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Separation of ^{32}P-labeled 3′,5′-bisphosphate nucleotides of polycyclic aromatic hydrocarbon *anti*-diol-epoxides and derivatives☆

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Abstract

^{32}P-Postlabeling-HPLC is a highly sensitive analytical method for identification of chemical-modified DNA adducts isolated from samples obtained from experimental animals or humans exposed to carcinogenic chemicals. To determine optimal ^{32}P-postlabeling-HPLC conditions for efficient separation, we report here the use of ten diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotides derived from benzo[*a*]pyrene (BaP), nitrated BaP, and related compounds. After testing ODS-modified, C_4-modified, phenyl-modified, diphenyl-modified, and cyclodextrin-bonded reversed-phase HPLC columns, we found that the Vydac diphenyl-modified column can efficiently separate these 3′,5′-bisphosphate deoxynucleotides. The results suggest that ^{32}P-postlabeling-HPLC is a potentially useful methodology for detecting environmental carcinogens that can be metabolized to diol-epoxides. The relationships between the structures of *anti*-diol-epoxides and HPLC retention order are also discussed.

1. Introduction

In the USA about 95% of the current energy consumption is fossil fuel [1]. Combustion of fossil fuel is never complete and produces polycyclic organic matter (POM) as by-products. It has been well documented that POM causes air pollution and poses a threat to human health and the environment [2,3]. POM contains polycyclic aromatic hydrocarbons (PAHs) and nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) [4–7], both of which are carcinogenic chemicals [4,5,8,9]. The POMs extracted from the ambient air, coal, chimney soot, diesel and gasoline engines, industrial carbon black, oil shale soot, coke oven emissions, and roofing tar emissions all induce tumors in experimental animals [4]. Consequently, it is important to assess human health risk posed by air pollution from different sources.

Benzo[*a*]pyrene (BaP) has been used as a biomarker for measurement of PAHs extracted from the POM. It has been found that POM extracted from diesel soot contains a relatively

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high concentration of nitro-PAHs compared to the parent PAHs [6,10]. For example, diesel soot extract contains 30 and 202 ppm pyrene and 1-nitropyrene, respectively, and contains BaP, 1-nitrobenzo[*a*]pyrene (1-NBaP), 3-nitrobenzo[*a*]pyrene (3-NBaP), and 6-nitrobenzo[*a*]pyrene in quantities of 6.4, 3, 6, and 14 ppm, respectively [6]. Both PAHs and nitro-PAHs require metabolic activation to covalently bind to DNA and to exert their mutagenic and tumorigenic activities [4,5,8,9,11]. It has been established that binding of the reactive *anti*-diol-epoxide metabolites to DNA, resulting in the formation of N^2-deoxyguanosinyl adducts, is the principal metabolic activation of PAHs in vivo [1,8]. ^{32}P-Postlabeling has been established as a sensitive analytical method for identification of chemical-modified DNA adducts isolated from samples obtained from experimental animals or humans exposed to carcinogenic chemicals [6,7,12–16]. Pfau and Phillips [16] developed an efficient ^{32}P-postlabeling-HPLC methodology for the separation of ten ^{32}P-labeled nucleoside 3′,5′-bisphosphate adducts derived from *anti*-diol-epoxides of PAHs, including benzo[*a*]pyrene (BaP) *anti*-diol-epoxide. The optimal HPLC conditions for separation of these adducts were discussed in detail. Recently, King et al. [7] reported the separation of several ^{32}P-labeled 3′,5′-bisphosphate adducts of *anti*-diol-epoxides of PAHs and a series of nitro-PAH derived adducts.

1-NBaP and 3-NBaP are potent mutagens in *Salmonella* [17,18]. In vitro microsomal metabolism of 1- and 3-NBaP-DE both produce the corresponding bay-region *anti*-diol-epoxides as metabolites [17,18], which upon interaction with calf thymus DNA result in the formation of N^2-deoxyguanosinyl adducts [19,20]. Since both BaP and NBaPs are present in diesel soot extract in substantial quantities [6], it is relevant to determine whether or not the ^{32}P-labeled nucleoside 3′,5′-bisphosphate adducts derived from *anti*-diol-epoxides of BaP and NBaPs are separable. We report in this paper the separation of the ^{32}P-labeled nucleoside 3′,5′-bisphosphate adducts derived from ten PAH and nitro-PAH *anti*-diol-epoxides. The relationships between the structures of *anti*-diol-epoxides and HPLC retention order are also discussed.

