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^{32}P-Postlabeling high-performance liquid chromatographic improvements to characterize DNA adduct stereoisomers from benzo[*a*]pyrene and benzo[*c*]phenanthrene, and to separate DNA adducts from 7,12-dimethylbenz[*a*]anthracene

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Abstract

Single compounds can generate complex DNA adduct patterns by reactions through different pathways, with different target nucleotides and through different configurations of the products. DNA adduct analysis by ^{32}P-HPLC was improved by adding an isocratic plateau in an otherwise linear gradient, thereby enhancing resolution of predictable retention time intervals. This enhanced ^{32}P-HPLC technique was used to analyze and at least partly resolve 14 out of 16 available benzo[*c*]phenanthrene deoxyadenosine and deoxyguanosine adduct standards, 8 out of 8 available benzo[*a*]pyrene deoxyadenosine and deoxyguanosine adduct standards, and 51 peaks from 7,12-dimethylbenz[a]anthracene–calf thymus DNA reaction products. The same type of gradient modifications could be used to enhance resolution in analyses of other complex DNA adduct mixtures, e.g., in vivo in humans.

Keywords: DNA; Benzo[*a*]pyrene; Benzo[*c*]phenanthrene; 7,12-Dimethylbenz[*a*]anthracene

1. Introduction

Increased DNA adduct levels have been found in human populations with increased exposure to suspected carcinogens and with known or suspected risks of developing cancer [1]. Many suspected carcinogens have also been shown to form DNA adducts in vitro or in vivo [2,3]. Several substances have been shown to form a variety of DNA adducts, both by different paths of activation [4] and reaction with various nucleotides or via different stereoisomers [5]. This can result in very complex DNA adduct patterns even from single substances [6]. It is known that different stereoisomers of the same compound exhibit varying adduct-forming, mutagenic and carcinogenic properties [7]. It is therefore of value to be able to distinguish between DNA adducts of different conformations. This can often be troublesome, since many stereoisomers exhibits similar chemical and chromatographic properties [8].

Several methods for DNA adduct analysis combining the sensitivity of ^{32}P-postlabeling and the resolution of high-performance liquid chromatography have been developed [2,9–17]. Recently, a ^{32}P-postlabeling high-performance liquid chromatography (^{32}P-HPLC) method was developed and applied to

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various DNA adducts. These included DNA adducts formed in vivo in rats after administration of 2-nitrofluorene and its metabolites [18,19]. The ^{32}P-HPLC method was capable of resolving and analyzing a wide variety of ^{32}P-postlabeled compounds, from polar substances like orthophosphates, normal nucleotides and methylated nucleotides [20], to bulky and non-polar DNA adducts of polycyclic aromatic hydrocarbons (PAHs) like chrysene and benzo[*a*]pyrene (BaP), formed in vitro [20]. A wide variety of 365 DNA and nucleotide adducts formed in vitro from 35 different substances were analyzed [6]. Seasonal variations of 16 different DNA adducts were also recently analyzed in human lymphocytes and granulocytes [21]. The sensitivity of the ^{32}P-HPLC method was comparable to that of the commonly used ^{32}P-TLC [18], while the chromatographic resolution in most cases was superior.

The aim of this study was to develop the ^{32}P-HPLC system further, allowing increased resolution of selected retention time intervals, and application of this development to the analyses of complex mixtures of similar DNA adducts. In vitro reaction products from calf thymus DNA and 7,12-dimethylbenz[*a*]anthracene (DMBA) were used as a model for developing the system which was then applied to DNA adduct stereoisomer standards from benzo[*a*]pyrene (BaP) and benzo[*c*]phenanthrene (BcPh).

2. Experimental

2.1. Reagents and chemicals

All solvents and salts were of analytical grade and purchased from the following sources; T4 polynucleotide kinase (PNK) (U.S. Biochemical, Cleveland, OH, USA) and adenosine 5′-[gamma-^{32}P]triphosphate ([^{32}P]ATP) with an original specific activity of approximately 3000 Ci/mmol (Amersham, Little Chalfont, UK). 1,2,3,4-Tetrahydro-3,4-dihydroxy-1,2-epoxybenzo[*c*]phenanthrene (BcPhDE) and 7,8,9,10 -tetrahydro-7,8-dihydroxy-9,10-epoxybenzo-[*a*]pyrene (BaPDE)-nucleoside 3′-phosphate standards, prepared as described previously [22–24], were kindly provided by Dr. Anthony Dipple (ABL – Basic Research Program, NCI – Frederick Cancer Research and Development Center, Frederick, MD, USA). Structures and the nomenclature used in this study for these standards are shown in Fig. 1.

