Mutagenic Chemistry of Heteroaromatic Amines and Mitomycin C

YUICHI HASHIMOTO, KOICHI SHUDO,* and TOSHIHIKO OKAMOTO

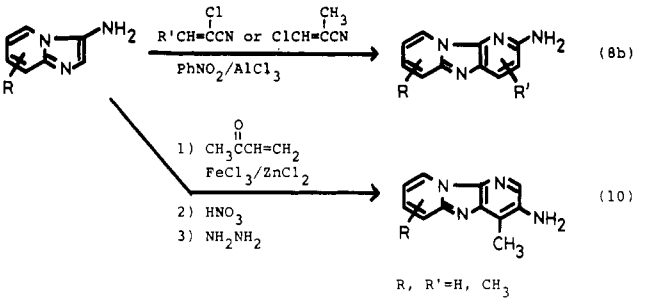
Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Received April 19, 1984 (Revised Manuscript Received August 13, 1984)

Elucidation of the chemical events caused by chemical carcinogens in the body is essential for the effective prevention and treatment of cancer. Since Miller's pioneering studies on acetylaminofluorene and methylaminoazobenzene, which established the metabolic activation of carcinogens to electrophiles (so-called ultimate carcinogens) and the covalent reaction of the electrophiles with DNA,[1] many investigators have been studying the chemical events caused by carcinogens. Though the chemical modification of DNA is believed to be the initial step of chemical carcinogenesis, very little unambiguous information has been obtained so far. Only benzo[*a*]pyrene, 4-nitroquinoline *N*-oxide, aflatoxin B1, and naphthylamine have been extensively studied and their modes of binding with DNA after metabolic activation have been established.[2] In these cases, the structures of the modified nucleotides have been determined on the basis of chemical information. Quite similar events are believed to be caused by many antitumor agents whose targets are nucleic acids. Therefore, we have also been interested in the chemical study of antitumor agents, in particular mitomycin C (MMC), which is one of the most clinically useful antitumor antibiotics.

On the other hand, a huge number of carcinogens are believed to be present in our environment, many of which should be detectable as mutagens by Ames assay using bacteria and mammalian metabolic enzyme systems (S-9 mix).[3] Another important problem is the presence of carcinogens in cooked foods.[4] It was shown that cooked foods such as broiled beefsteak, broiled fish, and hamburger contain strong mutagens formed by heating during the cooking process. These mutagens were found to be formed by thermal reactions of the food components, especially proteins,[5] or amino acids and sugars.[6] One group of these carcinogens consists of heteroaromatic amines isolated from pyrolysates of amino acids and cooked foods (Table I).[5] All these heteroaromatic amines possess a fused tricyclic aromatic system and are potent mutagens toward *Salmonella typhimurium* TA98. Among these compounds, 3-amino-5*H*-pyrido[4,3-*b*]indoles (Trp-P's)[7] and 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazoles (Glu-P's) proved to be carcinogenic.[4b] The structures of these compounds were deduced from spectral data and X-ray analysis results and finally confirmed by synthesis.[8] These carcinogens were found to exist in cooked foods such as broiled cuttlefish,[9] grilled beef,[9] broiled sardine,[5b] and beef extracts.[5e] They seem likely to be distributed widely in cooked foods and might play an important role in human carcinogenesis. Quantitative evaluation of the cancer risk due to these mutagens still requires extensive chemical, analytical, and biological studies. After the establishment of the synthesis of

Scheme I



Yuichi Hashimoto was born in 1955. He graduated from the University of Tokyo and is an Assistant Professor at the University. His research has dealt with the chemistry of carbocations, and this Account is a part of his doctoral research.

Koichi Shudo was born in 1940 and received his Doctorate from the University of Tokyo. Following a postdoctoral research associateship at Ohio State University (with Prof. P. Gassman) and at the University of British Columbia (with Prof. A. Rosenthal), he returned to Tokyo, where he succeeded Professor Okamoto as chair. His current interests include phenoxenium ions and benzenium–iminium dications and the differentiation of cancer cells.

Toshihiko Okamoto is Emeritus Professor of the University of Tokyo. A student of the late Prof. E. Ochiai and associate of Prof. J. Bunnett at the University of North Carolina (1954–1956), he was appointed Professor of Chemistry on the Faculty of Pharmaceutical Sciences in 1959. His research interests include the chemistry of aconite alkaloids, plant growth regulators, and mutagen–carcinogens.

(1) For reviews, see: (a) J. A. Miller, *Cancer Res.*, **30**, 559 (1970); (b) J. A. Miller and E. C. Miller, *Int. Rev. Biochem.*, **27**, 3041 (1975).

(2) For reviews, see: (a) *ACS Monogr.*, **173**, (1976); (b) P. D. Moore et al., *ACS Symp. Ser.*, *44*, 127 (1977); (c) E. C. Miller, *Cancer Res.*, **38**, 1479 (1978).