2. Experimental

2.1. Materials

BaP diol-epoxide, *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BaP-DE), was prepared as previously described [21]. The following five *anti*-diol-epoxides were previously prepared in our laboratory: *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydro-1-nitrobenzo[*a*]pyrene (1-NBaP-DE) [22], *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydro-2-nitrobenzo[*a*]pyrene (2-NBaP-DE) [23], *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydro-3-nitrobenzo[*a*]pyrene (3-NBaP-DE) [19], *cis*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*cis*-BaP-DE) [24], and *trans*-9,10-dihydroxy-*anti*-7,8-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (9,10-BaP-DE) [24]. Following the established procedures for synthesis of the diol-epoxides described above [19–22], *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydro-7-methylbenzo[*a*]pyrene (7-MBaP-DE) was synthesized by epoxidation of *trans*-7,8-dihydroxy-7,8-dihydro-7-methylbenzo[*a*]pyrene [25]. *Trans*-8,9-dihydroxy-*anti*-10,11-epoxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (8,9-BA-DE), *trans*-10,11-dihydroxy-*anti*-8,9-epoxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (10,11-BA-DE), and *trans*-10,11-dihydroxy-*anti*-12,13-epoxy-10,11,12,13-tetrahydrodibenz[*a*,*c*]-anthracene (DB[*a*,*c*]A-DE) were similarly prepared.

2.2. Chemical synthesis of DNA adducts

The DNA adducts of BaP-DE, 3-NBaP-DE, *cis*-BaP-DE, and 7-MBaP-DE were prepared according to previously described procedures [19,24,26]. The DNA adducts of 1- and 2-NBaP-DE were prepared in a manner similar to the 3-NBaP-DE DNA adduct [19]. The DNA adducts of the remaining diol-epoxides were prepared by a standard procedure: a solution of a

diol-epoxide (5.0 mg, dissolved in 200 μl of freshly distilled tetrahydrofuran) in 10 m*M* Tris buffer (20 ml, pH 7.5) was incubated with purified calf thymus DNA (20 mg) at 37°C for 4 h. The reaction mixture was extracted three times with an equal volume of *n*-butanol saturated with 10 m*M* Tris buffer (pH 7.5). The aqueous layer was extracted three times with 20 ml of water-saturated isoamyl alcohol. An amount of 2 ml of 5 *M* sodium chloride was added to the aqueous phase and the DNA was precipitated with 22 ml of ice-cold 95% ethanol. The DNA concentration was determined spectrophotometrically by measuring the UV absorbance at 260 nm.

For structural identification of the adducts, each of the modified DNA was enzymatically digested to nucleosides by incubating with DNase I, followed by incubation with alkaline phosphatase and snake venom phosphodiesterase I.

2.3. ^{32}P-postlabeling-HPLC identification of 3′5′-bisphosphates deoxyribonucleotides

The modified DNA (10 μg) suspended in 20 μl of 5 m*M* Bis-Tris, 0.1 m*M* EDTA, pH 7.1 buffer was hydrolyzed to deoxyribonucleoside 3′-monophosphate at 37°C for 4 h with 30 μl of a mixture containing micrococcal nuclease (125 U/ml) and spleen phosphodiesterase (12.5 U/ml) in 20 m*M* sodium succinate and 10 m*M* calcium chloride (pH 6.0) following the procedure of Gupta [27]. DNA adducts were enriched by the *n*-butanol extraction method. The resulting deoxyribonucleoside 3′-monophosphates were dissolved in 25 μl of water and [γ-^{32}P] phosphorylated with 5 μl of PNK mix containing 200 μCi [γ-^{32}P]ATP and 1 U/μg DNA of T4 polynucleotide kinase at 37°C for 40 min. The residual [γ-^{32}P]ATP was destroyed by the addition of 1.5 μl of potato apyrase (30 μU), followed by incubation at 37°C for 30 min.

To separate the modified 3′,5′-bisphosphate deoxyribonucleotides from the unmodified nucleotides, thin-layer chromatography was performed using Machery–Nagel PEI cellulose plates (3 × 5 cm, Alltech Associates, Deerfield, IL, USA) with 0.65 *M* sodium phosphate, pH 6.8, onto a 15-cm Whatman 1 paper wick that had been attached to the top of the plate (D_1 direction only). The material from the origin of the plates was scraped, transferred to 1.5-ml polypropylene microcentrifuge tubes, and extracted with 500 μl of 4 *M* pyridinium formate, pH 4.2 at room temperature for 4 h with stirring on a mechanical mixer. After centrifugation for 10 min, the supernatant was collected and centrifuged again for 10 min to remove fine particles. The supernatants were dried under reduced pressure and redissolved in 0.5 *M* sodium phosphate buffer (pH 2.0) for HPLC analysis. The radioactivity was determined by liquid scintillation counting.

