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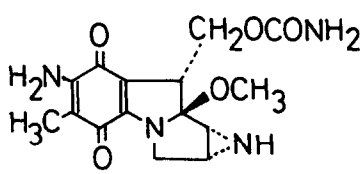
STRUCTURES OF MODIFIED NUCLEOTIDES ISOLATED FROM CALF THYMUS DNA ALKYLATED WITH REDUCTIVELY ACTIVATED MITOMYCIN C

Yuichi Hashimoto, Koichi Shudo, and Toshihiko Okamoto
Faculty of Pharmaceutical Sciences, University of Tokyo
Hongo, Tokyo, JAPAN

Summary: The structures of modified nucleotides isolated from calf thymus DNA alkylated with reductively activated mitomycin C were determined.

Mitomycin C (MMC) is a potent antibiotic and is also used clinically as an anticancer agent. It is well established that MMC acts as a bioreductive alkylating agent of biological macromolecules including DNA,[1] and this alkylation is presumably, at least in part, responsible for its effectiveness as an anticancer agent. Though there have been several studies on the sites of binding of MMC and DNA, no unambiguous evidence for the chemical structure of the modified DNA has been obtained. Recently, we described the alkylation of 5'-guanylic acid (5'-GMP) by reductively activated MMC and showed that the structure of MMC bound 5'-GMP was 1,2-cis-2,7-diamino-(5'-guanylyl)-mitosene.[2] The other nucleic acid derivative modified with MMC is a uridyl mitosene.[3] In this paper, we describe the structures of modified nucleotides isolated from calf thymus DNA alkylated with reductively activated MMC.

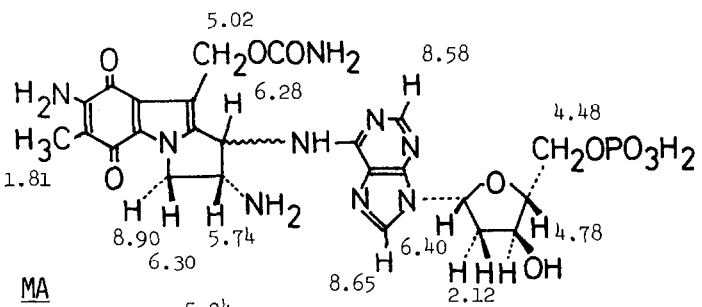


MMC

Active MMC bound DNA was prepared by catalytic reduction of MMC (0.5 mg/mL) in the presence of 5% Pd-C (0.1 mg/mL) and calf thymus DNA (1 mg/mL) in water (pH 7.5) with H_2 gas. Modified DNA was purified by EtOH-water precipitations and gel filtration column chromatography. The amount of MMC bound to DNA by reduction for 10 min was estimated as one molecule per 200-300 nucleotides from the uv-spectrum. No significant binding was observed in the absence of Pd-C or H_2. The MMC-bound DNA thus obtained was hydrolysed

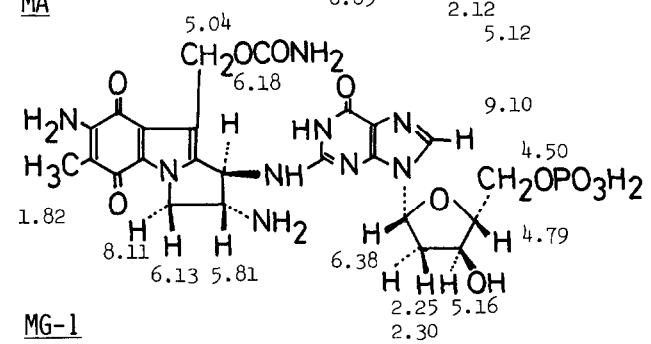
enzymatically to 5'-deoxynucleotides by nuclease P1 at pH 5. During the hydrolysis, a dark red precipitate (probably MMC bound nucleic acid derivatives) was formed, and the amount of the precipitate increased when the DNA used was modified by a longer reduction time (0.5-1 hr). Analysis of the nucleotide mixture by high performance liquid chromatography (hplc) showed the presence of three modified nucleotides, named MA, MG-1 and MG-2. These three products were isolated by semi-preparative hplc (Polygosil $_5C_{18}$, 8.0ø x 250 mm, 7% CH_3CN-0.3% NH_4Cl aq). The fractions containing MA, MG-1 and MG-2 were lyophilized and desalted by precipitation of the nucleotides from aqueous DMSO. The modified nucleotides, MA, MG-1 and MC-2, thus obtained were found chromatographically pure. Their uv-spectra (nm, in water) under neutral, acidic and basic condi-

Chart 1. Structures of MA, MG-1, and MG-2



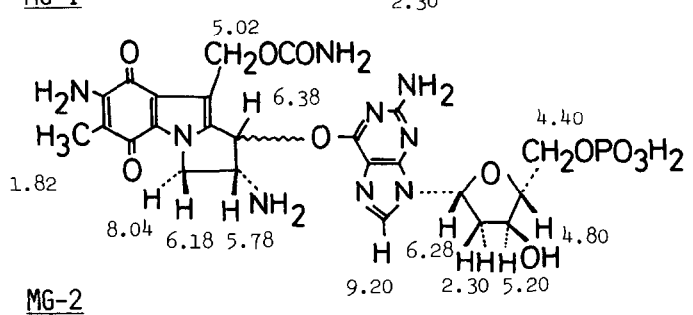
MA

uv		
pH 1	pH 7	pH 10
258	258	258
310	310	312



MG-1

uv		
pH 1	pH 7	pH 10
260	255	255
275(sh)	277(sh)	270(sh)
312	310	312

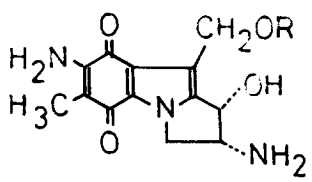


MG-2

uv		
pH 1	pH 7	pH 10
260	255	255
310	275(sh)	275(sh)
	308	310

tions, and nmr spectra (δ, ppm from TMS) in 5%CF_3COOD-d^6DMSO were shown in Chart 1. The structures of MA, MG-1 and MG-2 were deduced to be as shown in Chart 1 from the results described below.