(3) (a) B. N. Ames, W. E. Durston, E. Yamasaki, and F. D. Lee, *Proc. Natl. Acad. U.S.A., Sci.* **70**, 2281 (1973); (b) T. Yahagi, M. Nagao, Y. Seino, T. Matsushima, T. Sugimura, and M. Okada, *Mutat. Res.*, **48**, 121 (1977).

(4) (a) T. Kawachi, M. Nagao, T. Yahagi, Y. Takahashi, T. Sugimura, S. Takayama, T. Kosuge, and K. Shudo, *Adv. Mod. Oncol., Res. Educ., Proc. Int. Cancer Congr., 12th*, 1 (1979); (b) T. Sugimura, *Cancer*, **49**, 1970 (1982).

(5) (a) T. Sugimura, T. Kawachi, M. Nagao, T. Yahagi, Y. Seino, T. Okamoto, K. Shudo, T. Kosuge, T. Tsuji, K. Wakabayashi, Y. Iitaka, and A. Itai, *Proc. Jpn. Acad.*, **53**, 58 (1977); (b) T. Yamamoto, K. Tsuji, T. Kosuge, T. Okamoto, K. Shudo, K. Takeda, Y. Iitaka, K. Yamaguchi, Y. Seino, T. Yahagi, and T. Sugimura, *Proc. Jpn. Acad.*, **54**(*B*), 248 (1978); (c) D. Yoshida, T. Matsumoto, R. Yoshimura, and T. Matsuzaki, *Biochem. Biophys. Res. Commun.*, **83**, 915 (1978); (d) H. Kasai, Z. Yamaizumi, K. Wakabayashi, M. Nagao, T. Sugimura, S. Yokoyama, T. Miyazawa, N. E. Spingarn, J. H. Weisberger, and S. Nishimura, *Proc. Jpn. Acad., Ser. B*, **56**, 278 (1980); (e) H. Kasai, Z. Yamaizumi, T. Shiomi, S. Yokoyama, T. Miyazawa, K. Wakabayashi, M. Nagao, T. Sugimura, and S. Nishimura, *Chem. Lett.*, 485, (1981); (f) B. Commoner, A. Vithayathil, P. Dolora, S. Nair, P. Madyastha, and G. C. Cuca, *Science*, **201**, 913 (1978).

(6) M. Jagerstad, K. Olsson, S. Grivas, C. Negishi, K. Wakabayashi, M. Tsuda, S. Sato, and T. Sugimura, *Mutat. Res.*, in press.

(7) N. Matsuura, T. Kawachi, K. Morino, H. Ohgaki, T. Sugimura, and S. Takayama, *Science*, **213**, 246 (1981).

(8) (a) K. Takeda, T. Ohta, K. Shudo, T. Okamoto, K. Tsuji, and T. Kosuge, *Chem. Pharm. Bull.*, **25**, 2145 (1977); (b) K. Takeda, K. Shudo, T. Okamoto, and T. Kosuge, *Chem. Pharm. Bull.*, **26**, 2924 (1978); (c) C.-S. Lee, Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **30**, 1857 (1982).

(9) (a) K. Yamaguchi, K. Shudo, T. Okamoto, T. Sugimura, and T. Kosuge, *Gann*, **70**, 743, 745 (1982); (b) K. Yamaguchi, H. Zenda, K. Shudo, T. Okamoto, T. Kosuge, and T. Sugimura, *Gann*, **71**, 849 (1979).

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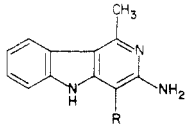
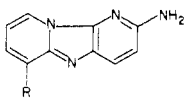
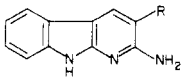
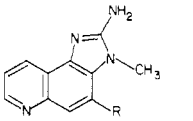
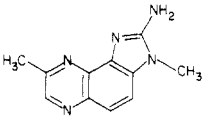
Table I
Mutagenic Heteroaromatic Amines Isolated from Food Pyrolysates

compd	R	mutagenicity,[a] revertants/μg	source
	R = H (Trp-P-2) R = CH_3 (Trp-P-1)	104 000 39 000	pyrolysate of L-tryptophan[5a]
	R = H (Glu-P-2) R = CH_3 (Glu-P-1)	500 19 000	pyrolysate of L-glutamic acid[5b]
	R = H R = CH_3	300 200	pyrolysate of globulin[5c]
	R = H (IQ) R = CH_3 (MeIQ)	433 000 661 000	broiled sardine[5d]
		145 000	fried beef[5e]

[a] Mutagenicity toward *Salmonella typhimurium* TA98 in the presence of S-9 mix.[3b]

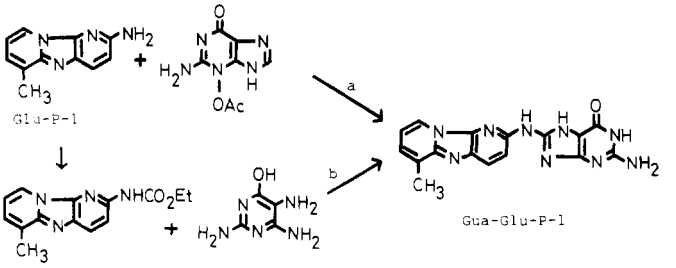
these compounds in 1979, chemical studies on these carcinogens started. This Account reviews from the viewpoint of organic chemistry our work on the metabolic activation of heteroaromatic amines (Glu-P-1, Trp-P-2, and MMC) and on the modification of DNA by these compounds.

