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Chemical Modification of DNA with Muta-Carcinogens. II. Base Sequence-Specific Binding to DNA of 2-Amino-6-methyl-dipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1)

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2-Amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1) binds covalently to DNA after metabolic activation to give 2-(C^8-guanyl)amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Gua-Glu-P-1). The importance of the intercalative ability of the Glu-P-1 skeleton into DNA base pairs for this reaction is emphasized. The reactive form of Glu-P-1, *N*-acetoxy-Glu-P-1 (N-OAc-Glu-P-1), reacts preferentially at the C^8 position of guanine residues in G-C-rich regions of DNA.

Introduction

The pathway of DNA modification (the initial chemical events caused by the carcinogen) with carcinogenic 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1) was established (*1,2*) as described in the previous article. Glu-P-1 is metabolically activated to the corresponding hydroxylamine (N-OH-Glu-P-1). N-OH-Glu-P-1 is further activated by cytosol to the reactive ultimate form, *N*-acyloxy-Glu-P-1, which reacts with DNA. The structure of the modified nucleic acid base is 2-(C^8-guanyl)amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Gua-Glu-P-1). The problems which should be elucidated next are the physical interaction(s) of the reactive ultimate form of Glu-P-1 with DNA prior to the covalent reaction, the nature of the modified DNA after the reaction with Glu-P-1, and the base sequence specificity of the covalent binding of Glu-P-1 to DNA.

Glu-P-1 as an Intercalator

A candidate for the ultimate form of Glu-P-1 is *N*-acetoxy-Glu-P-1 (N-OAc-Glu-P-1), as mentioned previously. N-OAc-Glu-P-1 reacts efficiently with double-stranded DNA and a self-complemental nucleotide dimer, GpC, to give Gua-Glu-P-1 after hydrolysis of the modified nucleic acids in yields of about 2% from guanine residues in DNA (*4*). GpC is known to exist in part as a dimer in solution. On the other hand, N-OAc-Glu-P-1 reacts with single-stranded DNA, guanylic acid, guanosine, or a mixture of guanylic acid and cytidylic acid to give Gua-Glu-P-1 in very low yields (Table 1) (*4*). The results suggest that the intercalation of the Glu-P-1 skeleton into nucleic acid base pairs is essential for efficient binding of Glu-P-1 at the C^8 position of guanine residues in nucleic acids. The intercalative ability of Glu-P-1 was established by means of spectroscopic methods, a flow dichroism method (*5*) and unwinding experiments (*6*). The physical binding constant of Glu-P-1 to DNA is typically about 1×10^4 M^{-1} (*5*). The orientation of Glu-P-1 physically bound to DNA is parallel with the DNA base pairs (*5*). The unwinding angle is typically 20° (*6*). The conformation of the intercalated complex formed from Glu-P-1 and the dimer of GpC was simulated by the use of the Giglio function (unpublished results). In the most stable conformation, the reaction sites (N^2 of Glu-P-1 and C^8 of guanine residues) are oriented in very close proximity.

Nature of the Glu-P-1-Modified DNA

The reactive ultimate form of Glu-P-1 is considered to bind covalently to DNA via intercalation as mentioned above. The next problem is the orientation of the covalently bound Glu-P-1 moiety in the DNA. The fluorescence of DNA modified with Glu-P-1 (the chromophore is the Glu-P-1 skeleton) is quenched by Hg^{2+} cation but not by I^- anion (unpublished results). The

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Table 1. Yields of Gua-Glu-P-1 in the reactions of N-OAc-Glu-P-1 with nucleic acids.

Reactant	Yield of Gua-Glu-P-1 from guanine, %
Guanosine	*0.01*
5'-Guanylic acid	0.01
Mixture of 5'-guanylic acid and 5'-cytidylic acid	0.01
Poly G	0.02
DNA	2.3
GpC	2.5

results suggest that the covalently bound Glu-P-1 moiety is inside the DNA double helix, and the modified guanine residues are outside the helix (*7*). The conformation of the left-handed Z-form DNA might also be compatible with the observations.

In addition, the orientation of the covalently bound Glu-P-1 is established to be parallel with the other base pairs from flow dichroism experiments (unpublished results). From the above results, covalent binding of Glu-P-1 is considered to be an intercalative covalent binding. Intercalative covalent binding was also proposed in the case of acetylaminofluorene by Miller et al. (*8*).