a) Mitosene moiety: The uv-spectra of MA, MG-1 and MG-2 have absorption maxima at ca. 310 nm, characteristic of a mitosene chromophore. The chemical shifts of protons at position 10 of the mitosene rings of these nucleotides are nearly the same (δ5.02-5.04) and singlet. Hydrolysis of these modified nucleotides under mild conditions (silica gel-nPrOH-MeOH-NH_4OH) gave 1 in yields of 20-30%.[4] MG-2 gave 2 as well as 1 on treatment with 0.5 N HCl at 0° for 20 min., though the yield of 2 was poor.[4] These results suggest that the mitosene moieties bind at the C^1-positions and that a carbamoyl group is present at position 10. The configuration at positions 1 and 2 of mitosene of MG-1 was expected to be trans from the small coupling constant (less than 2 Hz) between the protons at positions 1 and 2. The signals of the protons at positions 1 and 2 of mitosene of MA and MG-2 were rather broad, presumably because of a steric factor.



1: R=H
2: R=CONH2

b) Identification of nucleic acid bases: MA, MG-1 and MG-2 were hydrolysed completely by treatment with 1 N HCl at 100° for 1 hr and the hydrolysates were analysed by hplc. MA yielded adenine quantitatively.[4] MG-1 gave guanine (45%) and xanthine (15%), and MG-2 gave guanine quantitatively.[4]

c) Binding sites of nucleic acid base moieties: The nmr spectra and the results on hydrolysates of MA, MG-1 and MG-2 suggest that the binding sites of nucleic acid base moieties are hetero-atoms of purine rings. These modified nucleotides are all rather stable under acidic conditions (pH 2, 50°, 30 min.). When three nucleotides were hydrolysed with 1 N HCl after methylation with trimethylsulfoxonium iodide (20 eq. for MA and 6 eq. for MG) in DMSO, adenine (trace),[5] 1-methyladenine (14%), 3-methyladenine (11%), and 7-methyladenine (0.5%)[5] were obtained from MA, and guanine (4-10%), 3-methylguanine (1%), 1-methylguanine (2-3%), and 7-methylguanine (4-5%) from MG-1 and MG-2.[4] MG-1 also yielded xanthine (4%). These results suggest that the 1, 3-, 7- and 9-

positions of the purine rings are not the binding sites. Therefore, the binding site of MA is the N^6 atom of adenine, and those of MG-1 and MG-2 are the O^6 or N^2 atoms of guanines. Chemical transformation of the guanine moieties was used to determine the binding sites of the guanine moieties of MG-1 and MG-2 unambiguously. Acid hydrolysis of MG-1 and MG-2 after treatment with P_2S_5 (10^3 eq) in pyridine gave 6-thioguanine[4] from MG-1 (30%), but not from MG-2. In addition, MG-1 and MG-2 were hydrolysed with 1N HCl after treatment with $NaNO_2$ (10^3eq) in dil. HCl (pH 2). Guanine (10%) together with xanthine (20%) was obtained from MG-1, but only xanthine (30%) was obtained from MG-2.[4] From these results, the binding sites of MG-1 and MG-2 were determined to be the N^2 and O^6 atoms, respectively.

d) Phosphomonoester group: When these modified nucleotides were treated with alkaline phosphatase at pH 9 at 40° for 1 hr, less polar products were obtained quantitatively. This finding and the observation that these nucleotides were all resistant to phosphodiesterase suggest that these compounds have a monophosphate group at the 5'-position.

In this work we showed that enzymatic hydrolysis of DNA alkylated with reductively activated MMC gave MA, MG-1 and MG-2, the structures of which were determined to be as shown in Chart 1. Similar reactions should occur in vivo. In fact, MG-2 was identified[4] in an enzymatic hydrolysate of DNA from a rat liver homogenate treated with MMC in vitro. Products whose retention times were consistent with those of MG-1 and MA were also recognized, though in small amounts. The present findings contribute to the understanding of the molecular basis of the action of MMC as a bioreductive alkylating agent.

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References and notes:

1) J.W.Lown, in Bioorganic Chemistry, vol. III, pp 95-121, Academic Press, New York, (1977)
2) Yuichi Hashimoto, Koichi Shudo and Toshihiko Okamoto, Chem. Pharm. Bull., 28,1961,(1980)
3) M.Tomasz and R.Lipman, J.Amer.Chem.Soc., 101, 6063, (1979)
4) Compounds were identified by comparing their retention times on hplc and uv-spectra with those of authentic samples. The yields were estimated from the peak heights on hplc.
5) Compounds were identified by comparing their retention times on hplc with those of authentic samples.

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