2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]-imidazole (Glu-P-1)

2-Amino-6-methyl- and 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazoles (Glu-P-1 and Glu-P-2), which are potent mutagens toward *Salmonella typhimurium* TA98, were isolated from a pyrolysate of L-glutamic acid.[5b] They were found to be carcinogenic.[4b] These carcinogens are novel aza analogues of aminofluorene, and their chemical nature is different from that of aminofluorene: the aromatic ring system of Glu-P-1 is electron deficient. Synthesis of Glu-P derivatives was reported by Takeda et al.[8b] and Saint-Ruf et al.[10] as shown in Scheme I. The synthetic method is simple enough for the preparation of Glu-P's on a gram scale, making possible detailed chemical and biological studies of Glu-P's.

Glu-P-1 shows high mutagenicity toward bacteria only in the presence of S-9 mix.[3b,5b] In accordance with this results, Glu-P-1 bound to DNA in vitro only in the presence of rat liver microsomal fraction:[11] the covalent reaction of Glu-P-1 with DNA, as well as the expression of its mutagenicity, requires metabolic activation. The amount of Glu-P-1 covalently bound to DNA in vitro is typically one molecule of the carcinogen per 2–3 × 10^3 nucleotides in DNA. The hydrolysis of DNA modified with Glu-P-1 gives only one major modified nucleic acid base, which was purified by high-performance liquid chromatography (HPLC).[11] The hydrolysis of the Glu-P-1-modified base with aqueous alkali gave Glu-P-1 and 8-hydroxyguanine. The above results and

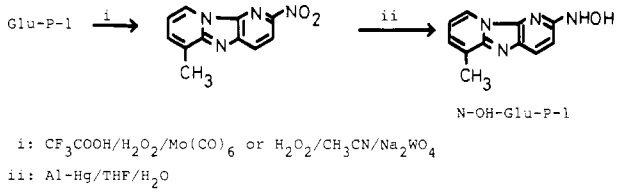
Scheme II



analysis of spectroscopic data of the Glu-P-1-modified base suggest that the modified base is a Glu-P-1–guanine adduct whose binding sites are the 8-position of guanine and the nitrogen atom of the 2-amino group of Glu-P-1. To confirm the proposed structure, 2-(8-guanylamino)-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Gau-Glu-P-1) was synthesized as shown in Scheme II. The synthesis was performed by the nucleophilic substitution of 3-acetoxyguanine by Glu-P-1 (path a)[12] and by a ring-condensation reaction of a carboethoxy derivative of Glu-P-1 with 2,4,5-triamino-6-hydroxypyrimidine (path b).[13] The methods are useful to prepare guanines modified with mutagenic amines at the 8-position, which seems to be a general site of attack by chemical carcinogens, and nucleic acid bases modified with Trp-P-2,[14] methylaminoazobenzene,[15] and naphthylamines[16] were synthesized by these methods. The nucleic acid base modified with Glu-P-1 in vitro was identified with the synthetic Gua-Glu-P-1.[11,17] The

(10) G. Saint-Ruf, B. Loukakou, and C. N. Zouzi, *J. Heterocycl. Chem.*, **18**, 1565 (1981).
(11) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **27**, 2532 (1979).
(12) N. J. M. Birdsall, J. C. Parham, U. Woelke, and G. B. Brown, *Tetrahedron*, **28**, 3 (1972).
(13) W. W. Zoebach and R. S. Tipson, "Synthetic Procedures in Nucleic Acid Chemistry", Interscience, New York, 1968, Vol. 1.
(14) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **27**, 1058 (1979).
(15) J.-K. Lin, B. Schmall, I. D. Sharpe, I. Miura, J. A. Miller, and E. C. Miller, *Cancer Res.*, **35**, 832 (1975).
(16) Y. Murofushi, Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **29**, 2730 (1981).
(17) Y. Hashimoto, K. Shudo, and T. Okamoto, *J. Am. Chem. Soc.*, **104**, 7637 (1983).

