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STRUCTURE OF NUCLEIC ACID BASES IN CALF THYMUS DNA MODIFIED BY THE POTENT MUTAGEN, 10-AZABENZO[a]PYRENE-4,5-OXIDE

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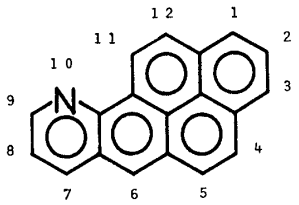
Modified nucleic acid bases were isolated from the hydrolysates of calf thymus DNA bound with 10-azabenzo[a]pyrene-4,5-oxide. The structures of two modified cytosines were proposed.

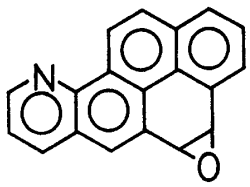
KEYWORDS —— 10-azabenzo[a]pyrene; carcinogen; mutagen; modification of DNA; modified nucleotide; cytosine; 10-azabenzo[a]pyrene-4,-5-oxide; arene oxide

Arene oxides are the active metabolites in the process of carcinogenesis induced by polyaromatic hydrocarbons. The covalent binding of the arene oxides to nucleic acid is essential for the carcinogenic process. "Bay region"[1] diol epoxides are claimed to be the major active metabolites of benzo[a]pyrene,[2] benz[a]anthracene[3] and 5-methylchrysene.[4]

10-Azabenzo[a]pyrene (10-ABP), which is a potent carcinogen,[5] has a nitrogen atom in the position of the bay region. We reported that 10-ABP-4,5-oxide is a strong mutagen without metabolic activation[6] and is the major metabolite in the presence of microsomal protein.[7] Since the formation of the diol epoxide at the 9,10 positions of 10-ABP is very unlikely, the role of mutagenic 10-ABP-4,5-oxide is of critical importance in muta-carcinogenicity of 10-ABP. The present paper describes the structure of the adducts isolated from calf thymus DNA modified with 10-ABP-4,5-oxide.



10-ABP



10-ABP-4,5-oxide

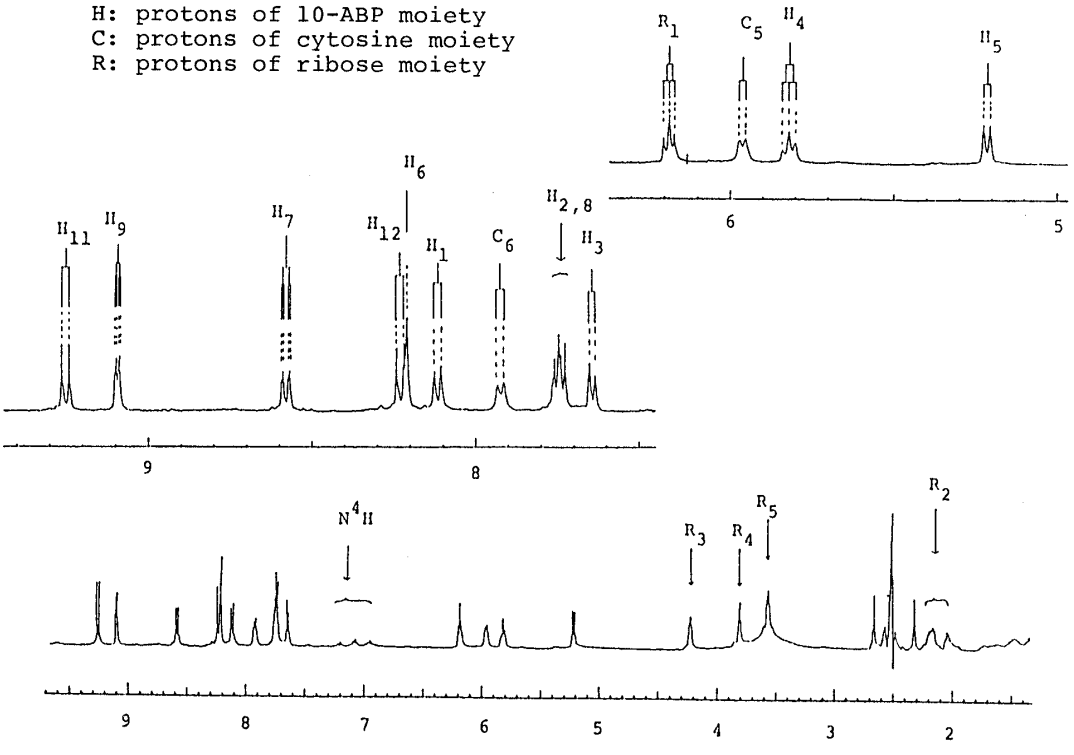
DNA modified with 10-ABP-4,5-oxide was prepared as follows. For an effective binding the solution is weakly acidic, though a binding was observed in the neutral medium. Thus, one gram of 10-ABP-4,5-oxide in 500 ml of acetone was added slowly to a solution of calf thymus DNA (2g) in 1000 ml of ammonium acetate buffer (pH 4.2). The mixture was incubated under argon atmosphere at 37°C for 24 h in the absence of light. Unbound 10-ABP-4,5-oxide and its decomposition products were removed by repeated dichloromethane extractions. The modified DNA was purified by ethanol-water reprecipitation and washed with acetone. The amount of 10-

