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^{32}P-postlabelling of N^6-adenine adducts of epoxybutanediol in vivo after 1,3-butadiene exposure

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Abstract

Epoxybutanediol is one of the epoxide metabolites of butadiene (BD). A pair of diastereomeric *N*-1-adenine adducts were formed by reacting epoxybutanediol with deoxyadenosine 5′-monophosphate (5′-dAMP). These two *N*-1-adenine adducts rearranged in a base-catalysed reaction to an N^6-trihydroxybutyl-adenine adduct, which was characterized by UV and mass spectroscopy. Using the ^{32}P-postlabelling/HPLC assay the same adducts were detected in diepoxybutane (DEB)-treated DNA in vitro and in liver DNA samples from rats exposed to BD by inhalation. Adenine adducts of epoxybutanediol are probably suitable for monitoring BD exposure.

Keywords: Epoxybutanediol; ^{32}P-postlabelling; DNA adducts; HPLC

1. Introduction

1,3-Butadiene (BD) is mainly used in the manufacture of polymers and copolymers. Rats and mice exposed to BD develop tumours at multiple sites (Melnick et al., 1987; Owen et al., 1987). BD is metabolized by cytochrome P450 to 1,2-epoxy-3-butene (EB) (Malvoisin et al., 1979), which may also be further oxidized to diepoxybutane (DEB). Epoxybutanediol may be formed via hydrolysis of EB followed by oxidation, or via hydrolysis of DEB (Malvoisin and Roberfroid, 1982). The epoxide metabolites of BD are DNA alkylating agents. In vitro studies have shown the formation of several $N7$-guanine and N^6-adenine adducts from EB and DEB. The observation of a high frequency of A → G transitions in mice exposed to BD (Recio et al., 1993) suggests that adenine adducts might play an important role in the toxicity of BD.

In this paper *N*-1- and N^6-adenine adducts of epoxybutanediol were identified with the aid of rearrangement experiments, UV and mass spectroscopy. Using the synthesized standard adducts as UV markers and a ^{32}P-postlabelling/HPLC assay, these adducts were measured in DEB-ex-

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posed DNA in vitro and, for the first time, in liver DNA of rats exposed to BD by inhalation.

2. Materials and methods

Epoxybutanediol was prepared by hydrolysis of DEB in water. The remaining DEB was extracted with toluene. *N*-1-adenine adducts were formed by incubation of 5′-dAMP with the hydrolysate. Part of the mixture was treated with base at 37°C overnight to convert the *N*-1-adenine adducts into the corresponding N^6-adenine adducts by Dimroth rearrangement. Salmon testis DNA was reacted with DEB at 37°C for 24 h.

Rats were exposed to 300 ppm BD for 5 days, 6 h/day. DNA adducts were analyzed by the postlabelling procedure described by Randerath et al. (1989). The postlabelled sample mixed with the synthesized UV-marker, *N*-1-epoxybutanediol-dAMP, was injected into a Beckman HPLC. HPLC fractions containing ^{32}P-labelled *N*-1-adenine adducts were collected and treated with base at 37°C overnight. The corresponding N^6-adenine adducts were further analyzed by HPLC. Radioactivity was measured on-line with a Beckman 171 Radioisotope detector.

3. Results and discussion

In the reaction of epoxybutanediol with dAMP two major products were formed (Fig. 1). The two adducts (peaks a and b) had λ_{max} at 259 nm (pH 1), 270 nm (pH 7) and 270 nm (pH 13). These UV spectra at different pH were similar to those of other reported *N*-1-adenine adducts. The later eluting adduct (peak c) had UV spectra at different pH identical to those of authentic N^6-adenine adducts. After incubation of the purified peaks a and b at pH 13 and 37°C overnight, they were fully converted to a new product (peak c). This suggests that peaks a and b are diastereomeric *N*-1-adenine adducts. The mass spectroscopic analysis of peak c showed a molecular ion at m/z 434.6, deriving from 331 (dAMP) + 105 (trihydroxybutyl group). This N^6-(2,3,4-trihydroxybut-1-yl)adenine adduct has previously been identified in DEB-treated DNA (Tretyakova et al., 1997).

Using the ^{32}P-postlabelling/HPLC method, the same adducts were analyzed in DEB-treated DNA and rat liver DNA samples. The synthesized *N*-1-alkyl- and N^6-alkyl-5′-dAMP were used as UV markers in HPLC. As shown in Fig. 2A, two *N*-1-adenine adducts of epoxybutanediol were detected in DEB-treated DNA. N^6-adenine adducts in DEB-treated DNA and rat liver DNA samples (Fig. 2B–D) were analyzed by HPLC after rearrangement of the collected labelled *N*-1-adenine adduct fractions. Since much less radioactivity was injected into HPLC and since N^6-adenine adducts eluted at a later retention time than *N*-1-adenine adducts, the sensitivity was increased. The approximate detection limit was 1 adduct/10^9 nucleotides using 10 μg DNA. The N^6-adenine adduct level in rat liver was on average 4.5/10^9 nucleotides, and nil in control rat liver.