Scheme III

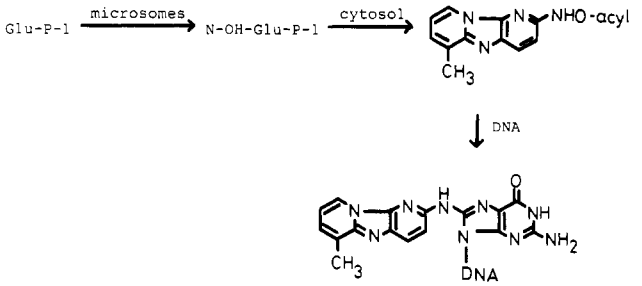


same modified base, Gua-Glu-P-1, was also obtained as the major adduct from the hydrolysate of the liver DNA of rats treated with Glu-P-1.[18] The biological significance of the formation of Gua-Glu-P-1 is not clear, but it is considered that a large substituent at the 8-position of guanine residues in DNA would induce mispairing, repairing, or B to Z DNA structural alteration and might effect DNA replication and transcription.[19]

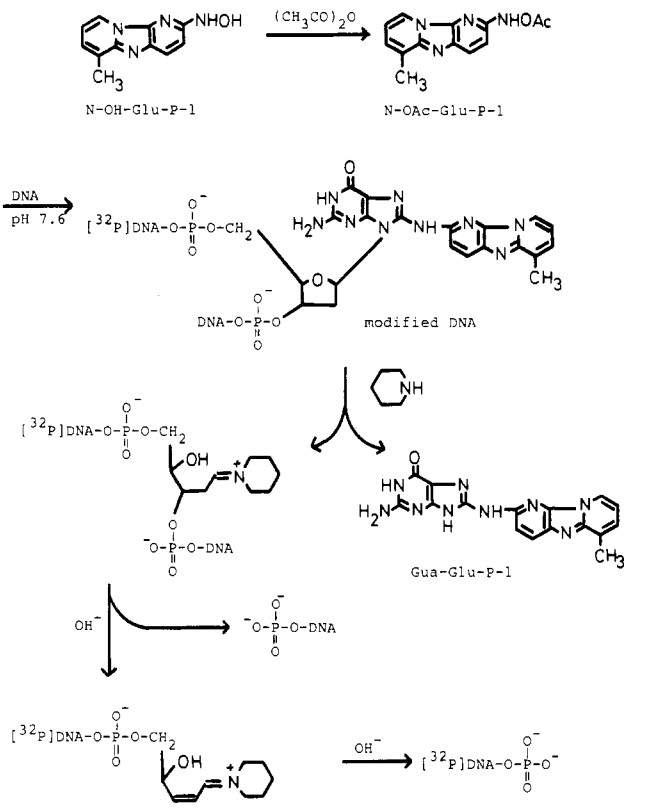
The structure of the modified nucleic acid base Gua-Glu-P-1 suggests the involvement in the reaction of a metabolically activated, electrophilic form(s) of Glu-P-1. Conversion of the nucleophilic aromatic amino group to an electrophilic group can be achieved by the oxidation of the amino group to a hydroxyamino group: the N–O bond of an aromatic hydroxyamino group can be heterolytically cleaved to form a strong electrophile.[20] In addition, N-oxidation is a common metabolic pathway. Therefore, we supposed that the metabolically activated form of Glu-P-1 might be the corresponding hydroxylamine, N-OH-Glu-P-1.[11] In fact, treatment of Glu-P-1 with rat liver microsomes gave N-OH-Glu-P-1 as the only primary metabolite.[21] The structure of the metabolite was confirmed by comparison with the authentic hydroxylamine synthesized as shown in Scheme III.[21] The oxidation methods adopted in the synthesis are of general applicability in the oxidation of aromatic amines to nitro aromatics.[22] Kato et al. also reported the formation of N-OH-Glu-P-1 as the active metabolite of Glu-P-1.[23]

The reaction of N-OH-Glu-P-1 with DNA was investigated next. N-OH-Glu-P-1 itself does not bind covalently to DNA. However, when the hydroxylamine was O-acetylated, the resulting *N*-acetoxy-Glu-P-1 (N-OAc-Glu-P-1) undergoes efficient covalent binding with DNA to give Gua-Glu-P-1 after hydrolysis of the modified DNA.[17,21] It is well established that O-acylation of hydroxylamines facilitates the heterolytic cleavage of the N–O bond.[24] As might be expected from these chemical results, the direct mutagenicity of N-OH-Glu-P-1 is not very high.[25] However, addition of cytosol fractions which contain many esterifying enzymes enhances the direct mutagenicity of N-OH-Glu-P-1 drastically. Therefore, N-OH-Glu-P-1 is a so-called

Scheme IV



Scheme V



proximate form of Glu-P-1, and the ultimate carcinogen is the corresponding *N*-acyloxy derivative, though the acyloxy group in vivo has not been specified: a possible acylation catalyzed by cytosol is acetylation, aminoacylation, sulfonation, or phosphorylation. Similarly, the aromatic hydroxylamine and its *O*-acyl derivative are also the metabolically activated forms of aminofluorenes, methylaminoazobenzenes, naphthylamines, etc.

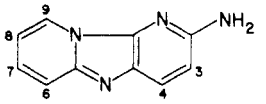
The reactions of N-OAc-Glu-P-1 with guanine nucleotides were also investigated.[17,21] A model compound of the ultimate form of Glu-P-1, N-OAc-Glu-P-1, reacts efficiently with double-stranded DNA and self-complemental guanylyl(3′–5′)cytidine, which is known to exist partially as a dimer in solution, and gives Gua-Glu-P-1 after hydrolysis. However, reactions of N-OAc-Glu-P-1 with single-stranded DNA, guanine, guanosine, guanylic acid, poly G, or a mixture of guanylic acid and cytidylic acid gave Gua-Glu-P-1 in very low yields. The results suggest that intercalation of the reactive form of Glu-P-1 into G–C base pairs is important for the covalent reaction at the 8-position of the guanine residues. The path of DNA modification with Glu-P-1 is summarized in Scheme IV.

