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INTERACTION OF MUTAGENS ISOLATED FROM L-GLUTAMIC ACID PYROLYSATE WITH DNA.

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SUMMARY:
The interaction of Glu-P-1 (2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole) and Glu-P-2 (2-aminodipyrido[1,2-a:3',2'-d]-imidazole) with DNA were studied. Agarose gel electrophoresis of closed-circular DNA treated with an excess of DNA-relaxing enzyme in the presence of increasing amounts of Glu-P-1 or Glu-P-2 revealed that Glu-P-1 and Glu-P-2 intercalated into DNA. Correlation with the binding parameters, measured by optical titrations, showed that Glu-P-1 and Glu-P-2 caused about 20° unwinding of the DNA double helix.

INTRODUCTION:

Glu-P-1 (2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole) and Glu-P-2 (2-aminodipyrido[1,2-a:3',2'-d]imidazole) (1) are potent mutagens isolated from a pyrolysate of L-glutamic acid and are found in casein pyrolysate (2) and charred dried squid (3). Glu-P-1 and Glu-P-2 have the same heterocyclic ring, the only difference between them being that Glu-P-1 has a methyl group at position 6. We are interested in the great difference in the mutagenicities of Glu-P-1 and Glu-P-2. Recently, we proved that the potent mutagen is metabolically activated to a reactive intermediate, an ester of N-hydroxy Glu-P-1, which react covalently with DNA (4,5). In this case, the intercalative interaction seems to be important for the covalent reaction, because the activated

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compounds reacts far more efficiently with a complimentary dinucleo tide, guanyl cytidine, than guanylic acid (6). In way of modelling the possible intercalation of the reactive activated forms, the intercalation of Glu-P-1 and its analogs with DNA was studied spectroscopically (7). The results have shown that Glu-P-1 and Glu-P-2 both have physicochemical affinity with DNA and suggested that their mode of interaction with DNA is intercalation. This paper reports further studies on these interactions, and determination of the unwinding-angle induced by Glu-P-1 and Glu-P-2.

MATERIALS AND METHODS:

Glu-P-1·H_2O·HBr and Glu-P-2·HBr were prepared by Takeda of our laboratory by reported method (8). E.coli A 745 was a gift from Dr. Y. Sakakibara, National Institute of Health, Tokyo, and closed-circular colicin E1 DNA was prepared by his reported method (9). Untwisting Extract (UE) was prepared from cultured mouse mammary carcinoma cells FM3A. One µl of UE converts 0.35 µg of Col E1 DNA form I to form Ir in 15 min. at 37°C. Treatment of Col E1 DNA with UE was carried out in 100 µl of reaction mixture containing 140 mM sodium phosphate (pH 7,5), 6 mM Tris·HCl, 6 mM NaCl, 0.6 mM Na_2EDTA, 44 mM sucrose, and appropriate amount of DNA relaxing enzyme, 2.9 µg of Col E1 DNA, and Glu-P-1 at concentration of 8.99×10^{-5} to 2.67×10^{-4} or Glu-P-2 at concentration of 2.60×10^{-4} to 7.80×10^{-4}M. After incubation at 37°C for 15 min, the reaction mixture was extracted twice with phenol and once with chloroform. The aqueous phase was recovered, mixed with 20 µl of Tris-buffer (40 mM Tris.HCl-0.4 mM Na_2EDTA-0.4 M NaCl-20%sucrose-2% SDS pH 8.0) and incubated at 45°C for 15 min. Electrophoresis of DNA was performed in 1.4% agarose (Dōjin Agarose I) in a vertical slab gel electrophoresis apparatus of 0.2 × 12 × 12 cm. Samples of 20 µl per slot were applied and a constant voltage of 4V/cm was applied at 20°C for 16 hrs. The gel was formed in electrophoresis buffer consisting of 36 mM Tris·HCl-30 mM NaH_2PO_4-1 mM Na_2EDTA(pH 7.8). After electrophoresis, the gels were incubated for 1hr in 500 ml of 0.5 µg/ml of ethidium bromide and photographs of fluorescent DNA bands were taken with Kodak Tri-X film.

RESULTS AND DISCUSSION:

A very useful and conclusive method for demonstration of intercalation, developed by Keller, is estimation of superhelical turns caused by intercalation of a chemical into closed-circular DNA (10). This method was applied to Glu-P-1 and Glu-P-2. Closed-circular DNA, Col E1, was treated with relaxing enzyme in the presence of various concentrations of Glu-P-1 and -2, and after

removing the chemicals, the DNA was subjected to electrophoresis. The number of turns, corresponding to the number of turns unwound by the chemicals, could be deduced from the results. Fig.1 shows the electrophoretic pattern of Col E1 DNA containing increasing number of superhelical turns. Sample 8 was untreated Col E1 DNA form I, and sample 1 was a control mixture which was incubated without Glu-P-1 or Glu-P-2 (Col E1 DNA form Ir). The photographic