2.4. HPLC analysis

HPLC analysis was performed with a Waters Associates instrument consisting of two Model 510 pumps, a Model 680 solvent programmer, a Model U6K injector, and in line with a Radiomatic FLO-ONE/Beta A-515 system with the samples measured in a 500-μl flow cell. Samples were eluted at 1.2 ml/min with a 3-step linear gradient as follows: (1) a linear gradient from 10% of solvent A (methanol–500 m*M* sodium phosphate buffer, pH 2.0, 9:1) in solvent B (500 m*M* sodium phosphate buffer, pH 2.0) to 30% of solvent A in solvent B for 10 min; (2) a linear gradient for 30 min from 30% of solvent A in solvent B to 35% solvent A in solvent B; and (3) a 20 min linear gradient from 35% solvent A in solvent B to 90% solvent A in solvent B. The columns used were: Zorbax ODS (250 × 4.6 mm I.D.); Zorbax phenyl (250 × 4.6 mm I.D.) (DuPont Medical Products, Wilmington, DE, USA); Vydac 201TP54 ODS (250 × 4.6 mm I.D.); Vydac 219 diphenyl (250 × 4.6 mm I.D.) (The Separations Group, Hesperia, CA, USA); MetaChem C4/E (250 × 4.6 mm I.D.) (MetaChem Technologies, Redondo Beach, CA, USA); Phenomenex IB-SIL 5 phenyl column (250 × 4.6 mm I.D.) (Phenomenex, Torrance, CA, USA); Waters μBondapak ODS (300 × 3.9 mm I.D.) (Waters Associates, Milford, MA, USA); Beckman ODS (250 × 4.6 mm I.D.) (Beckman Instru-

ments, San Ramon, CA, USA); Astec Cyclobond II (γ); Supelcosil LC-18-DB (250 × 4.6 mm I.D.) (Supelco, Supelco Park, Bellefonte, PA, USA); Hypersil ODS (250 × 4.6 mm I.D.) (Sigma-Aldrich, Aldrich, Milwaukee, WI, USA). All columns have a particle size of 5 μm. To eliminate the possible UV photolytic decomposition of the compounds, the laboratory was equipped with UV absorbing films placed above the light diffusion panels.

3. Results and discussion

3.1. Selection of diol-epoxides for study

To determine optimal ^{32}P-postlabeling-HPLC conditions for efficient separation of diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotides derived from PAHs and nitro-PAHs, we employed ten structurally related PAH and nitro-PAH diol-epoxides and a number of different types of HPLC columns for study. The structures and abbreviations of the diol-epoxides used in this study are shown in Fig. 1. The reason for choosing these diol-epoxides is based on the following considerations: BaP-DE, 1-NBaP-DE, 3-NBaP-DE, and DB[*a*,*c*]A-DE are the ultimate metabolites of BaP, 1-NBaP, 3-NBaP, and DB[*a*,*c*]A [28], respectively. BA *trans*-8,9-dihydrodiol and BA *trans*-10,11-dihydrodiol are the predominant metabolites of BA in vitro [29]. Metabolism of these two BA dihydrodiols may result in the formation of 8,9-BA-DE and 10,11-BA-DE. 7-MBaP is a possible environmental contaminant, and metabolism of 7-MBaP forms