(18) Y. Hashimoto, K. Shudo, and T. Okamoto, *Mutat. Res.*, **105**, 9 (1982).
(19) P. L. Grover, Ed., "Chemical Carcinogens and DNA", CRC Press Inc., Boca Raton, FL, 1979, Vol. 1 and 2.
(20) (a) T. Okamoto and K. Shudo, *Tetrahedron Lett.*, 4533, (1973); (b) T. Okamoto, K. Shudo, and T. Ohta, *J. Am. Chem. Soc.*, **97**, 7184 (1975).
(21) Y. Hashimoto, K. Shudo, and T. Okamoto, *Biochem. Biophys. Res. Commun.*, **92**, 971 (1980).
(22) Y. Hashimoto and K. Shudo, *Chem. Pharm. Bull.*, **32**, 1992 (1984).
(23) K. Ishii, Y. Yamazoe, T. Kamataki, and R. Kato, *Chem.*-Biol. *Interact.*, **38**, 1 (1981).
(24) (a) T. Ohta, K. Shudo, and T. Okamoto, *Tetrahedron Lett.*, 1939 (1978); (b) Y. Hashimoto, T. Ohta, K. Shudo, and T. Okamoto, *Tetrahedron Lett.*, 1611, (1979).
(25) M. Nagao et al., unpublished results.

Scheme VI
Base-Sequence-Selective Modification of a Fragment of Plasmid DNA (pBR 322) with N-OAc-Glu-P-1[a]

[a] * = relative probability of modification with N-OAc-Glu-P-1. Numbers are the base numbers in pBR 322.

Table II.
Mutagenicity of Glu-P-1 Derivatives



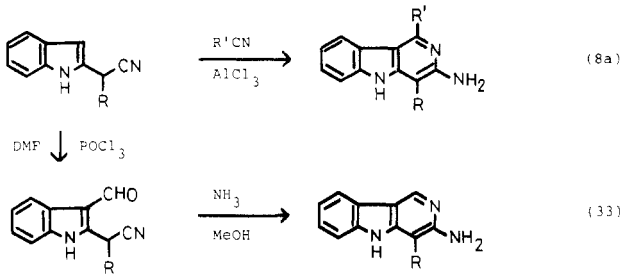
compd	mutagenicity (revertants/μg)[a]
Glu-P-2	1030
3-Me-Glu-P	7
4-Me-Glu-P	12900
6-Me-Glu-P	18600
7-Me-Glu-P	2720
8-Me-Glu-P	3610
9-Me-Glu-P	1350

[a] Mutagenicity toward *Salmonella typhimurium* TA98 in the presence of rat liver microsomes.

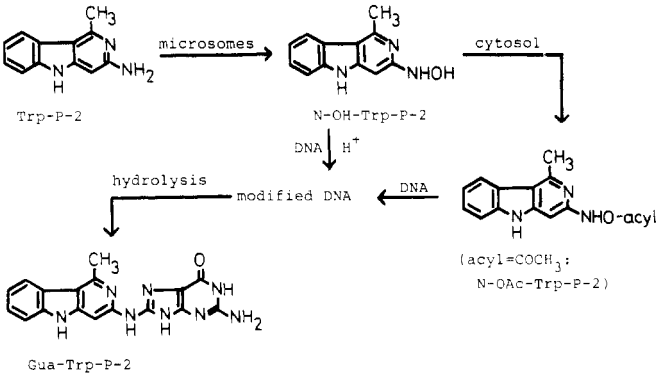
The glycosidyl bond of the Glu-P-1-modified guanine residue in DNA is so labile that heating of the modified DNA causes quantitative liberation of Gua-Glu-P-1 to leave an aguanylic acid residue.[17] Aguanylic acid containing DNA is known to be cleaved under basic conditions at the aguanylic sites.[26] Therefore, it is possible to determine the sites of modification with Glu-P-1 by the use of 5′-end ^{32}P-labeled DNA in conjunction with base-sequence-analyzing gel electrophoresis (Scheme V).[26,27] The reactive form of Glu-P-1 was found to react more frequently at the G–C-rich regions in DNA (Scheme VI).[27] The higher stability of the intercalated complex formed at G–C-rich regions is presumably one of the reasons for the base sequence selectivity of the DNA modification with Glu-P-1.

The possible importance of intercalation of the reactive form of Glu-P-1 into DNA for the covalent reaction to give Gua-Glu-P-1 was suggested above. We demonstrated by flow dichroism analysis[28] and by unwinding experiments with closed-circular supercoiled DNA[29] that Glu-P-1, a structural model compound of

Scheme VII



Scheme VIII



the reactive form of the carcinogen, does intercalate into double-stranded DNA. The association constant of Glu-P-1 with DNA was calculated to be about 10^3 M^{-1}, and the unwinding angle was typically 20–25°.