Another important characteristic of DNA modified with Glu-P-1 is the lability of the glycosidyl bond of the Gua-Glu-P-1 moiety in DNA. Gua-Glu-P-1 can be liberated quantitatively from the modified DNA by heat treatment (pH 7.2, 100°C, 30 min) or by treatment with aqueous acetic acid (pH 4.0, 25°C, 30 min) (unpublished results).

Sequence-Specific Binding of Glu-P-1 to DNA

Modification of DNA with N-OAc-Glu-P-1 is guanine-specific, and heating of the modified DNA causes quantitative liberation of Gua-Glu-P-1 to give aguanylic acid, as mentioned above. Aguanylic acid is known to be cleaved at the aguanylic site under basic conditions (Fig. 1). The established results suggested that the sequence

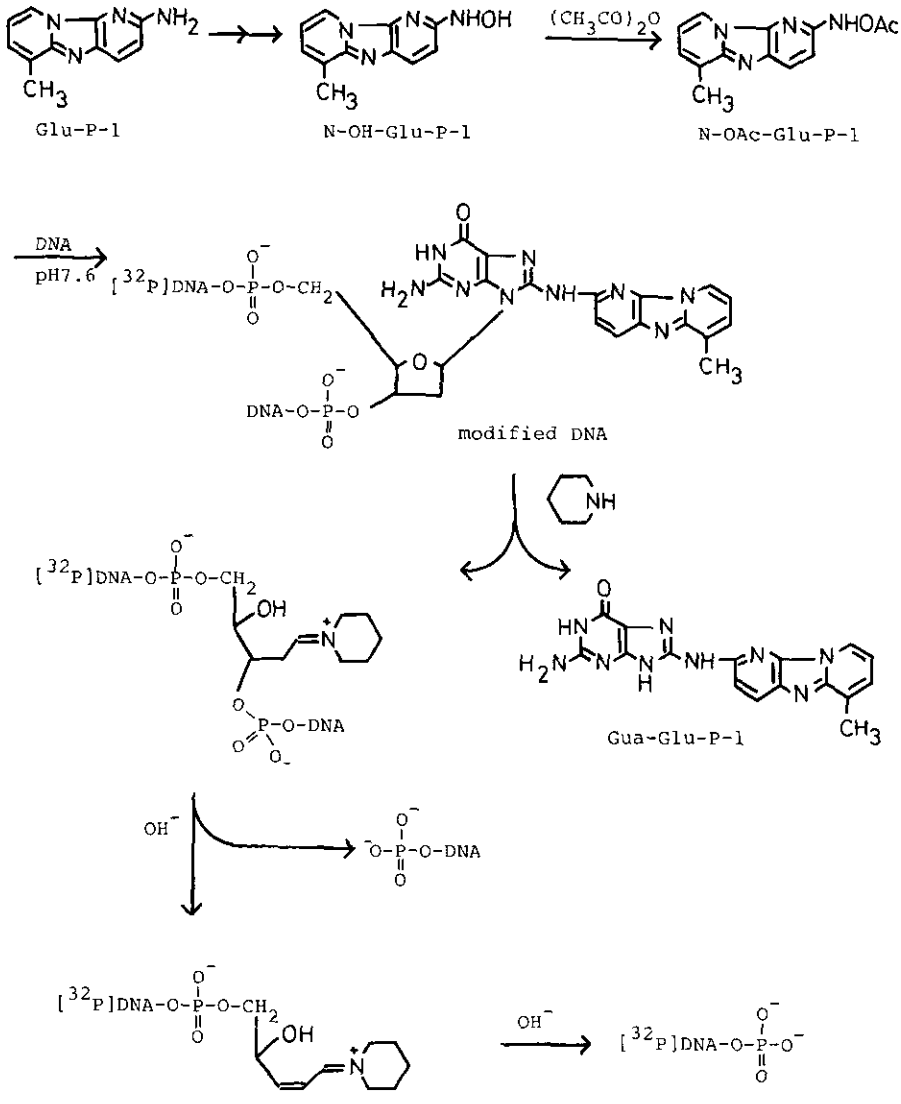

FIGURE 1. Sequence-specific modification of DNA by N-OAc-Glu-P-1.