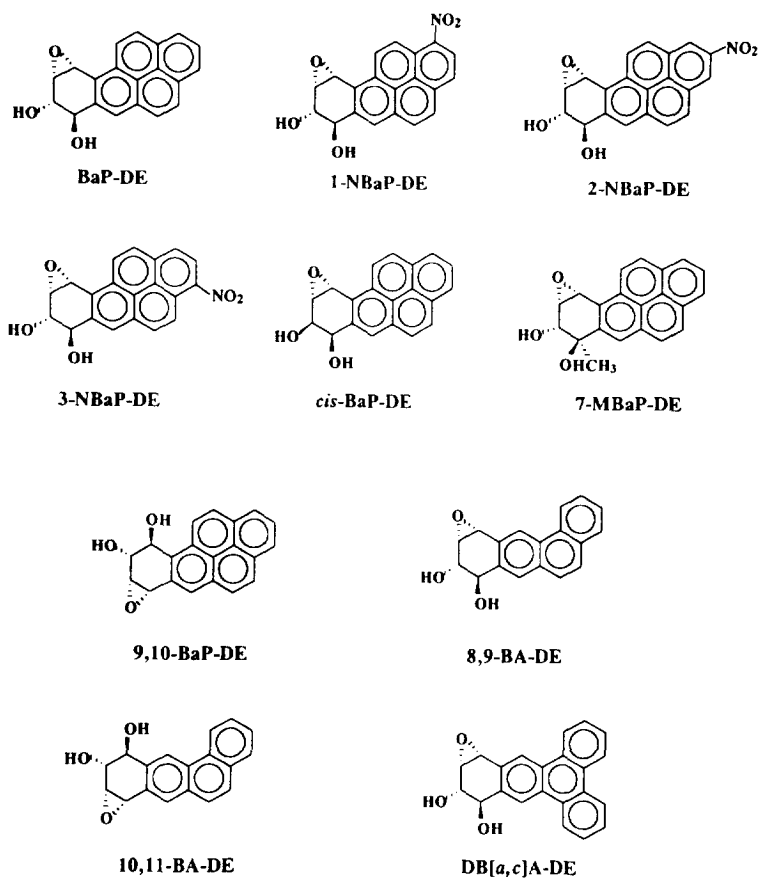

Fig. 1. Structures and abbreviations of *anti*-diol-epoxides of BaP, 1-NBaP, 2-NBaP, 3-NBaP, and related PAHs.

7-MBaP *trans*-7,8-dihydrodiol [30]. With an additional methyl group at the C_7 position compared with that of BaP-DE, study of this compound will enable us to determine the effect of a methyl group on HPLC retention. Since BaP *trans*-7,8-dihydrodiol is a metabolite of BaP, 9,10-BaP-DE is also possibly formed. The use of *cis*-BaP-DE will enable us to determine the effect of the *cis* configuration on elution order.

With the exception of 9,10-BaP-DE and *cis*-BaP-DE, all the other diol-epoxides employed for this study are *anti*-diol-epoxides. As reported previously, diol-epoxides of *cis*-BaP-DE and 9,10-BaP-DE are a mixture of the *syn*- and *anti*-diastereoisomers [24].

The diol-epoxide-modified DNA adducts, prepared by reacting diol-epoxides with purified calf thymus DNA, were hydrolyzed to deoxyribonucleoside 3′-monophosphates with micrococcal nuclease and spleen phosphodiesterase following the published standard procedure [12]. [5′-^{32}P]Phosphorylation of 3′-monophosphates resulted in diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotides [12] which were enriched by the *n*-butanol extraction procedure [14] and analyzed by HPLC.

3.2. Separation of diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotide adducts by HPLC

To compare separation efficiency among different types of columns (monomeric and polymeric; octadecyl-modified and phenyl-modified etc.), eleven reversed-phase HPLC columns [six ODS columns (Zorbax, Vydac, μBondapak, Supelcosil LC-18-DB, Ultrasphere, and Hypersil), one C_4-modified column (MetaChem), two phenyl-modified columns (Zorbax and Phenomenex IB-SIL), one diphenyl-modified column (Vydac), and one cyclobond column (Astec Cyclobond II(γ))] were used. The Zorbax ODS, μBondapak C_{18}, and Vydac ODS columns have a conventional bonding between the silicate hydroxy groups and the substituents. However, the bonded phase of the Zorbax and μBondapak ODS columns is monomeric and the Vydac ODS is polymeric. The MetaChem C_4 column is packed with a uniform matrix of crosslinked polysiloxane functional groups. The phenyl and diphenyl columns, which are recommended for polypeptides, particularly those containing aromatic sidechains, have an aromatic phenyl or diphenyl group chemically bonded to silica that is capped with trimethylsilane to prevent adsorption of polar compounds. The Cyclobond II(γ) column consists of eight glucopyranose units arranged in the shape of a hollow truncated cone with the interior cavity of 9.5 Å in diameter. Separation of compounds by the Cyclobond column is in general governed by the molecule's ability to fit the cavity of the cyclodextrin, although the polarity of the molecule is also a factor. Thus, comparison of the separation efficiencies of the columns and the retention order of the compounds will provide a better understanding of the mechanisms of interaction between the bonded phase and the diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotide solutes.