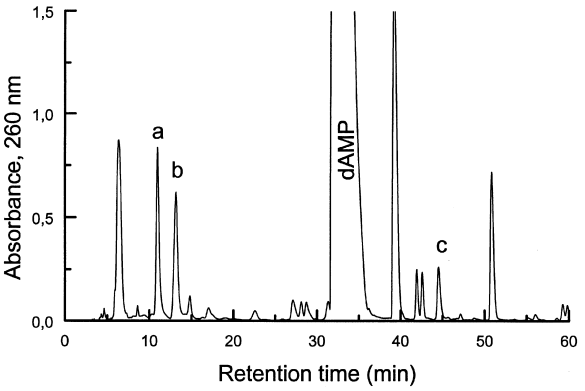

Fig. 1. HPLC separation of products formed by 5′-dAMP treated with epoxybutanediol. Peaks a and b are *N*-1-adenine adducts and c is an N^6-adenine adduct. UV detection at 260 nm.

N^6-adenine adducts are chemically stable and efficiently labelled. In addition, recent studies have found substantially higher levels of epoxybutanediol–haemoglobin adducts than those of EB in both BD-exposed rats and humans (Licea Pérez et al., 1997). Therefore, N^6-adenine adducts of epoxybutanediol might be a useful biomarker of BD exposure.

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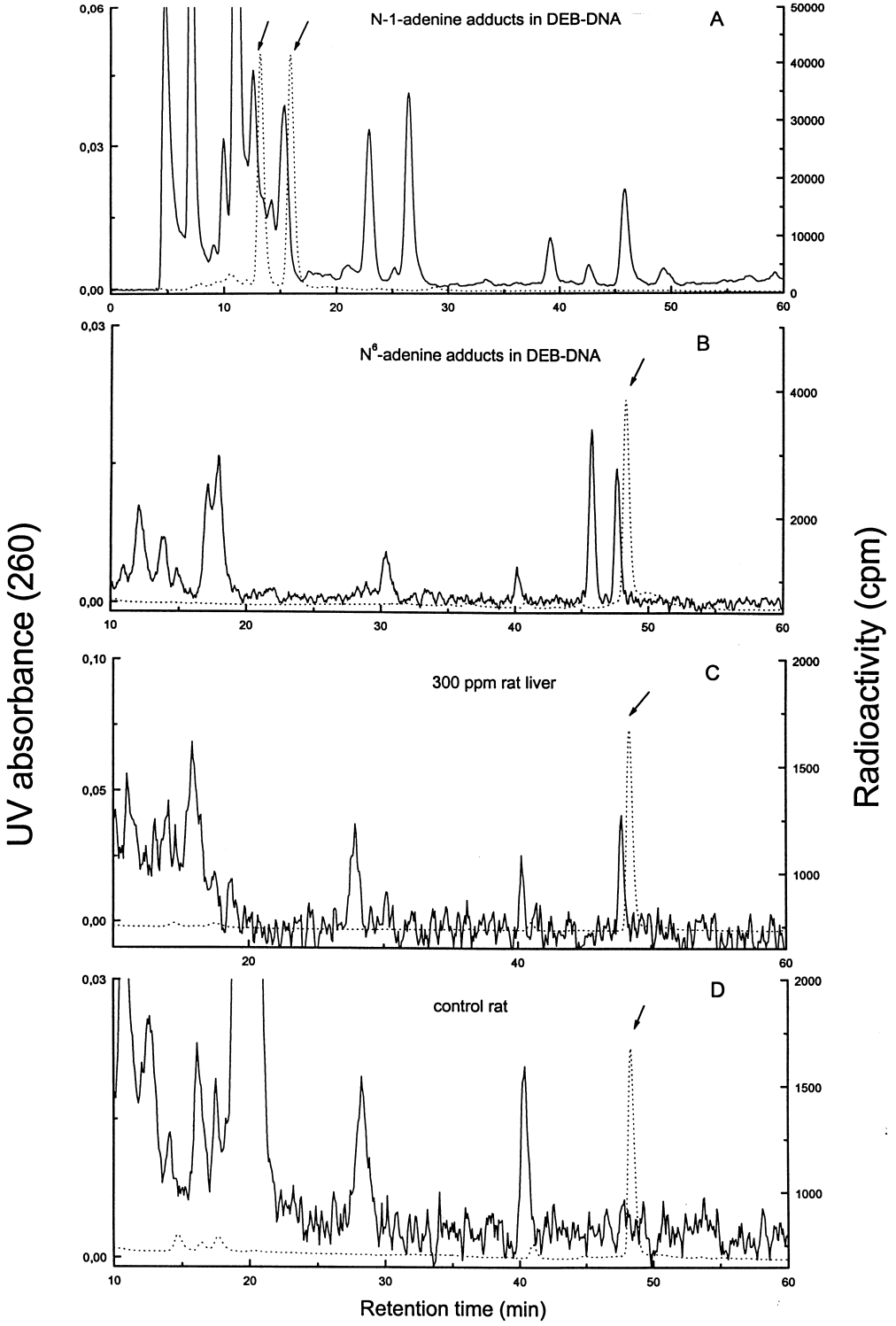

Fig. 2. HPLC chromatograms of ^{32}P-postlabelled DEB-treated DNA and rat liver DNA analysed with radioisotope and UV detectors. (A) *N*-1-adenine adducts in DEB-treated DNA, (B) N^6-adenine adducts in DEB-treated DNA, (C) an exposed rat liver sample, (D) a control rat liver sample. The positions of the adducts are indicated with arrows.——— radioactivity; … UV. Note that the UV detector is installed 0.6 min after the radioactivity detector.

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