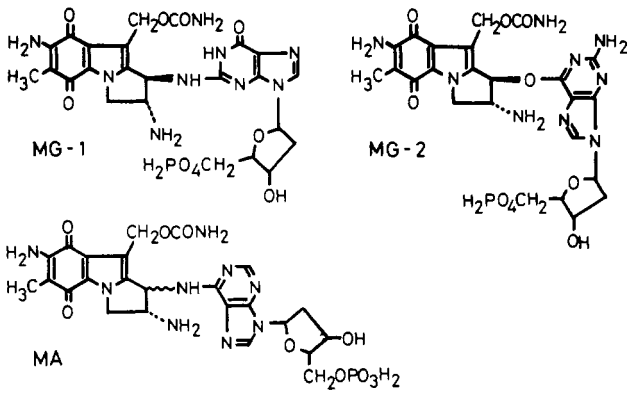
Methyl-substituted isomers of Glu-P-1 were synthesized by the methods shown in Scheme I and tested for mutagenicity (Table II).[30] The mutagenicity of the methyl derivatives of Glu-P-1 depends greatly on the site of substitution, though the rate of metabolic activation, association constant with DNA and unwinding angle do not alter much.[28,29] The conformation of the intercalated complex (the distance between the 8-position of the guanine residue and the nitrogen atom of the 2-amino group of the Glu-P-1 derivative) might be an important factor determining the mutagenic activity (effectiveness of covalent binding). Simulations of the conformations of the intercalated complexes of these Glu-P-1 derivatives with cytidyl(3′–5′)guanosine calculated by use of the Giglio function[31] supported the view that the conformation is greatly influenced by the position of methyl substitution.[32]

3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2)

3-Amino-1,4-dimethyl- and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indoles (Trp-P-1 and Trp-P-2) are very strong mutagens isolated from a pyrolysate of L-tryptophan[5a] and are also carcinogenic.[7] The aromatic ring system of Trp-P's is electron rich. A very simple one-step synthesis of Trp-P's was reported by Takeda et al. (Scheme VII).[8a] The method is applicable to the synthesis of many alkylated derivatives of Trp-P's.[33] Another synthesis was reported by Akimoto et al. (Scheme VII).[34]

(26) A. M. Maxam and W. Gilbert, *Methods Enzymol.*, **65**, 499 (1980).
(27) Y. Hashimoto and K. Shudo, *Biochem. Biophys. Res. Commun.*, **116**, 1100 (1983).
(28) M. Imamura, K. Takeda, K. Shudo, T. Okamoto, C. Nagata, and M. Kodama, *Biochim. Biophys. Res. Commun.*, **96**, 611 (1980).
(29) M. Imamura, K. Shudo, T. Okamoto, and T. Andoh, *Biochim. Biophys. Res. Commun.*, **97**, 968 (1980).
(30) K. Takeda, K. Shudo, T. Okamoto, M. Nagao, K. Wakabayashi, and T. Sugimura, *Carcinogenesis*, **1**, 889 (1980).
(31) (a) S. C. Sanctis and E. Giglio, *Acta Crystallogr., Sect. B*, **B35**, 2650 (1979); (b) E. Giglio, *Nature (London)*, **222**, 339 (1969).
(32) K. Yamaguchi et al., unpublished results.
(33) M. Nagao, Y. Takahashi, T. Yahagi, T. Sugimura, K. Takeda, K. Shudo, and T. Okamoto, *Carcinogenesis*, **1**, 451 (1980).

Chart I



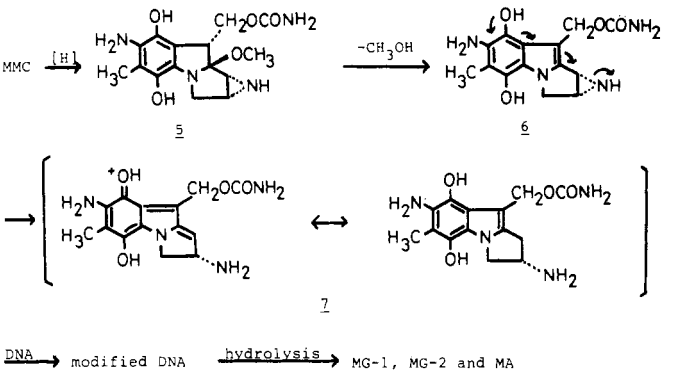
When the mutagenicity of various alkylated derivatives of Trp-P's was assayed,[33] it was found that substitution with larger alkyl groups decreases the mutagenicity of the derivatives, probably because of interference with the intercalation into DNA. Intercalative ability of the compounds seems to be essential for their mutagenic–carcinogenic activity. Similar results were reported by Pessuto et al.[35]