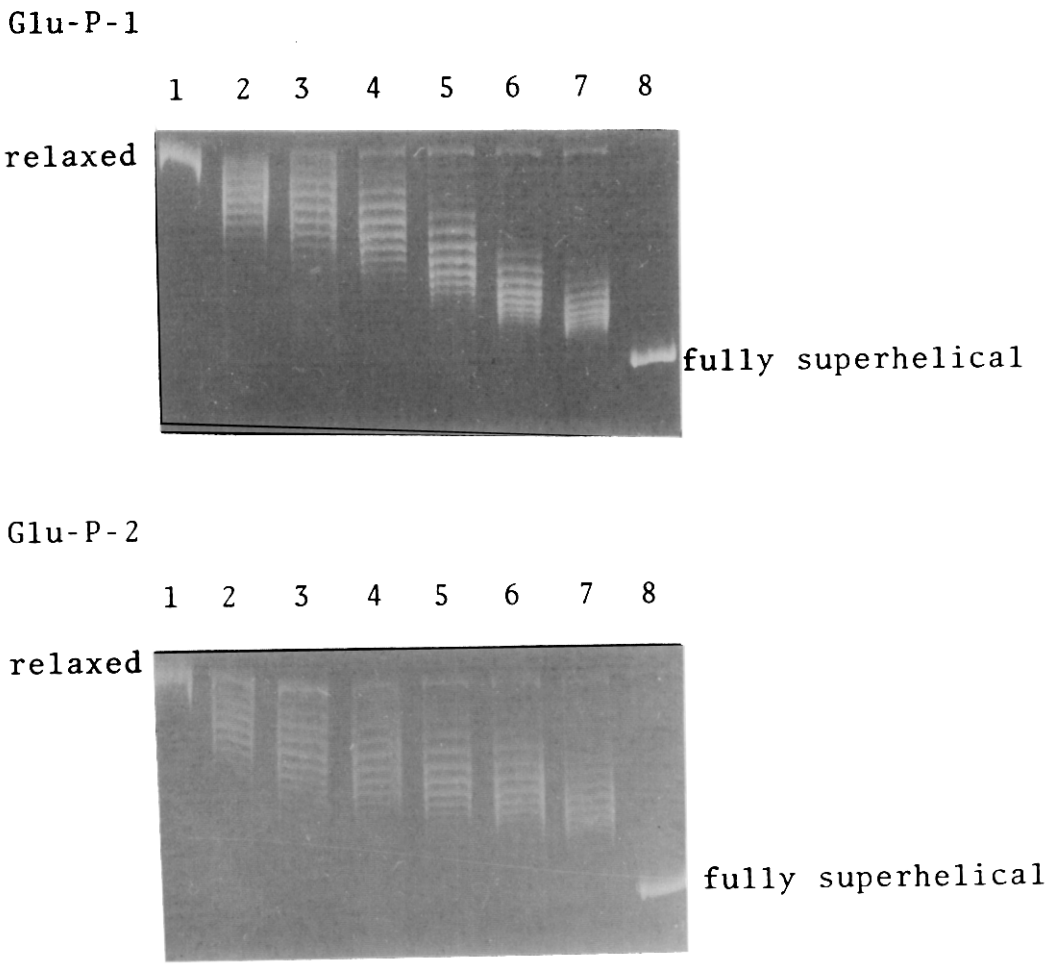

Figure 1. Agarose gel electrophoresis of Col E1 DNA containing increasing number of superhelical turns. Sample 8 was untreated Col E1 DNA I and sample 1 was a control mixture which was incubated without Glu-P-1 or Glu-P-2 (Col E1 DNA form Ir). The concentrations of Glu-P-1 were 0.899(2), 1.28(3), 1.61(4), 2.10(5), 2.51(6) and 2.67(7) $\times 10^{-4}$ M and the concentrations of Glu-P-2 were 2.60(2), 3.25(3), 3.90(4), 4.55(5), 5.20(6) and 6.50(7) $\times 10^{-4}$ M.

negatives of the DNA bands were traced with a microdensitometer and the middle point of each bands was determined from the band intensity. The difference in the mean number of superhelical turns ($\Delta \bar{\tau}$) in different DNA samples could be counted, and the values are shown in Fig.2 as a function of the concentration of Glu-P-1 or Glu-P-2. A higher concentration of Glu-P-2 than Glu-P-1 was required to obtained the same number of superhelical turns. This could be explained by the fact that the association constant of Glu-P-1 is about three times that of Glu-P-2 (7). Spectrophotomeric titration under condition for enzymatic relaxation (37°C UE-buffer) gave the K values of Glu-P-1 and Glu-P-2, 9.5×10^1 M^{-1} and 4.0×10^1 M^{-1}, respectively. These values were about 50 times less than those of Glu-P-1 and Glu-P-2 obtained at 20°C in 1 mM sodium phosphate buffer, 7.85×10^3 M^{-1} and 2.73×10^3 M^{-1}, respectively (7). The temperature and more particularly salt concentration affected the value considerably. The angular change in the DNA helix resulting from the intercalation of Glu-P-1 or Glu-P-2 can be deduced from the slope of the line in Fig.2 and the K values (10). The unwinding

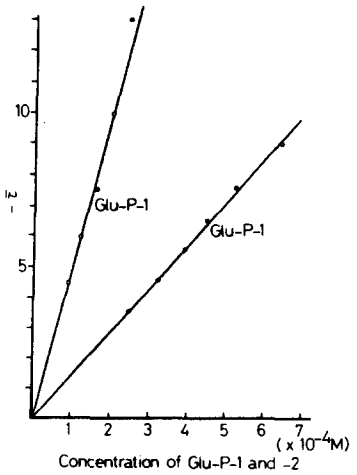

Figure 2. Plots of differences in the mean number of superhelical turns ($\Delta \bar{\tau}$) in DNA samples of Fig.1 as functions of Glu-P-1 and Glu-P-2 concentration.

angles caused by one molecule of Glu-P-1 and Glu-P-2 were calculated to be 20 ± 3° (Glu-P-1), 18 ± 3° (Glu-P-2) per intercalation, respectively. Thus although the association constants of Glu-P-1 and Glu-P-2 with DNA are different, these compounds caused the same extent of unwinding of the double helix on intercalation into DNA, and the values are almost the same as that of ethidium bromide. The present data show conclusively that Glu-P-1 and Glu-P-2 intercalate to DNA. This kind of interaction may be important prerequisite for the strong mutagenicities of these chemicals ; more correctly, the activated forms of these mutagens similarly interact with DNA before the covalent reaction(6).

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