After employing various solvent systems and HPLC conditions, it was found that, in general, the order of separation efficiency of these HPLC columns is: diphenyl-modified column ≥ phenyl-modified column > ODS column ≫ cyclobond column > C_4-modified column. Separation of each of the ten diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotides on a Vydac diphenyl-modified reversed-phase HPLC column is shown in Fig. 2. The optimal HPLC conditions employed a solvent system similar to that reported by Pfau and Phillips [16], which consists of buffer A, 0.5 *M* sodium dihydrogen orthophosphate and 0.5 *M* orthophosphoric acid (pH 2.0) and buffer B, a mixture of methanol in buffer A (9:1, v/v). With the exception of 9,10-BaP-DE and *cis*-BaP-DE, all the diol-epoxides generated one major adduct. The major adducts account for the following percentages of total adduct formation: 10,11-BA-DE, 79%; 8,9-BA-DE, 78%; BaP-DE, 90%; 1-NBaP-DE, 56%; 2-NBaP-DE, 75%; 3-NBaP-DE, 73%; 7-MBaP-DE, 61%; and DB[*a*,*c*]A, 72%. Due to being a mixture of *syn*- and *anti*-diol-epoxides, both 9,10-BaP-DE and *cis*-BaP-DE generate multiple adducts (Fig. 2) and are not suitable to combine with other adducts for HPLC separation. There-

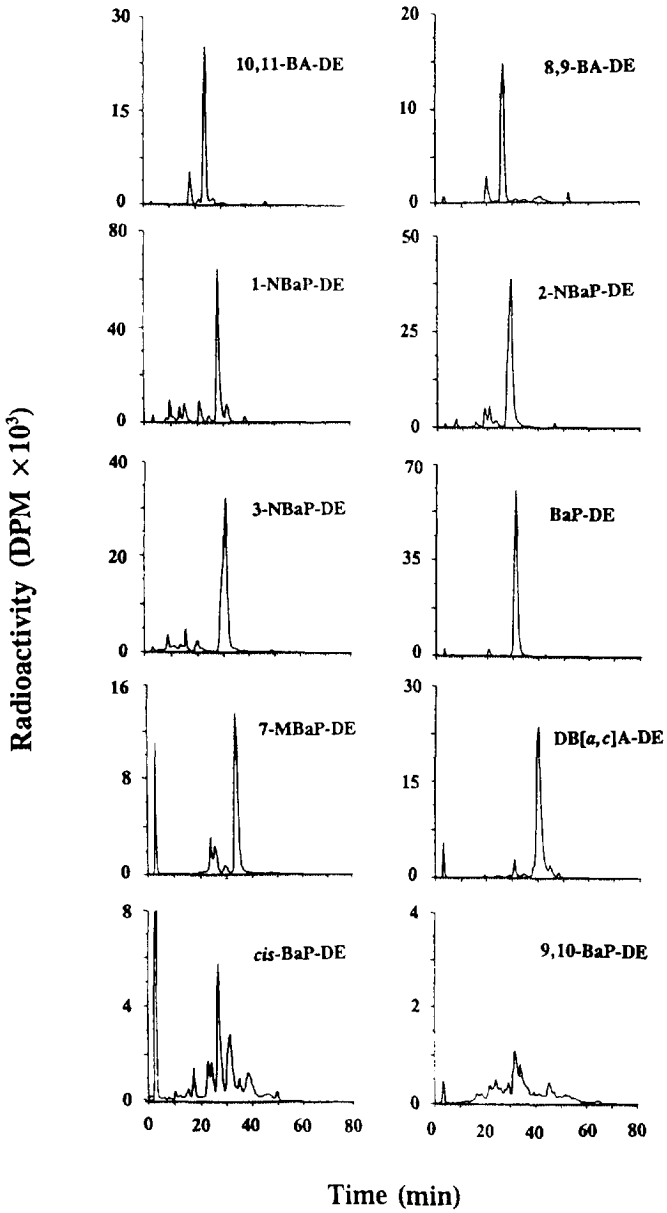

Fig. 2. Separation of each of the ten diol-epoxide-modified 3',5'-bisphosphate deoxynucleotides on a Vydac diphenyl-modified reversed-phase HPLC column. For chromatographic conditions, see Section 2.4.

fore, only eight diol-epoxide-modified 3',5'-bisphosphate deoxynucleotides were employed for determining HPLC separation efficiency (Fig. 3). For comparison, the retention times of the 9,10-BaP-DE and *cis*-BaP-DE adducts are marked in Fig. 3.