2.2. Instrumentation

The HPLC system consisted of a 600E multisolvent delivery system with a 484 tuneable absorbance detector (both Waters Chromatography Division, Millipore, Milford, MA, USA). Radioactivity was measured on-line with a Flo-One\Beta A270 radioactivity detector using a 0.5-ml cell and scintillation fluid Flo-Scint IV (both Radiomatic Instruments and Chemical, Tampa, FL, USA). The energy window was set to 8–600 keV with a counting efficiency of 60% for ^{32}P. Counting was performed in 12-s cycles. The analytical system had a pre-column NewGuard holder with an RP-18, 7 μm cartridge (both Brownlee Laboratories, Santa Clara, CA, USA) and as main column a Deltapak 5 μm, C18-100 A, 150×3.9 mm I.D. (Waters Chromatography Division) was used.

2.3. In vitro DNA adduct synthesis

DMBA-DNA adducts were prepared by incubating calf thymus DNA and DMBA with S9 metabolic activation, as previously described [6]. The DNA was precipitated with sodium chloride and ethanol, washed with ethanol and the purity controlled by UV. This procedure has previously been described in detail [6].

2.4. ^{32}P-Postlabeling and chromatography

DNA adduct standards were diluted 1:100 with water and 1-μl aliquots were mixed with PNK buffer (200 m*M* bicine, 100 m*M* DTT, 10 m*M* spermidine, 100 m*M* magnesium chloride (pH 9.6)) (0.1 μl/μl sample), PNK (0.4 μl/μl sample of 10 U/μl), [^{32}P]ATP (0.7 μl/μl sample of 10 μCi/μl) to a total volume of 2.2 μl/μl sample. The reaction mixture was incubated for 30 min at 37°C. The excess of [^{32}P]ATP was converted to [^{32}P]orthophosphate by incubation with apyrase (1.0 μl/μl sample of 30 mU/μl) for 15 min at 37°C.

HPLC analyses of ^{32}P-postlabeled DNA adducts were performed by injection of the total ^{32}P-post-

