Adducts from in Vivo Action of the Carcinogen 4-Hydroxyaminoquinoline 1-Oxide in Rats and from in Vitro Reaction of 4-Acetoxyaminoquinoline 1-Oxide with DNA and Polynucleotides

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ABSTRACT

In vivo 4-hydroxyamino[2-3H]quinoline 1-oxide-modified DNA and in vitro 4-acetoxyamino[2-3H]quinoline 1-oxide-modified DNA were enzymatically hydrolyzed, and the hydrolysates were analyzed by high-performance liquid chromatography. The two patterns were compared, and we showed that all of the high-performance liquid chromatography peaks which were recovered from in vivo-modified DNA were present in the hydrolysate of in vitro-modified DNA. Therefore, we used the in vitro 4-acetoxyamino[2-3H]quinoline 1-oxide-modified DNA to investigate the quinoline-purine adducts which are characteristics of the mode of action of the carcinogen 4-nitroquinoline 1-oxide. By comparison with the enzymatic hydrolysates of 4-acetoxyamino[2-3H]quinoline 1-oxide-modified covalent poly(deoxyadenylate-deoxycytidylylate)-poly(deoxyadenylate-deoxycytidylylate) and covalent poly(deoxyguanylate-deoxyctydylate)-poly(deoxyguanylate-deoxyctydylate) three nitroquinoline adducts were enumerated on the modified DNA. One of them was previously characterized as a C8-guanyl adduct. We proved that the two other are a guanine and an adenine adduct, respectively. A quinoline derivative was identified in the hydrolysates of the in vivo- and in vitro-modified DNAs as 4-aminoquinoline 1-oxide, the origin of which was postulated to be a degradation compound of one (or more) adduct(s). Moreover, the presence of two degradation compounds of the C8-guanyl adduct was shown in mild alkaline conditions. We suspected an imidazole ring-opened form.

INTRODUCTION

It is generally thought that the covalent reaction between DNA and ultimate carcinogens may constitute an important event in the carcinogenic process (12). In the case of 4-NQO2 (for reviews, see Refs. 13 and 15), 4-HAQO has been considered as the proximate carcinogen, and Ac-4-HAQO was proved to be an attractive model to study in vitro the carcinogenesis by this compound (1, 5).

Concerning the NQO adducts, Ikegami et al. (8) and after them Tada and Tada (17, 18) showed the presence of guanine and adenine adducts after enzymatic or chemical degradation of in vivo 4-NQO-modified DNA. It is now possible with more sensitive separation and detection techniques (e.g., HPLC) to reinvestigate the NQO adducts in order to define their complete chemical characterization. Indeed, we recently studied the in vitro DNA modification (5); after enzymatic hydrolysis of the modified DNA, we enumerated some different products the structure of one of them being totally elucidated (1). This C8-guanyl adduct, which we call dGuo-C8-AQO, accounts for about 30% of the total modification of the native DNA (6).

This paper is first devoted to comparison between the HPLC profiles of enzymatic hydrolysates of the in vivo- and in vitro-modified DNAs. Two modified DNAs were considered, the in vivo 4-HA[2-3H]HO-modified DNA from ascites cells (H. Z. line) and the in vitro Ac-4-HA[2-3H]HO-modified DNA. All the peaks obtained from the in vivo-modified DNA were recovered from the in vitro-modified DNA. The second part of this paper gives some information on the main HPLC compounds using a comparison between the enzymatic hydrolysates of 3 samples modified in vitro by Ac-4-HA[2-3H]HO: native DNA; poly(dA-dT)-poly(dA-dT); and poly(dG-dC)-poly(dG-dC). The dGuo-C8-AQO that we characterized previously (1) was recovered, and we observed its degradation toward 2 products in mild alkaline conditions. The existence of another guanine adduct, which we called dGuo-2-AQO, and of an adenine adduct was established. Finally, another compound was characterized as a quinoline derivative, 4-AQO, which probably arose from the degradation of one (or more) adduct(s).