The nature of DNA modification with Trp-P-2 is similar to that of Glu-P-1, as shown in Scheme VIII. Trp-P-2 binds covalently to DNA only in the presence of a microsomal fraction.[36] The active metabolite of Trp-P-2 is the corresponding hydroxylamine, N-OH-Trp-P-2.[37] N-OH-Trp-P-2 itself does not bind covalently to DNA under neutral conditions but does bind to DNA under slightly acidic conditions.[38] When N-OH-Trp-P-2 was O-acetylated, the resulting *N*-acetoxy-Trp-P-2 (N-OAc-Trp-P-2) was found to bind very efficiently to DNA to give 3-(8-guanylamino)-1-methyl-5*H*-pyrido[4,3-*b*]indole (Gua-Trp-P-2).[14] The same modified nucleic acid base was obtained in vivo.[18]

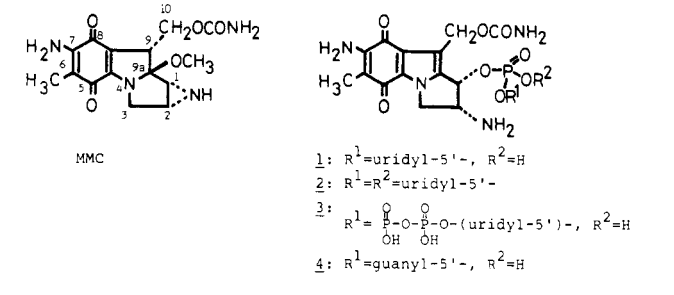
Mitomycin C (MMC)

MMC[39] is a clinically useful antitumor agent. It is well established that MMC acts as a bioreductive alkylating agent of DNA.[40] Elucidation of the molecular mechanism of this alkylation of DNA by MMC should provide basic information for the development of more effective antitumor agents. Moore proposed a mechanism for the alkylation of DNA and suggested that positions 1 and 10 of MMC are the binding sites.[41] Another site, position 9a, was also suggested as a binding site of MMC by Hornemann et al.[42] There have been several other studies on the sites of binding

Scheme IX



of MMC and DNA. However, no unambiguous evidence for the chemical structure of the modified DNA has yet been obtained. Recently, Tomasz and Lipman reported the structures of uridylic acid adducts of MMC (**1**–**3**).[43] They obtained the products (**1**–**3**) by reaction of MMC with uridylic acid derivatives under acidic conditions without reductive activation of MMC. The reaction of reductively activated MMC with guanylic acid was also investigated.[44] MMC reacted with guanylic acid after reduction of MMC with hydrogen gas in the presence of palladium on charcoal to give 1,2-*cis*-2,7-diamino-1-(5′-guanylyl)mitosene (**4**).[44] The reduction system used is a good model of the biological reduction of MMC according to Tomasz and Lipman.[45] The structure of the guanylic acid adduct **4** was deduced from its ^{1}H NMR spectrum and from the formation of 5′-guanylic acid and 1,2-*cis*-2,7-diamino-1-hydroxymitosene upon enzymatic hydrolysis of the phosphodiester bond. This was the first characterization of a nucleotide alkylation product formed by reductively activated MMC, but the alkylation of the phosphate group is not believed to be very important for the biological activity of MMC. Therefore, the reaction of a reductively activated MMC with DNA was investigated.



MMC binds to DNA after reductive activation with hydrogen gas in the presence of palladium on charcoal.[46] The DNA modified with MMC can be enzymatically hydrolyzed to 5′-nucleotides with Nuclease P1. Three MMC-modified nucleotides MG-1, MG-2, and MA were obtained in pure form.[46,47] These modified nucleotides were hydrolyzed with hydrochloric acid to give guanine from MG-1 and MG-2 and adenine from MA; thus,

(34) H. Akimoto, A. Kawai, H. Nomura, M. Nagao, T. Kawachi, and T. Sugimura, *Chem. Lett.*, 1061 (1977).
(35) J. M. Pessuto, P. P. Lau, Y. Luh, P. D. Moore, G. N. Wogan, and S. Hecht, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 1427 (1980).
(36) Y. Hashimoto, K. Takeda, K. Shudo, T. Okamoto, T. Sugimura, and T. Kosuge, *Chem.-Biol. Interact.*, **23**, 137 (1978).
(37) Y. Hashimoto, K. Shudo, and T. Okamoto, *Biochem. Biophys. Res. Commun.*, **96**, 355 (1980).
(38) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, in press.
(39) Very recently, a revised structure of MMC (which is antipodal to the previously proposed structure) was proposed on the basis of X-ray analysis (K. Shirahata and N. Hirayama, *J. Am. Chem. Soc.*, **105**, 7199 (1983)).
(40) For reviews, see: (a) R. W. Frank, *Fortsch. Chem. Org. Naturst.*, **38**, 1 (1979); (b) J. W. Lown in "Bioorganic Chemistry", E. E. van Tamelen, Ed., Academic Press, New York, 1977, Vol. 3.
(41) H. W. Moore, *Science*, **197**, 527 (1977).
(42) U. Hornemann, Y.-K. Ho, J. K. Mackey, and S. C. Srivastava, *J. Am. Chem. Soc.*, **98**, 7069 (1976).
(43) M. Tomasz and R. Lipman, *J. Am. Chem. Soc.*, **101**, 6063 (1979).
(44) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **28**, 1961 (1980).
(45) M. Tomasz and R. Lipman, *Biochemistry*, **20**, 5056 (1981).
(46) Y. Hashimoto, K. Shudo, and T. Okamoto, *Nucl. Acid. Res., Spec. Publ.*, **10**, 217 (1981).
(47) Y. Hashimoto, K. Shudo, and T. Okamoto, *Tetrahedron Lett.*, **23**, 677 (1982).