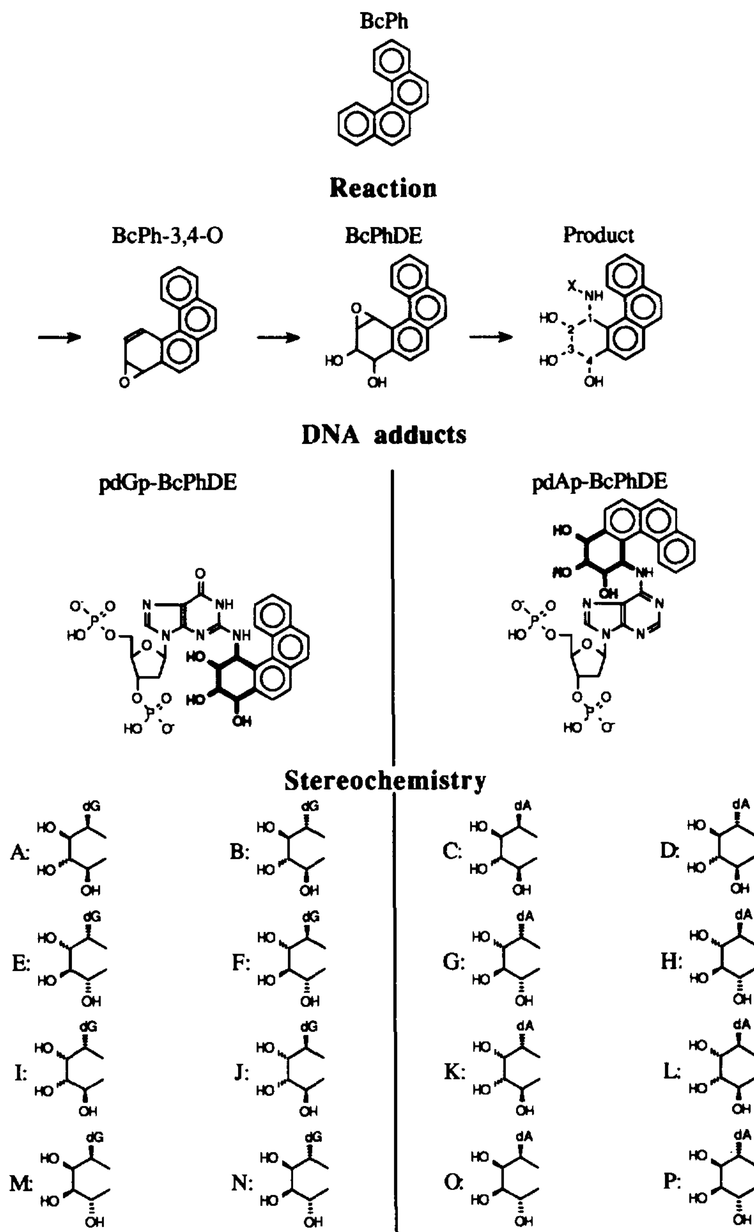

Fig. 1. (a) Structures, reactions and stereochemistry of analyzed BcPh-DNA adduct standard. The stereoisomers were (A) 1*S*,2*R*,3*S*,4*R*-, (B) 1*R*,2*R*,3*S*,4*R*-, (E) 1*R*,2*S*,3*R*,4*S*-, (F) 1*S*,2*S*,3*R*,4*S*-, (I) 1*R*,2*S*,3*S*,4*R*-, (J) 1*S*,2*S*,3*S*,4*R*-, (M) 1*S*,2*R*,3*R*,4*S*- and (N) 1*R*,2*R*,3*R*,4*S*-N^2-[1-(1,2,3,4-tetrahydro-2,3,4-trihydroxybenzo[*c*]phenanthrenyl)]-2′-deoxyguanosine 3′,5′-bisphosphate and (C) 1*S*,2*R*,3*S*,4*R*-, (D) 1*R*,2*R*,3*S*,4*R*-, (G) 1*R*,2*S*,3*R*,4*S*-, (H) 1*S*,2*S*,3*R*,4*S*-, (K) 1*R*,2*S*,3*S*,4*R*-, (L) 1*S*,2*S*,3*S*,4*R*-, (O): 1*S*,2*R*,3*R*,4*S*- and (P) 1*R*,2*R*,3*R*,4*S*-N^6-[1-(1,2,3,4-tetrahydro-2,3,4-trihydroxybenzo[*c*]phenanthrenyl)]-2′-deoxyadenosine 3′,5′-bisphosphate. (b) Structures, reactions and stereochemistry of analyzed BaP-DNA adduct standard. The stereoisomers were (1) 10*R*,7*R*,8*S*,9*S*,10*R*-, (2) 10*S*,7*R*,8*S*,9*S*,10*S*-, (5) 10*S*,7*S*,8*R*,9*R*,10*S*- and (6) 10*R*,7*S*,8*R*,9*R*,10*R*-N^2-[10*R*-(7,8,9,10-tetrahydro-7*S*,8*R*,9*R*-trihydroxybenzo[*a*]pyrenyl)]-2′-deoxyguanosine 3′,5′-bisphosphate and (3) 10*R*,7*R*,8*S*,9*S*,10*R*-, (4) 10*S*,7*R*,8*S*,9*S*,10*S*-, (7) 10*S*,7*S*,8*R*,9*R*,10*S*- and (8) 10*R*,7*S*,8*R*,9*R*,10*R*-N^6-[10*R*-(7,8,9,10-tetrahydro-7*S*,8*R*,9*R*-trihydroxybenzo[*a*]pyrenyl)]-2′-deoxyadenosine 3′,5′-bisphosphate.

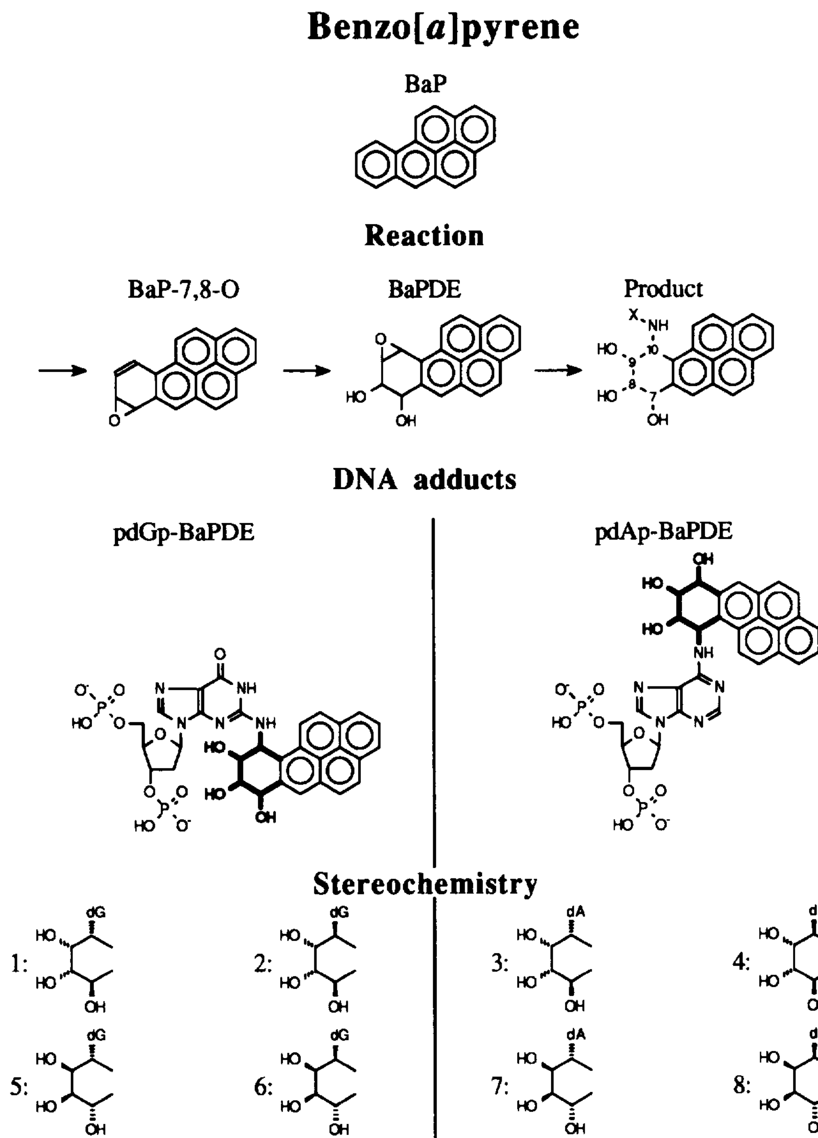

Fig. 1. (*continued*)