Two approaches are used to determine the relative HPLC retention times of each adduct. The first is to compare the retention time of the individual adduct. The second is to repeat the HPLC separation by increasing the concentration of one or two components. For example, as shown in Fig. 3B, when the quantities of the 10,11-BA-DE and BaP-DE adducts were in-

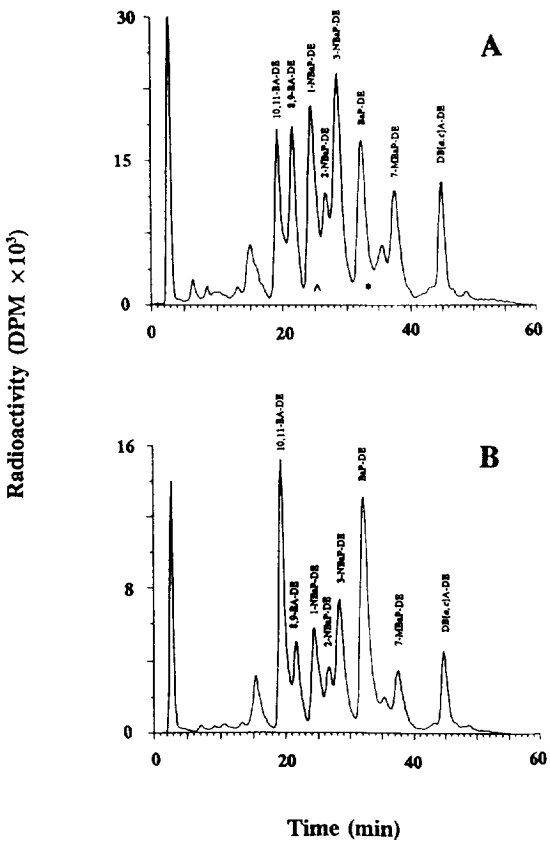

Fig. 3. (A) Separation of a mixture containing eight diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotides on a Vydac diphenyl-modified reversed-phase HPLC column. The retention times from the major adducts of 9,10-BaP-DE and *cis*-BaP-DE are marked (* and A, respectively). (B) The quantities of 10,11-BA-DE and BaP-DE adducts in (B) are increased compared to those in (A). For chromatographic conditions, see Section 2.4.

creased, the intensity of the corresponding chromatographic peaks increased (compared with those shown in Fig. 3A).

Pfau and Phillips [16] previously reported the use of a phenyl-modified Zorbax column for efficient separation of ten PAH *anti*-diol-epoxides, including the adducts of BaP-DE and DB[*a*,*c*]A that we also used. In our study of *anti*-diol-epoxide adducts, separation by Zorbax and another phenyl-modified HPLC column (Phenomenex IB-SIL) resulted in less satisfactory separation efficiency of 1-, 2- and 3-NBaP-DE, although the HPLC retention order is in general similar to that from the Vydac diphenyl-modified column (data not shown). The adducts of 1-NBaP-DE, 2-NBaP-DE, 3-NBaP-DE, and BaP-DE eluted within a very narrow range without having a baseline separation. Nevertheless, similar to the results reported by Pfau and Phillips [16], the adduct of DB[*a*,*c*]A eluted last (data not shown).

Separation of the adducts by the six ODS HPLC columns was less successful. The C_4 reversed-phase column provided the worst separation affording all the adducts eluted within a narrow range. The Cyclobond II column did not result in satisfactory separation either. Several of the diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotide adducts had a very short retention time, eluting closely with the unmodified deoxynucleotides (data not shown). Separation by the Cyclobond II(γ) column is in general governed by the molecule's ability to fit the cavity of the cyclodextrin, although polarity of the molecule can be considered as a secondary factor. Thus, these results indicate that part of the adduct molecules do not fit the cavity.

3.3. Relationships between structures of the adducts and HPLC retention order

We previously employed more than 100 PAHs, nitro-PAHs, and their derivatives for study of relationships between structures and HPLC retention order [31–33]. It is found that when a compound is eluted from an HPLC column, polarity and size of the molecule are important factors in determining the HPLC retention time. Polarity is largely related to the type, number, and location of the functional group(s) in the molecule. Thus, a functional group, such as a nitro, hydroxyl, or a methyl group, would also alter polarity and therefore affect HPLC retention times of nitro-PAHs. However, all diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotide adducts contain two highly polar phosphate groups attached to the deoxy ribose and three hydroxyl and one secondary amino groups linked to the saturated terminal benzo ring of the aromatic moiety. As a consequence, due to similar strong polarity, these adducts are not well

separated by the ODS reversed-phase HPLC columns. On the other hand, these adducts can be better separated by diphenyl- or phenyl-modified reversed-phase columns, which are recommended for separation of aromatic-ring-containing peptides. Therefore, it is evident that the determining factor in the separation of these adducts is the number of aromatic rings contained in the modified 3′,5′-bisphosphate deoxynucleotide adducts. This can well explain why 10,11-BA-DE and 8,9-BA-DE eluted first, followed by the NBaP-DEs, BaP-DE, and 7-MBaP-DE, and DB[*a*,*c*]A-DE last (Fig. 3).