MG-1 and MG-2 are MMC–guanylic acid adducts, and MA is an MMC–adenylic acid adduct. The structures of these modified nucleotides were deduced from the ^{1}H NMR spectra to be as shown in Chart I.[47-49] The binding sites in these modified nucleotides were confirmed by chemical transformations: acid hydrolysis, methylation–hydrolysis, thioketonization–hydrolysis, and diazotization–hydrolysis.[48] The structure of MG-2 was confirmed by Tomasz et al. and they determined the configuration at the positions 1 and 2 to be trans.[49] These three modified nucleotides, MG-1, MG-2, and MA, are also obtained by reaction of MMC with DNA in vitro (in the presence of rat liver microsomes) and in vivo (from liver DNA of rats treated with MMC) in a similar ratio (MG-1:MG-2:MA = 1:2:1).[48]

The mechanism of formation of these modified nucleotides was not established unequivocally, but in view of previous results, we propose that the mechanism of the reaction is as shown in Scheme IX.[50] MMC is reduced to the corresponding hydroquinone **5**. This reduction enhances the electron-donating ability and aids elimination of the 9a-methoxy group. In the demethoxy product **6**, cleavage of the aziridine ring is facilitated: position 1 is now benzylic and is activated by many electron-donating groups.[50] The carbonium ion **7** formed by the opening of the aziridine ring possesses a planar structure which can intercalate into DNA and can thus approach position 2 or 6 of the purine bases. Covalent binding then occurs between position 1 of mitosene and the heteroatom at position 2 or 6 of the purine bases. The products might be oxidized by excess MMC or MMC derivatives (or by other cellular components in vitro and in vivo) or during the workup procedures to give MG-1, MG-2, and MA. The formation of these three modified nucleotides (by reductive activation of MMC and alkylation of nucleotides in DNA) seems to play an important role in the carcinostatic or carcinogenic mechanism of MMC.

Conclusions

In this account we have explored the initial chemical events involved in mutagenesis by heteroatomatic amines (Glu-P-1 and Trp-P-2) isolated from cooked foods. These mutagenic heteroatomatic amines are metabolically oxidized to the corresponding hydroxylamines and bind to DNA at the 8-position of guanine residues. The methodology used, i.e., (i) reaction of mutagens with DNA in vitro and purification of modified nucleic acid components by high-performance liquid chromatography (HPLC), (ii) synthesis of modified nucleic acid bases and active metabolites for identification purposes, (iii) reaction of synthetic active metabolites with DNA, and (iv) reaction of mutagens in vivo, should be applicable to a wide range of studies on DNA modification with drugs. Similar approaches have been used to investigate the bioreductive alkylation of DNA with MMC and also to study the chemical actions of 7-N^7-(*p*-hydroxyphenyl)mitomycin C, which is an interesting new antitumor MMC derivative.[51] The methodology has also been applied to studies of the action of 2-amino-1-methylimidazo[4,5-*f*]quinoline (IQ)[52] and naphthylamines,[16] for example.

In the covalent reactions of Glu-P-1, Trp-P-2, and MMC with DNA, the importance of intercalation of the reactive forms of these compounds is paramount. One of the developments from these studies is the synthesis of Glu-P derivatives which possess a high affinity toward DNA[53] and a strong DNA-cleaving ability.[54]

(48) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **31**, 861 (1983).
(49) M. Tomasz, R. Lipman, J. K. Snyder, and K. Nakanishi, *J. Am. Chem. Soc.*, **105**, 2059 (1983).
(50) An S_N2 type reaction of the intermediate **6** with DNA is not rigorously eliminated as the alternative pathway.
(51) Y. Hashimoto, K. Shudo and T. Okamoto, *Chem. Pharm. Bull.*, **30**, 2644 (1982).
(52) T. Okamoto, K. Shudo, Y. Hashimoto, T. Kosuge, T. Sugimura, and S. Nishimura, *Chem. Pharm. Bull.*, **29**, 590 (1981).
(53) C.-S. Lee, Y. Hashimoto, T. Ohta, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **30**, 3046 (1982).
(54) (a) Y. Hashimoto, C.-S. Lee, K. Shudo, and T. Okamoto, *Tetrahedron Lett.*, **24**, 1523 (1983); (b) Y. Hashimoto, H. Iijima, and K. Shudo, *Gann*, **75**, 467 (1984).