labeling mixture into the HPLC, using a gradient of acetonitrile in ammonium formate buffer. The ammonium formate buffer was prepared by dissolving ammonium formate salt in water to 2 *M* and adjusting pH with formic acid to the indicated value. The analyses were performed at room temperature, 21–22°C. Two gradient systems were used. The gradient names are the ones commonly used at our laboratory and in previous papers on the ^{32}P-HPLC method. Gradient III: 0.5 ml/min of 2 *M* ammonium formate, 0.4 *M* formic acid (pH 4.5) with a linear gradient of 0 to 35% acetonitrile (0 to 70 min) for phosphorylated nucleotides and adducts. Gradient IV.*N*: 0.5 ml/min of 2 *M* ammonium formate, 0.4 *M* formic acid (pH 4.5) with a linear gradient of 0 to N% acetonitrile (0 to 2×*N* min), an isocratic plateau of *N*% acetonitrile (2×*N* to 30+2×*N* min), and a continued linear gradient of *N* to 35% acetonitrile

($30+2\times N$ to 100 min) for high resolution of various phosphorylated compounds. As an example, the gradient used for high resolution of BcPh with $N=14$ has a linear gradient of 0 to 14% acetonitrile (0 to 28 min), an isocratic plateau of 14% acetonitrile (28 to 58 min), and a continued linear gradient of 14 to 35% acetonitrile (58 to 100 min).

3. Results

3.1. General

The ^{32}P-HPLC system showed very good stability and obtained reproducible results. Retention times between analyses did not vary more than 0.4 min despite some analyses of the same sample were performed with up to three months interval. The same column, which had been in continuous use for more than two years, was used for all analyses in this study.

3.2. DNA–DMBA reaction products

The reaction between calf thymus DNA and DMBA resulted in several products that could be detected after ^{32}P-postlabeling (Fig. 2a: gradient III). These products covered almost the entire retention time interval from 0 to 75 min when analyzed with gradient III. When a 30 min long, isocratic plateau was used in the otherwise linear gradient it affected the elution of the ^{32}P-postlabeled compounds with retention times above 16 min (Fig. 2a, chromatograms IV.0–IV.30). Compounds with short retention times, i.e., polar compounds, were affected when the isocratic plateau was set at a low level of acetonitrile (Fig. 2a: lower part), while compounds with long retention times, i.e., lipophilic compounds, were also affected by plateaus at higher levels of acetonitrile (Fig. 2a: upper part).

In total, 108 different peaks, each with a size corresponding to more than 2 adducts/10^8 nucleotides (200 amol) or detectable over a range of gradients, were detected with various gradients. Of these, 51 peaks were detected within the retention time interval where PAH–DNA adducts are normally seen (Fig. 2a: gradient III, 45–75 min). Most of the remaining peaks were detected within the retention time interval where compounds from DNA, not reacted with adduct forming substances, are normally seen (Fig. 2a: gradient III and Control, 3–45 min).

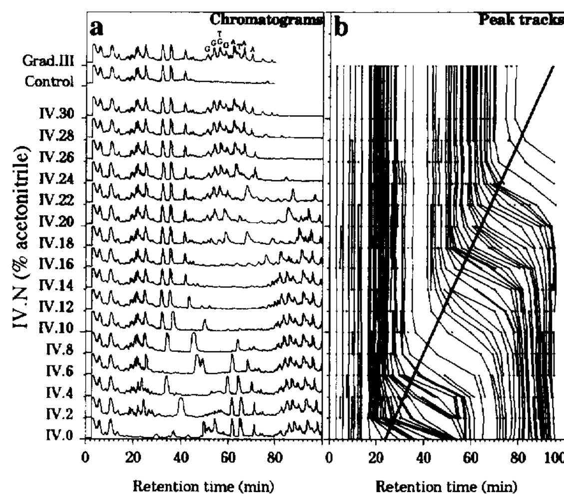

Fig. 2. ^{32}P-HPLC chromatograms of DMBA–DNA adducts at different acetonitrile levels in the isocratic plateau (a) and a control DNA sample (a, Control). High peaks have been partly suppressed to allow viewing of lower peaks. Some previously characterized DMBA adduct peaks have been labeled with their target nucleotides: A=2′-deoxyadenosine, G=2′-deoxyguanosine, T=thymidine (a, Gradient III). The gradients used were 0.5 ml/min of 2 *M* ammonium formate, 0.4 *M* formic acid (pH 4.5) with linear gradients (IV.*N*) of 0 to *N*% acetonitrile (0 to $2\times N$ min), an isocratic plateau of *N*% acetonitrile ($2\times N$ to $30+2\times N$ min) and a continued linear gradient of *N* to 35% acetonitrile ($30+2\times N$ to 100 min) (Gradient III and Control). The retention times of single peaks as a function of acetonitrile level in the isocratic plateau is more clearly demonstrated right (b). The diagonal line is the maximum resolution retention time, t(min) as a function of plateau acetonitrile concentration, N(%), $t=24+2\times N \equiv N=(t-24)/2$.