We have found that, when a diphenyl-modified HPLC column is used, the elution order of the adducts, as shown in Fig. 3, is in accordance with those of the parent PAHs, nitro-PAHs, and their derivatives. Comparison of the retention times between the adducts of BaP-DE and 1-, 2-, and 3-NBaP-DEs indicates that the presence of a polar nitro functional group results in a shorter retention time (Fig. 3). This finding is similar to the results previously reported for the HPLC separation of the parent PAHs and nitro-PAHs [31]. On the other hand, the increase of retention time by adding an additional methyl group to the molecule (e.g. the adduct of 7-MBaP-DE vs. that of BaP-DE) is also consistent with our previous report [31]. Thus, the molecular size, the geometric shape of the molecule, and the substituted nitro and methyl groups should be factors in determining HPLC retention of the modified 3′,5′-bisphosphate deoxynucleotide.

3.4. Separation of diol-epoxide-modified 3′,5′-bisphosphate deoxynucleotide adducts derived from ambient air

Both PAHs and nitro-PAHs are contaminants present in ambient air, diesel soot extract, and other environmental sources. Since BaP, 1-NBaP, and 3-NBaP have been detected in diesel soot extract [7], it is highly possible that more bay-region-containing PAHs and nitro-PAHs are present in a variety of environmental samples. When humans are exposed to these compounds, these compounds may be enzymatically converted into the corresponding diol-epoxides. For hazard prevention, biomonitoring of their presence in the human is, therefore, timely and important. Our study indicates that the BaP-DE adduct can be separated from the other diol-epoxide adducts (Fig. 3). These results are in accordance with the findings by Pfau and Phillips [16] and by King et al. [7] that the BaP-DE is separable from the other adducts. Furthermore, our results also indicate that the diol-epoxide adducts derived from the mutagenic environmental 1-NBaP and 3-NBaP are also separable (Fig. 3). Thus, the results presented in this paper, together with those reported by others, also suggest that the ^{32}P-postlabeling-HPLC system is a potentially useful methodology for detection of environmental carcinogens that can be metabolized to diol-epoxides. Nevertheless, it is worthy to note that due to exposure to various environmental carcinogenic chemicals, a large number of chemical-modified DNA adducts are present in human tissues. It is possible that some of the diol-epoxide modified 3′,5′bisphosphate deoxynucleotide adducts may co-elute together, and may co-elute with the adducts derived from other chemicals. Therefore, it is essential to use more than one analytical technique for identification and quantitation of DNA adducts contained in human samples [34–36].

References

[1] H.V. Gelboin and P.O.P. Ts'o (Editors), Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology, and Environment, Vol. 1, Academic Press, New York, NY, 1978.
[2] US EPA, The Air Toxics Problem in the United States: An Analysis of Cancer Risks for Selected Pollutants, EPA-450/1-85-001, May 1985.
[3] B. Holberg and U. Ahlborg (Editors), Proceedings of Symposium: Mutagenicity and Carcinogenicity of Air Pollutions, Environ. Health Perspect., 47 (1983) 1.
[4] J. Lewtas, Environ. Health Perspect., 100 (1993) 211.
[5] P.C. Howard, S.S. Hecht and F.A. Beland (Editors), Nitroarenes: Occurrence, Metabolism and Biological Impact, Vol. 40, Plenum Press, New York, NY, 1990.
[6] J. Gallagher, U. Heinrich, M. George, L. Hendee, D.H. Phillips and J. Lewtas, Carcinogenesis, 17 (1994) 1291.

