) demonstrates that one large (peak 4) and two smaller (peaks 1 and 2) adduct peaks formed in MCF-7 cells are also formed by the treatment with DB[a/I]P-11,12-diol. This suggested that DB[a,I]P was activated through formation of an 11,12-diol 13,14-epoxide.

DNA was reacted with synthetic syn- and anti-DB[a,I]PDE. The adducts formed were postlabeled, and their elution times compared with those of the DB[a,I]P-DNA adducts formed in cells. The HPLC profile of the [35S]DB[a,I]P-DNA adducts for MCF-7 cells is shown in Fig. 3A, and the HPLC profiles of the syn- and anti-DB[a,I]PDE-DNA adducts are shown in Fig. 3, B and C, respectively. The large DB[a,I]P-DNA adduct peak retained on the boronate column, peak 4, is identical to the major peak present in the anti-DB[a,I]PDE reacted DNA sample (Fig. 3C). The two smaller adduct peaks observed in the retained fraction from the immobilized boronate chromatography of the DB[a,I]P and DB[a,I]P-11,12-diol-DNA adducts (peaks 1 and 2) were also present in the anti-DB[a,I]PDE-DNA adduct profile (Fig. 3C). The three DB[a,I]P-DNA adduct peaks not retained by the boronate column (peaks 3, 5, and 6; Fig. 1B) had identical elution characteristics to adducts formed by reaction of syn-DB[a,I]PDE with DNA (Fig. 3B). The ratio of the amount of material in the two larger peaks, 3 and 6, was similar in both the DNA for DB[a,I]P-treated cells (Figs. 1B and 3A) and in the DNA reacted with syn-DB[a,I]PDE (Fig. 3B). The DB[a,I]P-DNA adduct peaks coeluted with DB[a,I]PDE-adduct standards in the above chromatographic system. Thus, all six DB[a,I]P-DNA adducts detected in MCF-7 cells resulted from activation of DB[a,I]P to DB[a,I]P-11,12-diol-13,14-epoxide; peaks 1, 2, and 4 were formed by the anti-isomer, and peaks 3, 5, and 6 by the syn-isomer. The synthetic diol epoxides also reacted with DNA to form additional adduct peaks not observed in DB[a,I]P-treated cells including a syn-DB[a,I]PDE-adduct at 85 min (Fig. 3B) and an anti-DB[a,I]PDE-adduct at 45 min (Fig. 3C). Their identity has not been established, but they may result from enantiomers of DB[a,I]PDE not formed in MCF-7 cells.

**Discussion**

Due to the structure of DB[a,I]P, three regioisomers of stericly hindered diol-epoxides can be formed, namely: a bay-region 1,2-diol 3,4-epoxide and the two fjord-region 3,4-diol 1,2-epoxide and 11,12-diol 13,14-epoxide. Furthermore, each of these diol-epoxides can be metabolically produced in a variable ratio of syn- and anti-diasteroisomers. Based on the relatively large PAH ring system of DB[a,I]P, an additional number of higher hydroxylated derivatives of the above diol-epoxides could possibly be formed in cells. Hughes and Phillips (19) examined the DNA adducts formed in DB[a,I]P-treated mouse skin by 32P-postlabeling and demonstrated the presence of at least six adducts in skin and four in lung by thin-layer chromatography. Two adducts each accounted for more than 25% of the total, two represented about 8 to 10%, and two accounted for 1 to 2%. To identify the metabolites responsible for DB[a,I]P-DNA binding, we treated the human mammary carcinoma cell line MCF-7 with DB[a,I]P and analyzed the DNA adducts by 32P-postlabeling, immobilized boronate chromatography, and HPLC. The results demonstrate that DB[a,I]P is metabolically activated in MCF-7 cell cultures to DNA-binding metabolites and that both the syn- and anti-isomers of DB[a,I]PDE are formed (Fig. 4). As observed by Hughes and Phillips (19), two adducts were present in larger amounts than the other four. We detected large amounts of one syn- and one anti-DB[a,I]PDE adduct, an intermediate amount of one syn-DB[a,I]PDE adduct, and smaller amounts of one syn- and two anti-DB[a,I]PDE adducts. No other adducts formed by unidentified metabolites were observed, although the possibility that the peak of radioactivity eluting in the breakthrough volume could contain some very highly polar adducts along with the unincorporated [35S]phosphorothioate cannot be ruled out. Cultures treated with DB[a,I]P-11,12-diol, the proximate carcinogenic metabolite of DB[a,I]PDE, formed only anti-DB[a,I]PDE-DNA adducts, whereas those treated with the parent hydrocarbon formed adducts from both diol-epoxide isomers. One possible explanation was that the enzymatic method used to produce the DB[a,I]P-11,12-diol resulted in selective formation of a single enantiomer that was metabolized only to anti-DB[a,I]PDE. However, treatment with synthetic racemic DB[a,I]P-11,12-diol produced a similar DNA adduct profile. One potential explanation is that DB[a,I]P pretreatment could induce a cytochrome-P450 isozyme that selectively activates DB[a,I]P-11,12-diol to the syn-DB[a,I]PDE, and this may not have been induced in cultures treated with DB[a,I]P-11,12-diol.

The finding that DNA-binding of DB[a,I]P is mediated by the metabolic formation of a single regiosomeric pair of diol-epoxides rather than a more highly oxidized derivative of a diol-epoxide contrasts with results obtained for a number of other five- or six-ring PAH such as dibenz[a,h]anthracene (14, 15) and dibenzo[a,h]pyrene (16). However, taking into account the related symmetry properties of the latter two PAH, they are significantly different from DB[a,I]P. Thus, the metabolic activation of DB[a,I]P appears to be more comparable to that of B[a]P. Liver microsomes from 3-methylcholanthrene-induced rats metabolized DB[a,I]P mainly to the 11,12- and the 8,9-diols (8). The structure of DB[a,I]P may make the oxidation of the 1,2,3,4-positions less favorable. The high level of DNA-binding
DB[a,l]P is activated to diol epoxides

The pathway proceeds efficiently in human cells leading to stable DNA adducts of this potent carcinogen, environmental exposure to DB[a,l]P may pose a risk to humans.

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References


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