3.3. Influence of gradient plateau

An increased separation of different parts of the chromatogram could be achieved, owing to the expansion of certain retention time intervals and depending on which acetonitrile level the isocratic plateau was set to. The maximum increase in resolution for a compound with gradient IV was achieved if the gradient plateau began 24 min earlier than the compound's retention time in gradient III. Resolution was enhanced for peaks within a 20 min

retention time interval surrounding the point of maximum enhancement (Fig. 2b).

An optimal resolution of BcPh-DNA adducts was achieved with an isocratic plateau at 14% acetonitrile in the gradient (gradient IV.14). This resulted in notable retention time differences between all of the BcPh-deoxyadenosine and BcPh-deoxyguanosine adducts, when run separately (Fig. 3 Table 1). In some cases the retention time differences were small, <1.5 min (Table 1: Adducts A/P, B/F, B/N, J/M and L/O), indicating that separation from a mixture could be difficult.

3.4. BcPh-nucleotide adducts

A mixture of the 16 BcPh adducts resulted in the resolution of 7 peaks and 2 shoulders in a ^{32}P-HPLC chromatogram analyzed on the standard gradient III (Fig. 4a). The same mixture resolved into 12 peaks and 2 shoulders in a chromatogram run on the optimized gradient IV.14 (Fig. 4b). The peaks were characterized by retention time comparisons and co-chromatography with single BcPh adduct standards (Table 1). BcPh adducts B, N, A and P (Fig. 1a) could not be resolved with this gradient. Adducts M and O formed shoulders on the peaks of adducts J and L, respectively. A few small, additional peaks co-chromatographed with impurities in the BcPh adduct standard The peak widths were widened by a

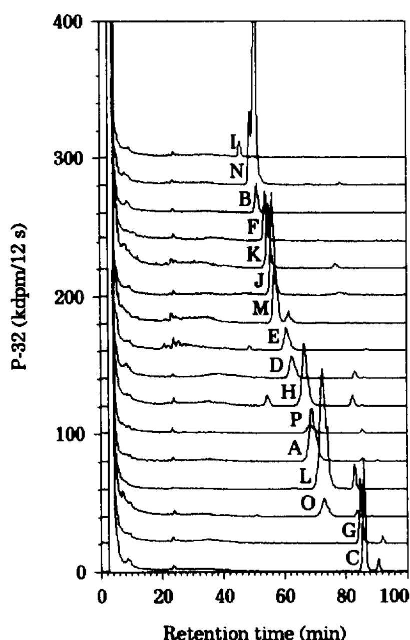

Fig. 3. ^{32}P-HPLC chromatograms from single (A–P) BcPh–DNA adduct standards using an optimized (gradient IV.14) HPLC gradient. Letters A–P correspond to the standards S.A–S.P (Fig. 1a). The label is placed to the left of the corresponding standard peak. The unit kdp12s means thousand ^{32}P-decays per 12 s.

Table 1
Retention time comparisons of stereoisomer DNA adduct standards, derived from ^{32}P-HPLC analyses of two mixtures of BcPh-DNA adduct standards (S.A–S.P) and BaP-DNA adduct standards (S.1–S.8), and co-chromatography and retention time comparisons with single standards in two gradient systems

BcPh-DNA adduct	Gradient III retention time (min)	Gradient IV.14 retention time (min)
A	53.2	67.0
B	49.0	50.4
C	58.8	85.6
D	52.4	61.4
E	51.6	59.8
F	49.0	51.2
G	58.0	84.2
H	53.2	64.4
I	45.2	45.0
J	50.6	55.6
K	50.6	54.0
L	54.2	70.2
M	51.6	56.8
N	48.4	49.6
O	54.2	71.6
P	53.2	66.4
BaP-DNA adduct	**Gradient III retention time (min)**	**Gradient IV.18 retention time (min)**
1	57.6	60.8
2	57.6	58.8
3	62.0	75.0
4	62.0	73.0
5	57.6	63.2
6	55.6	55.6
7	62.0	78.2
8	62.0	71.2

For explanation of abbreviations, see Fig. 1.