MATERIALS AND METHODS

Chemicals. 4-N[2-3H]HO (specific activity, 941 mCi/mmol) was obtained from Amersham. 4-HA[2-3H]HO was prepared as described previously (3) and exhibited a specific activity of 18.43 mCi/mmol. Ac-4-HA[2-3H]HO was prepared from an O,O'-diacetyl derivative of 4-HA[2-3H]HO (1, 4). 4-AQO was synthesized (as a reference standard compound) as described previously (14) by catalytic hydrogenation of 4-NQO with palladium-charcoal in alcohol. The compound exhibited the same characteristics (melting point, UV spectrum) as described in the literature by Sugimura et al. (16) and Hoshino et al. (7). dGuo-C8[2-3H]HO was prepared as described previously (1) by reaction between dGuo and Ac-4-HA[2-3H]HO.
Native DNA was obtained from chicken erythrocytes. It had the following characteristics: s<sub>20w</sub> 225; hyperchromicity at 260 nm, 41%. The polynucleotides poly(dG-dC)-poly(dG-dC) and poly(dA-dT)-poly(dA-dT) were purchased from PL Biochemicals. All the enzymes (bovine pancreatic DNase I, snake venom phosphodiesterase, calf spleen phosphodiesterase, alkaline phosphatase of calf intestine) were purchased from Boehringer-Mannheim, Mannheim, Federal Republic of Germany.

Preparation of in Vitro Ac-4-HA[2-3H]QO-modified DNA and Polynucleotides. Native DNA and polynucleotides were modified by the ultimate carcinogen Ac-4-HA[2-3H]QO in the following manner. The reactions were performed in 2 mM sodium citrate buffer (pH 7); approximately 1 mg of polymer (concentration, 0.5 mg/ml) was incubated at 37° for 0.25 hr under a nitrogen atmosphere, in the dark, and in the presence of the equivalent amount of Ac-4-HA[2-3H]QO solubilized in dimethyl sulfoxide. The modified DNA was purified by diethyl ether extractions and extensive ethanol precipitations (0.1 volume of 5 M NaCl and 2 volumes of precooled ethanol were added to each DNA solution. The DNA precipitated after shaking). The amount of bound quinoline ring was calculated as described previously (5). It was 2.5, 3.3, and 2% of modified bases for the modified DNA, poly(dG-dC)-poly(dG-dC), and poly(dA-dT)-poly(dA-dT), respectively.

Preparation of in Vivo 4-HA[2-3H]QO-modified DNA. The in vivo-modified DNA was prepared as follows. Approximately 10<sup>7</sup> ascites tumor cells (H.2 line) were injected i.p. into 3-month-old male Wistar rats weighing about 300 g. Seven days after transplantation, an acidic 0.9% NaCl solution of 0.034 m 4-HA[2-3H]QO in HCl was injected i.p. (0.5 ml/rat). The rats were killed by decapitation 1 or 2 hr later. The ascitic fluid was drained, and the cells were harvested by centrifugation (5 min at 1500 rpm). Cells were washed in a 0.25 M-0.88 M sucrose gradient containing 10% phosphate-buffered saline in order to remove contaminating RBC. Then DNA was extracted using the method of Marmur (11). We obtained a modified DNA sample with 0.04% modified base pairs (2 molecules of carcinogen for 10,000 nucleotides).

Enzymatic Hydrolysis of the Modified DNA to Nucleosides and Chromatographic Analysis of the Hydrolysates. In vitro Ac-4-HA[2-3H]QO- and in vivo 4-HA[2-3H]QO-modified DNAs were hydrolyzed under the following conditions. One mg of modified DNA was solubilized (concentration, 2 mg/ml) in a buffer of 5 mM Tris-HCl, 2 mM MgCl<sub>2</sub>, and 2 mM CaCl<sub>2</sub> (pH 7.4) and incubated at 37° for 2 hr with 45 μg of DNase I; then 45 μg of each phosphodiesterase were added for an additional incubation of 17 hr at 37°. The pH was adjusted to 8.0, 3.5 μg of alkaline phosphatase were added, and dephosphorylation of the hydrolysates was performed for 7 hr. Thus, the total incubation time was 26 hr.

Chromatography on Bio-Gel P-2 (minus 400 mesh) was performed using a column (0.6 x 60 cm) equilibrated with 2 mM sodium citrate buffer (pH 7). The flow rate was 4 ml/hr. Fractions of 1 ml were collected, and the radioactivity was measured. Each P-2 tritiated fraction was collected and submitted to HPLC analysis. Every other P-2 fraction was collected, concentrated, and submitted to HPLC analysis.