[7] L.C. King, M. George, J.E. Gallagher and J. Lewtas, Chem. Res. Toxicol., 7 (1994) 503.
[8] A. Dipple, R.C. Moschel and C.A.H. Bigger, in C.E. Searle (Editor), Chemical Carcinogens, ACS Monograph 182, American Chemical Society, Washington, DC, 1984, pp. 41–163.
[9] F.A. Beland and M.M. Marques, in K. Hemminki, A. Dipple, D.E.G. Shuker, F.F. Kadlubar, D. Segerback and H. Bartsch (Editors), DNA Adducts: Identification and Biological Significance, IARC Sci. Publ. No. 125, International Agency for Research on Cancer, Lyon, pp. 229–243, 1994.
[10] J.E. Gallagher, M.J. Kohan, M.H. George and J. Lewtas, Carcinogenesis, 12 (1991) 1685.
[11] P.P. Fu, Drug Metab. Rev., 22 (1990) 209.
[12] K. Randerath, M.V. Reddy and R.C. Gupta, Proc. Natl. Acad. Sci. USA, 78 (1981) 6126.
[13] M.V. Reddy and K. Randerath, Carcinogenesis, 7 (1986) 1543.
[14] A.C. Beach and R.C. Gupta, Carcinogenesis, 13 (1992) 1053.
[15] D.H. Phillips, M. Castegnaro and H. Bartsch (Editors), Postlabelling Methods for Detection of DNA Adducts, IARC Sci. Publ. No. 124, International Agency for Research on Cancer, Lyon, 1993.
[16] W. Pfau and D.H. Phillips, J. Chromatogr., 570 (1991) 65.
[17] M.W. Chou, R.H. Heflich and P.P. Fu, Carcinogenesis, 6 (1985) 1235.
[18] M.W. Chou, R.H. Heflich and P.P. Fu, Carcinogenesis, 7 (1986) 1837.
[19] P.P. Fu, Y.-S. Wu, L.S. Von Tungeln, J.-S. Lai, P.M. Chiarelli and F.E. Evans, Chem. Res. Toxicol., 6 (1993) 606.
[20] P.P. Fu, D. Herreno-Saenz, L.S. Von Tungeln, Y.-S. Wu, J.-S. Lai and F.E. Evans, Environ. Health Perspect., 102 (1994) 177.
[21] P.P. Fu and R.G. Harvey, Tetrahedron Lett., (1977) 2059.
[22] Y.-S. Wu, J.-S. Lai and P.P. Fu, J. Org. Chem., 58 (1993) 7283.
[23] Unpublished data.
[24] P.P. Fu, F.A. Beland and S.K. Yang, in A.J. Dennis and W.M. Cooke (Editors), Polynuclear Aromatic Hydrocarbons: Fifth International Symposium on Chemical Analysis and Biological Fate, Battelle Press, Columbus, OH, pp. 655–666, 1981.
[25] P.P. Fu, C.C. Lai and S.K. Yang, J. Org. Chem., 46 (1981) 220.
[26] T. Meehan and K. Straub, Nature, 277 (1979) 410.
[27] R.C. Gupta, Cancer Res., 45 (1985) 5656.
[28] A. Hewer, C.S. Cooper, O. Ribeiro, K. Pal, P.L. Grover and P. Sims, Carcinogenesis, 2 (1981) 1345.
[29] M. Mushtag, H.B. Weems and S.K. Yang, Arch. Biochem. Biophys., 246 (1986) 478.
[30] P.-L. Chiu, H.B. Weems, T.K. Wong, P.P. Fu and S.K. Yang, Chem. Biol. Interact., 44 (1983) 155–168.
[31] P.P. Fu, Y. Zhang, Y.-L. Mao, L.S. Von Tungeln, Y. Kim, H. Jung and M.-J. Jun, J. Chromatogr., 642 (1993) 107.
[32] J.-S. Lai, S.S. Hung, L.E. Unruh, H. Jung and P.P. Fu, J. Chromatogr., 461 (1989) 327.
[33] L.S. Von Tungeln and P.P. Fu, J. Chromatogr., 461 (1989) 315.
[34] D.K. Manchester, A. Weston, J.-S. Choi, G.E. Trivers, P.V. Fennessey, E. Quintana, P.B. Farmer, D.L. Mann and C.C. Harris, Proc. Natl. Acad. Sci. USA, 85 (1988) 9243.
[35] A. Weston, M.L. Rowe, D.K. Manchester, P.B. Farmer, D.L. Mann and C.C. Harris, Carcinogenesis, 10 (1989) 251.
[36] K. Alexandrow, M. Rojas, O. Geneste, M. Castegnaro, A.-M. Camus, S. Petruzzelli, C. Giuntini and H. Bartsch, Cancer Res., 52 (1992) 6248.