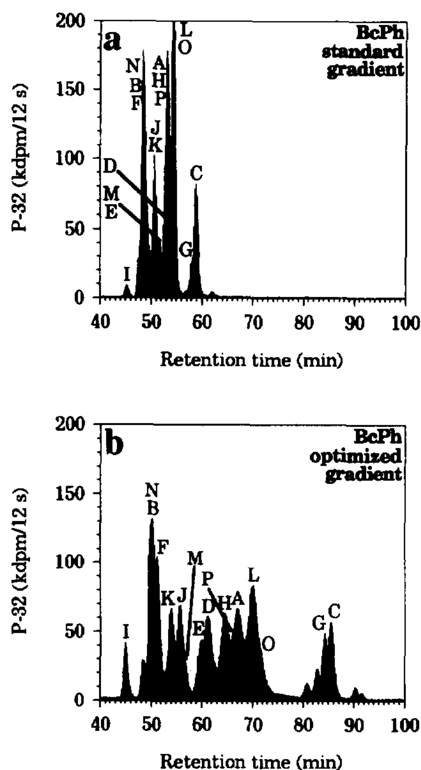

Fig. 4. ^{32}P-HPLC chromatograms from a mixture of BcPh–DNA adduct standards before (a, gradient III) and after (b, gradient IV.14) optimization of the HPLC gradient. Letters A–P correspond to the adduct standards A–P (Fig. 1a). The unit kdp12s means thousand ^{32}P-decays per 12 s.

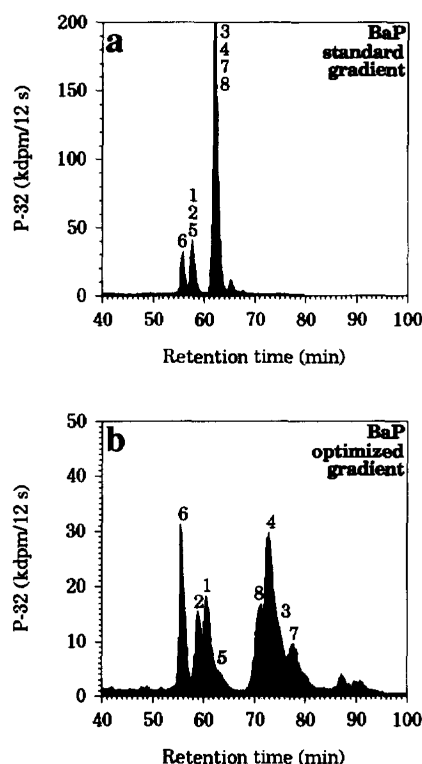

Fig. 5. ^{32}P-HPLC chromatograms from a mixture of benzo[*a*]pyrene-DNA adduct standards before (a, gradient III) and after (b, gradient IV.18) optimization of the HPLC gradient. Digits 1–8 correspond to the adduct standards 1–8 (Fig. 1b). The unit kdp12s means thousand ^{32}P-decays per 12 s.

factor of 1.5–2.5 while the retention time interval was expanded by an average factor of 3.0 (1.2–5.0).

3.5. *BaP–nucleotide adducts*

A mixture of 8 BaP adducts resulted in the resolution of 3 peaks in a ^{32}P-HPLC chromatogram run on the standard gradient III (Fig. 5a). The same mixture resolved into 6 peaks and 2 shoulders in a chromatogram run on the optimized gradient IV.18 (Fig. 5b). The peaks were characterized by retention time comparisons and co-chromatography with single BaP adduct standards (Table 1). Adducts 5 and 3 formed shoulders on the peaks of adducts 1 and 4, respectively. A few small, additional peaks co-chromatographed with impurities in the BaP adduct standards. The peak widths were widened by a factor of 2–3 while the retention time interval was expanded by an average factor of 3.5 (1.6–5.3).

4. Discussion

The ^{32}P-HPLC method has a high resolving power [20] and has been used to analyze more than 350 DNA adducts from various substances [6]. However, many of these DNA adducts have such similar chromatographic properties that the ^{32}P-HPLC would be unable to resolve most of them in a complex mixture.

The applied modification, with an isocratic plateau in an otherwise linear gradient, allowed for expansion of selected retention time, i.e., mainly polarity, intervals of special interest. A 10-min interval could be expanded to 30 to 40 min, improving the chromatographic resolution. However, peaks within the interval tended to widen in this process, so the actual improvement in resolution did not follow the expansion of the interval exactly. The span of acetonitrile levels, that would cause a substantial increase in resolution around a particular retention time, was usually a few percent (Fig. 2). The general rule was that the isocratic plateau should start approximately 24 min before the point of desired maximum resolution. However, this might sometimes need adjustments to reach the optimal resolution for the entire retention time interval of interest. The analyzed BcPh–DNA adducts were centered around 52 min retention time (Table 1, upper part) and required an isocratic plateau level of (52−24)/2=14% acetonitrile. The analyzed BaP-DNA adducts were centered around 60 min retention time (Table 1, lower part) and required an isocratic plateau level of (60−24)/2=18% acetonitrile.