In Vivo 4-HA[2-3H]QO-modified DNAs. Samples of in vivo 4-HA[2-3H]QO-modified DNA and in vitro Ac-4-HA[2-3H]QO-modified native DNA were incubated with the enzymes, at 37° for 26 hr. The hydrolysates were analyzed on a Bio-Gel P-2 minus 400 mesh column. The UV detection was at 254 nm (not shown) exhibited 4 peaks corresponding to the 4 deoxyribonucleosides as expected after the hydrolysis. The chromatographic radioactive patterns are shown in Chart 1. In both cases, similar profiles were obtained. There were 5 distinct tritiated fractions, which we called A, B, C, D, and E. They were corresponding to the same elution volumes for in vivo- and in vitro-modified DNA. Moreover, the percentage of each fraction was almost the same in both cases. The compound corresponding to Peak E was previously proved by HPLC to be the dGuo-C8-AQO (5).

In vitro- and in vivo-modified DNAs, every other P-2 fraction was collected, concentrated, and submitted to HPLC analysis. We used an absorbance detection at 2 wavelengths: 254 nm, the characteristic wavelength of both nucleosides and quinoline ring; and 365 nm, the characteristic wavelength of the quinoline moiety. The quinoline was also detected by tritiated radioactivity measurements of 500-μl aliquots. Fractions B and C, which were the quantitatively less important, were studied first. The study of P-2 Fraction B was difficult because its UV characterization at 254 nm was completely hindered by the 3 deoxyribonucleosides.
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(dAdo, dThd, and dCyd) which were present in this fraction. Some tentative separations of the unlabeled nucleosides on a Sephadex LH-20 column could not be effected. However, we observed that the 2 HPLC radioactive profiles (not shown) exhibited 2 peaks which were recovered, whatever the origin, in vivo or in vitro, of the modification of DNA. P-2 Fraction C of the in vivo- and in vitro-modified DNAs contained only one compound which was the same in both cases (HPLC profiles not shown). Its percentage was very weak: about 6 and 8% of the total radioactivity of in vivo- and in vitro-modified DNA, respectively.

Chart 2 shows the HPLC tritiated profiles of the quantitatively more important P-2 Fractions A and D for in vivo (Chart 2, a and c) and in vitro (Chart 2, b and d) modified DNAs. In the P-2 Fraction A, 2 identical compounds were recovered from the in vivo- and in vitro-modified DNAs, which we called A1 and A2. Another peak, A3, was detected only in the hydrolysate of the in vitro-modified DNA. P-2 Fraction D was composed in both cases of 4 major compounds, D1, D2, D3, and D4, which were well individualized on the UV profiles at 365 and 254 nm. On the tritiated profile, the 2 peaks corresponding to D3 and D4 were not as well separated, due to the very similar retention time of the 3 compounds, D2, D3, and D4. We also detected in this fraction 2 minor radioactive peaks with a retention time at about 15 min. Therefore, we can claim that all the HPLC radioactive peaks obtained from the hydrolysates of the in vivo-modified DNA were also recovered from the in vitro-modified DNA, with the same chromatographic characteristics (identical retention times on Bio-Gel P-2 and HPLC; identical ratios (Δ) of the absorbances at 254 and 365 nm in neutral and acidic conditions in HPLC). We will now focus our attention on the HPLC profiles obtained from the in vitro-modified DNA, and we will consider the more quantitatively important P-2 fractions, Fractions A and D. In the case of Fraction D, the corresponding subfractions will be studied by comparison with the HPLC profiles obtained with modified polynucleotides.

HPLC Analysis of the Hydrolysates of DNA and Polynucleotides Modified in Vitro by Ac-4-HA[2-3H]QO (P2 Fractions A and D). The 2 compounds A1 and A2 (see Chart 2) (which account for about 15% of the total radioactivity of DNA) were identified as 2 degradation products of the dGuo-C8-AQO. Indeed, when incubated at 37° during 48 hr under the conditions of the enzymatic hydrolysis, the dGuo-C8-AQO gave a P-2 chro-
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matographic tritiated pattern which exhibited 2 major peaks, the nonhydrolyzed dGuo-C8-AQO and an A fraction which contained A₁ and A₂. HPLC analysis showed that, whatever the conditions under which the HPLC profiles were recorded (neutral and acidic media), the 2 hydrolysis compounds were recovered at the same retention times as those of A₁ and A₂ when obtained from the DNA hydrolysate.