ABP-4,5-oxide bound to DNA was estimated as one molecule per 130 nucleotides from the intensity of the fluorescence of the modified DNA.

The 10-ABP bound DNA thus obtained was hydrolyzed enzymatically to 5'-deoxyribonucleotides by nuclease P_1 at 40°C for 2 h at pH 5. The hydrolysates were chromatographed on Sephadex LH-20 column (4 x 30 cm). Normal nucleotides were first eluted with 0.02 N aqueous ammonium bicarbonate, and then, 10-ABP bound nucleotides were eluted with 40% methanol-0.02 N aqueous ammonium bicarbonate. The modified nucleotides were hydrolyzed to nucleosides by alkaline phosphatase at 37°C for 3 h at pH 9. The modified nucleosides were submitted to high performance liquid chromatography (HPLC). Three main modified nucleosides named A-1, A-2 and A-3 were recognized. They were isolated by semi-preparative HPLC (Polygosil $_5C_{18}$, 8ø x 250 mm, 26% CH_3CN-5 mM KH_2PO_4).

Careful treatment of A-1 and A-2 with trifluoroacetic acid gave the modified bases named B-1 and B-2 resulting from elimination of deoxyribose from A-1 and A-2, respectively. Structural studies were carried out by acid hydrolysis, NMR spectral and mass spectral analyses. For the purpose of identification of the bases, A-1 and A-2 were hydrolyzed with 1 N HCl at 100°C for 1 h under argon atmosphere, and the major hydrolysates were identified as cytosine and uracil by comparison of the hplc retention times and uv spectra of the hydrolysates with those of authentic samples. It is possible that the 10-ABP moiety is attached to O^2, N-3 or N^4 of cytosine. The binding position of cytosine of A-1 and A-2 was deduced to be N^4 of cytosine from the analysis of NMR and mass spectra of the modified nucleosides. Fig. 1 shows the proton NMR spectrum of A-1. Doublets at δ 5.92 (5.98) and δ 7.92 (7.98) were assigned to C-5 and C-6 protons of cytosine of A-1

Fig. 1. NMR spectrum of A-1
(in DMSO, δ from TMS)
H: protons of 10-ABP moiety
C: protons of cytosine moiety
R: protons of ribose moiety



(data for A-2 in parentheses). The signal of C^4 proton of 10-ABP moiety of A-1 was double doublet (triplet-like, $J_{4,5}$=7.8 Hz, $J_{4,NH}$=7.8 Hz) at δ 5.82. Fortunately, the proton on N^4 of cytosine residue was found as a doublet-triplet absorption at δ 7.06, whose coupling constants were $J_{NH,4}$=7.8 Hz and $J_{N,NH}$=50 Hz. By complete D-H exchange by D_2O, the signal of C^4 proton became doublet and the NH proton disappeared.