DMBA is a potent DNA adduct forming agent. It forms a wide variety of DNA adducts in vitro [6,8,25]. Fifteen of these have been separated using ^{32}P-HPLC [6]. Several of the DMBA–DNA adducts are stereoisomers of the DMBA bay-region dihydrodiol epoxide reaction products with the exocyclic amines of 2′-deoxyadenosine 3′-monophosphate (dAp) and 2′-deoxyadenosine 3′-monophosphate (dGp) [8,25,26]. Therefore DMBA was considered a suitable model substance for developing an improved resolution of the ^{32}P-HPLC method, using an isocratic plateau in the otherwise linear gradient. This resulted in a total of 108 peaks visible at various gradients. 51 peaks, not present in control DNA (Fig. 2a: Control), were detected within the retention time interval where PAH–DNA adducts are normally found (Fig. 2a, 45–75 min in Gradient III) [6]. Some of these peaks were only seen with isocratic plateaus at certain acetonitrile levels (Fig. 2b). With isocratic plateaus at other acetonitrile levels the peaks became inseparable from adjacent peaks. All 51 peaks were not necessarily unique DMBA–nucleotide adducts. Some of the peaks might correspond to di- or oligo-nucleotide adducts, resulting from incomplete digestion of DNA. Others may be decay products of the expected nucleotide adduct 3′,5′-bisphosphates, e.g., nucleotide adduct 5′-monophosphate. Also, commercial calf thymus DNA may contain minor amounts of RNA, resulting in some minor DMBA–RNA reaction products. However, since the aim with using DMBA was developing and testing the improved resolution of ^{32}P-HPLC, the multitude of postlabeled reaction products was more important than the actual characteristics of them.

BcPh can form 16 different adducts with dAp and dGp from its bay-region dihydrodiol epoxide (Fig. 1a) [24]. The ^{32}P-HPLC system was able to resolve 14 out of 16 of these adducts into either peaks or distinct shoulders in the same analysis (Fig. 4), by using a gradient designed to give optimal resolution in the retention time interval of the adducts. This, together with the stability and reproducibility of the ^{32}P-HPLC system, allows for characterization of these DNA adducts by co-chromatography and retention time comparisons (Fig. 4b).

The BcPh–DNA adducts A, B, C and D result from the reaction of (−)-*syn* BcPhDE with dAp and dGp. These four adducts have been fully separated from each other with a modified, two-dimensional ^{32}P-TLC method [24], as they were with ^{32}P-HPLC in this study (Fig. 4b: peaks A, B, C, D). The BcPh–DNA adducts E, F, G and H result from the reaction of (+)-*syn* BcPhDE with dAp and dGp. The adducts E and G could not be fully resolved even with the modified ^{32}P-TLC method [24], while all of them were fully separated with ^{32}P-HPLC here (Fig. 4b: peaks E, F, G, H). The BcPh–DNA adducts I, J, K and L results from the reaction of (−)-*anti* BcPhDE with dAp and dGp. All four have been separated with the ^{32}P-TLC method [24], and with ^{32}P-HPLC (Fig. 4b: peaks I, J, K, L). The BcPh–DNA adducts M, N, O and P result from the reaction of (+)-*anti* BcPhDE with dAp and dGp. The adducts M and N co-migrated with the modified ^{32}P-TLC method [24], but all four were separated with ^{32}P-HPLC (Fig. 4b: peaks M, N, O, P).

BaP can form 8 different adducts with dAp and dGp from its bay-region dihydrodiol epoxide *anti* forms (Fig. 1b) [22]. The ^{32}P-HPLC system with an optimized gradient was able to resolve all 8 of these adducts into either peaks or distinct shoulders in the

same analysis (Fig. 5b). This allows for characterization of these adducts as well.

The BaP–DNA adducts 1, 2, 3 and 4 result from the reaction of (+)-*anti* BaPDE with dAp and dGp. These four adducts have been fully separated with a modified, two-dimensional ^{32}P-TLC method [22], as they were with ^{32}P-HPLC in this study (Fig. 5b: peaks 1, 2, 3, 4). The BaP–DNA adducts 5, 6, 7 and 8 result from the reaction of (−)-*anti* BaPDE with dAp and dGp. They have also been fully resolved with both the ^{32}P-TLC method [22] and ^{32}P-HPLC here (Fig. 5b: peaks 5,6,7,8). It is however doubtful if all eight BaP–DNA adducts could be resolved in the same ^{32}P-TLC analysis.

In cases where base line resolution between adducts are not achieved, visual resolution depends partly on ratios between the different postlabeled adduct. Two adducts neighbouring each other in the ^{32}P-HPLC chromatogram might be seen as separate peaks if they are similar in quantity but as a peak with an invisible shoulder if one is much more abundant than the other. The former is the case with adducts B/F, C/G (Fig. 4b) and 4/8 (Fig. 5b), while the latter could become the case with adducts D/E, J/M, L/O (Fig. 4b), 1/5 and 3/4 (Fig. 5b).

Although the peak width of BcPh–DNA and BaP–DNA adducts increased in the expanded retention time interval, this increase was relatively small compared to the average expansion of the retention time interval. The expansion also varied over the DNA adduct retention time interval with a maximum relative expansion close to the middle and a small relative expansion close to the outer peaks of the interval. This means that some additional peaks could be resolved by varying the exact acetonitrile content in the isocratic plateau slightly.