Three samples, native DNA, poly(dA-dT)-poly(dA-dT), and poly(dG-dC)-poly(dG-dC), were modified in vitro with Ac-4-HAQO, enzymatically hydrolyzed, and submitted to the chromatographic analysis. The HPLC profiles (absorbance at 365 nm, detection of the quinoline moiety) of the 3 P-2 Fractions D are shown in Chart 3. Chart 3a, which corresponds to the modified DNA, exhibits 4 peaks as mentioned previously, D₁, D₂, D₃, and another compound (D₅). By using the method of Dische (2), we showed that the 2 compounds D₁ and D₃ which contained the quinoline moiety also contained a deoxyribosyl residue, indicating that they are adducts. The D₅ component arose from the degradation of the adduct D₁; indeed, the stability of this isolated adduct was checked by HPLC and allowed to follow the appearance of this product with a retention time of about 15 min. D₂ was identified as a quinoline compound, 4-AQO. Indeed, D₂ comigrated with the reference standard (R₄ 10.5 min) with an identical ratio (Δ), which is 1.5 and 8.8 in near neutral and acidic media, respectively. The possible origin of 4-AQO in the DNA hydrolysates will be discussed below. We can only observe here that it was not recovered from modified poly(dA-dT)-poly(dA-dT). Any information was obtained about the D₄ compound (R₄ 11.8 min; Δ 2.3). The observation of the HPLC profile of the modified poly(dG-dC)-poly(dG-dC) (see Chart 3b) indicated that Compounds D₁, D₂, D₃, and D₅ were recovered from this modified polymer. Their retention times and their ratios Δ were identical to those of the equivalent compounds obtained from the DNA hydrolysate. Chart 3c indicates that P-2 Fraction D obtained after hydrolysis of the modified poly(dA-dT)-poly(dA-dT) contained only one component which was the D₅ compound that we recovered from the modified DNA. Indeed, the retention time and Δ values were exactly the same, and the 2 isolated D₅ compounds [either from modified poly(dA-dT)-poly(dA-dT) or from modified DNA] comigrated in HPLC.

As we mentioned previously D₁ and D₃ are quinoline adducts. We previously checked the reactivity of the 4 deoxyribonucleosides with Ac-4-HAQO, and we showed that any reaction was obtained with dCyd and dThd when positive reaction was obtained with dGuo and dAdo (1). Moreover, in their previous works about the NQO adducts, Ikegami et al. (8) and Tada and Tada

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Chart 3. HPLC elution profiles of P-2 Fractions D of hydrolysates of in vitro-modified DNA and polynucleotides. Absorbance detection at 365 nm. Chromatographies were carried out with a linear gradient (during 20 min) from water to 95% methanol-water. Each peak was characterized by its retention time R₄ and its ratio (Δ). a, DNA with 2.5% modified bases; b, poly(dG-dC)-poly(dG-dC) with 3.3% modified bases; c, poly(dA-dT)-poly(dA-dT) with 2% modified bases.
(17, 18) indicated that 4-NQO reacted preferentially with guanine and adenine in nucleic acids in vivo. Thus, we can claim that D1 is a guanyl adduct, dGuo-2-AQO, and that D3 is an adenyl adduct, dAdo-AQO.

DISCUSSION

In this study, an important observation was made. The main NQO adducts (the dGuo-C8-AQO, the dGuo-2-AQO, and the dAdo-AQO), which are formed on in vivo 4-HAQO-modified DNA, are recovered from the in vitro Ac-4-HAQO-modified DNA. The in vivo system that we used (ascite cells) is open to criticism. Nevertheless, in terms of metabolization of 4NQO, the fact that the same 3 main adducts were recovered from this in vivo system and from the in vitro material strengthens the choice of Ac-4-HAQO as a ultimate carcinogen model in our studies of chemical carcinogenesis by 4-NQO.

Another important feature is the identification of the 2 hydrolysis products arising from the decomposition of the dGuo-C8-AQO during enzymatic hydrolysis. With regard to the total radioactivity of the modified DNA, these 2 compounds were recovered at about 16% (in vitro) and 20% (in vivo). This finding explains the fact that the percentage of dGuo-C8-AQO (13%) was underestimated when determined from an enzymatic hydrolysis, as compared to the determination that we recently made from a spectrophotometric titration of the C8-guanyl adduct on a non-hydrolyzed modified DNA (6). Using this spectrophotometric determination, we found that a modified native DNA and a modified single-stranded DNA contained, respectively, 30 and 70% of dGuo-C8-AQO. Taking into account the degradation of this adduct in its titration after enzymatic hydrolysis, we obtain the same values of 30 and 70%. The nature of the hydrolysis products of dGuo-C8-AQO may be discussed in light of the results obtained previously by Kriek and Westra (10) with the C8-guanilyl adduct of aminofluorene. They observed its hydrolysis through guanine 7–8 bond at basic pH, in the presence of cationic ions such as Mg2+ and Mn2+. The presence of alkaline phosphatase in the medium increased the hydrolysis. Due to the chemical similarity between the C8-guanilyl adducts of 4-NQO and aminofluorene, an identical reaction may be possible with dGuo-C8-AQO during its hydrolysis since it is performed under the same conditions (basic pH, Mg2+, and alkaline phosphatase).