Mass spectra obtained by electron impact at 70eV failed to reveal the molecular ions. However, A-2 shows strong ions at m/e 268.1010 ($C_{19}H_{12}N_2$, Calcd. 268.1000) and at m/e 362.1150 ($C_{23}H_{14}N_4O$, Calcd. 362.1165). $C_{19}H_{12}N_2$ corresponds to the cleavage of the bond between N^4 and C^4 positions of deoxycytidine and subsequent loss of water from 10-ABP moiety. $C_{23}H_{14}N_4O$ corresponds to the protonated ion losing water and deoxyribose. Corresponding ions were observed for A-1 at m/e 268 and m/e 362. This result also supported that 10-ABP linked through N^4 of cytosine.

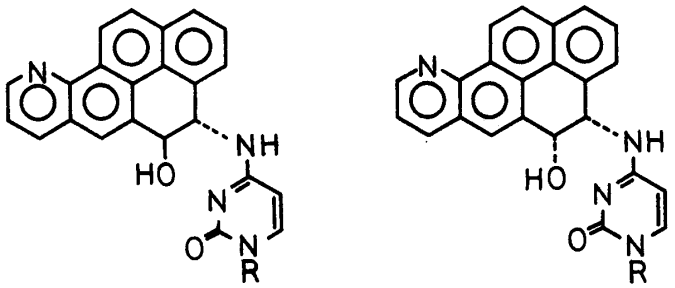
The stereochemistry of the cytosine moiety and the hydroxy group of 10-ABP of A-1 is trans and has an e.e. conformation, because the coupling constant between C^4H and C^5H is 7.8 Hz, while that of A-2 is cis and has an e.a. conformation ($J_{4,5}$=3 Hz). The cytosine residue must be equatorial in both compounds.

The position of the binding site of N^4 of cytosine was deduced to be the same position of 10-ABP for both A-1 and A-2, because the treatment of B-1 and B-2 with methanesulfonyl chloride gave a common dehydrated product, which, on further acid treatment, gave 4-hydroxy-10-ABP (no 5-hydroxy-10-ABP was detected).[8] The structural difference between A-1 and A-2 is that the hydroxy group of one of them is equatorial, while that of the other is axial. When the chemical shifts of proton NMR of 10-ABP moiety of A-1 and those of A-2 were compared, their chemical shifts were almost superimposable except C^6 protons. Only the C^6 proton of A-1 was shifted downfield by 0.06 ppm, which could be interpreted as the influence of the equatorial hydroxy group.[9] These results show that hydroxy group of 10-ABP moiety locates at the 5 position of 10-ABP, and the 4 position of 10-ABP attaches to the N^4 of cytosine. Combination of these chemical and spectral results led us to propose the structures of the cytosine bound products as shown in Fig.2.

Recent studies have demonstrated that activated polyaromatic hydrocarbons attack purine bases, especially guanine or ribose of guanosine.[10] An adduct with cytosine in DNA of (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]-pyrene has been reported.[11] In this paper we showed that A-1 and A-2 which were isolated from modified DNA with 10-ABP-4,5-oxide were the adducts resulting from the attack of N^4 of cytosine at C^4 of 10-ABP by trans opening of the epoxide for A-1 and by cis opening of the epoxide for A-2. We are studying the absolute stereochemistry of these two products as well as the structural study of an adenosine-bound product (A-3). Additionally, we wish to mention that these products could not be obtained in the reaction of 10-ABP-4,5-oxide and cytidylic acid, or cytosine monomer.

The present results may provide an important background for understanding of molecular basis and for further study on carcinogenesis by 10-ABP and other polyaromatic hydrocarbons.

Fig. 2.



A-1: R=deoxyribose
B-1: R=H

A-2: R=deoxyribose
B-2: R=H

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