With the approach used in this study, applying small variations to the basically same analytical system to enhance resolution selectively, it is possible to use one system with a total resolving power that would normally require several different analytical systems. This approach is also greatly simplified by being able to use one parameter, the ^{32}P-HPLC retention time in a standard gradient, to decide what variation is required. The above approach has been used to enhance resolution of stereoisomers in this study (Figs. 4 and 5), but as the example with DMBA showed (Fig. 2), it should be generally applicable to most lipophilic DNA adducts. This method with easily calculated gradient plateaus should also be applicable to enhance resolution in any similar HPLC system. This should greatly facilitate characterization of unknown DNA adducts, especially in analyses of DNA adducts from complex mixtures, e.g., in humans exposed to environmental genotoxins.

5. Conclusions

The resolving power of ^{32}P-HPLC in analyses of DNA adducts can be enhanced by gradient modifications. The required modifications can be determined by the retention time of unresolved DNA adducts in a standard gradient system. The enhanced resolving power can be used for analyses of DNA adducts with very similar chemical and chromatographic properties, e.g., stereoisomers. The enhanced ^{32}P-HPLC method should also be applicable to analyses of DNA adducts formed from complex mixtures and facilitate characterization of single adducts in these.

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References

[1] K. Hemminki, E. Grzybowska, M. Chorazy, K. Twardowska-Saucha, J.W. Sroczynski, K.L. Putman, K. Randerath, D.H. Phillips, A. Hewer, R.M. Santella and F.P. Perera, in H. Vainio, M. Sorsa and A.J. McMichael (Editors), Complex Mixtures and Cancer Risk, IARC Scientific Publications 104, Lyon, 1990, pp. 181–192.
[2] W. Pfau and D.H. Phillips, J. Chromatogr. B, 570 (1991) 65.
[3] A.C. Beach and R.C. Gupta, Carcinogenesis, 13 (1992) 1053.
[4] F.A. Beland and F.F. Kadlubar, Environ. Health Perspect., 62 (1985) 19.

[5] A. Dipple, M.A. Pigott, S.K. Agarwal, H. Yagi, J.M. Sayer and D.M. Jerina, Nature, 327 (1987) 535.
[6] M. Zeisig and L. Möller, Carcinogenesis, 16 (1995) 1.
[7] S.C. Cheng, B.D. Hilton, J.M. Roman and A. Dipple, Chem. Res. Toxicol., 2 (1989) 334.
[8] H. Schmeiser, A. Dipple, M.E. Schurdak, E. Randerath and K. Randerath, Carcinogenesis, 9 (1988) 633.
[9] G.N. Levy and W.W. Weber, Anal. Biochem., 174 (1988) 381.
[10] E.H. Weyand, J.E. Rice and E. LaVoie, Cancer Lett., 37 (1987) 257.
[11] N.J. Gorelick and G.N. Wogan, Carcinogenesis, 10 (1989) 1567.
[12] W.P. Watson and A.E. Crane, Mutagenesis, 4 (1989) 75.
[13] S. Amin, B. Misra, D. Desai, K. Huie and S.S. Hecht, Carcinogenesis, 10 (1989) 1971.
[14] R.A. Smith, D.S. Williamson and S.M. Cohen, Chem. Res. Toxicol., 2 (1989) 267.
[15] G. Talaska, K.L. Dooley and F.F. Kadlubar, Carcinogenesis, 11 (1990) 639.
[16] W. Pfau, H.H. Schmeiser and M. Wiessler, Carcinogenesis, 11 (1990) 1627.
[17] J.F. Mouret, F. Odin, M. Polverelli and J. Cadet, Chem. Res. Toxicol., 3 (1990) 102.
[18] L. Möller and M. Zeisig, Carcinogenesis, 14 (1993) 53.
[19] L. Möller, M. Zeisig, T. Midtvedt and J.-Å. Gustafsson, Carcinogenesis, 15 (1994) 857.
[20] L. Möller, M. Zeisig and P. Vodicka, Carcinogenesis, 14 (1993) 1343.
[21] L. Möller, E. Grzybowska, M. Zeisig, B. Cimander, K. Hemminki and M. Chorazy, Carcinogenesis, 17 (1995) 61.
[22] K. Canella, K. Peltonen and A. Dipple, Carcinogenesis, 12 (1991) 1109.
[23] H. Yagi, H, D.R. Thakker, Y. Ittah, M. Croist-Delcey and D.M. Jerina, Tetrahedron Lett., 24 (1983) 1349.
[24] K.A. Canella, K. Peltonen, H. Yagi, D.M. Jerina and A. Dipple, Chem. Res. Toxicol., 5 (1992) 685.
[25] J.A. Vericat, S.C. Cheng, and A. Dipple, Carcinogenesis, 10 (1989) 567.
[26] C.A. Bigger, J.E. Tomaszewski, A.W. Andrews and A. Dipple, Cancer Res., 40 (1980) 655.