Studies were carried out very recently in our laboratory about the NQO adducts, Tada and Tada (17, 18) showed that there were 3 guanine adducts, one of them being very quickly decomposed during the chemical hydrolysis of their modified DNA, leading to a release of 4-AQO. Although our hydrolysis conditions were milder than those of Tada and Tada, it is possible that this phenomenon occurred. It is also possible that more than one guanine adduct were decomposed to release 4-AQO during and after the enzymatic hydrolysis. For example, we think that, after the enzymatic hydrolysis and during the chromatographic P-2 separation, some interactions with Bio-Gel P-2 could lead to the emergence of degradation compounds (4-AQO and others) which were then recovered in the different P-2 fractions. We rapidly tested this hypothesis by making a direct HPLC analysis of the total enzymatic hydrolysate of an in vitro Ac-4-HAQO-modified DNA. 4-AQO was still detected but in a smaller amount, indicating a possible bad effect of the P-2 column chromatography.

By comparison between the amounts of the 3 adducts (evaluated by the intensities of the 3 HPLC peaks at 365 nm), we observed that the dGuo-2-AQO seemed to be the major adduct formed on the Ac-4-HAQO-modified DNA. The same direct HPLC analysis of an in vitro-modified poly(dG-dC)-poly(dG-dC) indicated the presence on this polymer of the 2 main guanine adducts, dGuo-C8-AQO and dGuo-2-AQO.

In conclusion, we point out that Ac-4-HAQO is a very adequate model for the study of the in vitro carcinogenesis by 4-NQO. The decomposition of different adducts was elucidated [the C8-guanilyl adduct led to ring-opened dGuo-C8-AQO; one (or more) guanine adduct(s) led to 4-AQO]. Moreover, we established the breaking of the quinoline-base bond. In their investigations on the NQO adducts, Tada and Tada (17, 18) showed that there were 3 guanine adducts, one of them being very quickly decomposed during the chemical hydrolysis of their modified DNA, leading to a release of 4-AQO. Although our hydrolysis conditions were milder than those of Tada and Tada, it is possible that this phenomenon occurred. It is also possible that more than one guanine adduct were decomposed to release 4-AQO during and after the enzymatic hydrolysis. For example, we think that, after the enzymatic hydrolysis and during the chromatographic P-2 separation, some interactions with Bio-Gel P-2 could lead to the emergence of degradation compounds (4-AQO and others) which were then recovered in the different P-2 fractions. We rapidly tested this hypothesis by making a direct HPLC analysis of the total enzymatic hydrolysate of an in vitro Ac-4-HAQO-modified DNA. 4-AQO was still detected but in a smaller amount, indicating a possible bad effect of the P-2 column chromatography.

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existence of a second guanine adduct, dGuo-2-AQO, which seemed to be a major one for in vitro-modified DNA. The yield of dGuo-2-AQO formation is secondary structure DNA dependent, like the dGuo-C8-AQO (6), but a greater extent of this guanine adduct was observed on the native DNA (5). In other words, the double helix facilitates its formation. The identification of the structure of the dGuo-2-AQO was just recently achieved in our laboratory, and all our data on this adduct strongly support its characterization as 3-(deoxyguanosin-8-yl)-4-aminoquinoline 1-oxide. We also investigated the structure of the dAdo-AQO and showed that it is the same compound as the adenine adduct QA II previously isolated by Kawazoe et al. (9), but while these authors proposed either the N1 or the N6 position of adenine our data strongly support the structure of dAdo-AQO as the 3-(deoxyadenosin-6-yl)-4-aminoquinoline 1-oxide. Chart 4 illustrates our actual knowledge about the DNA-NQO adducts.

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REFERENCES

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