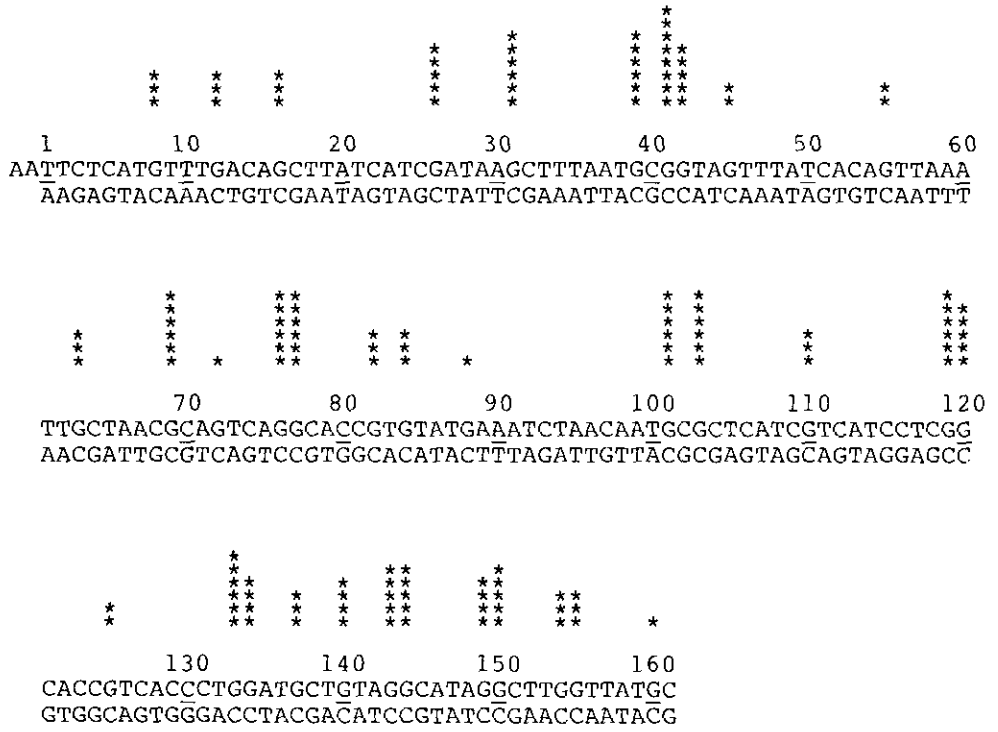

FIGURE 2. Sequence specificity of DNA modification with Glu-P-1. Base sequence-selective modification of a fragment of plasmid DNA (pBR 322) with N-OAc-Glu-P-1. The asterisks (*) denote relative probability of modification with N-OAc-Glu-P-1. Numbers are base numbers in pBR 322.

specificity of the reaction of N-OAc-Glu-P-1 with DNA could be analyzed by the use of ^{32}P-labeled DNA according to Maxam and Gilbert (*9*).

The 5'-end ^{32} P-labeled DNA was modified with N-OAc-Glu-P-1. The modified DNA was heated in aqueous piperidine to cleave the DNA at the site of the modified nucleotides after the release of Gua-Glu-P-1 (Fig. 1). The mixture was analyzed by sequence-analyzing gel electrophoresis (*9*). The autoradiograph of the samples showed ladder spots at the positions identical with those of Maxam and Gilbert's G-lane. This result confirmed our previous suggestion that the modification of DNA with N-OAc-Glu-P-1 is G-specific. The probability of release of guanines in the reaction (i.e., probability of modification of guanines by N-OAc-Glu-P-1) was deduced from the darkness of the spots on the autoradiograph as measured with a densitometer. The results are shown in Figure 2 (*10*). The spots of all G neighboring C or G (i.e., *G*GG, *C*GC, *C*GG, and *G*GC) are dark. On the other hand, the spots of all G neighboring A and T (i.e., *A*GT and *T*GA) are weak. The results suggested that the modification of guanine residues in DNA with N-OAc-Glu-P-1 occurs more frequently at the guanine residues in G-C clusterlike regions. The ability to attack the guanine residues in G-C clusterlike regions might explain the extremely high mutagenicity of N-OAc-Glu-P-1 toward *Salmonella typhimurium* TA98, because the hot spot of mutation of TA98 was reported to be the sites of G-C clusters in the DNA (*11*).

In conclusion, the sites of modified guanines in DNA treated with N-OAc-Glu-P-1 were determined. The guanine residues in G-C clusterlike regions are modified more frequently.

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