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# NON-COVALENT INTERACTION WITH DNA OF THE MUTAGENS 2-AMINO-DIPYRIDO[1,2-a:3',2'-d]IMIDAZOLE AND METHYL-SUBSTITUTED ISOMERS

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SUMMARY

The interactions of 2-amino-6-methyldipyrido[1,2-a:3',2'-d]-imidazole (Glu-P-1) and its analog 2-amino-dipyrido[1,2-a:3',2'-d]-imidazole (Glu-P-2) which are potent mutagens isolated from a L-glutamic acid pyrolysate, with calf thymus DNA were studied spectroscopically. Scatchard plots obtained by optical titration gave association constants, K, of 2.7 $\sim$ 17 $\times$ $10^3$ $M^{-1}$ for several derivatives. Flow dichroism studies showed that Glu-P-1 and other derivatives are oriented in parallel to the planes of base pairs of DNA. The correlation between the association constants and degrees of mutagenicity is discussed.

## INTRODUCTION:

Carcinogenic chemicals produced by pyrolysis of food components are significant hazards in our environment ( 1 ). Among these, 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole ( Glu-P-1 ) and 2-aminodipyrido[1,2-a:3',2'-d]imidazole ( Glu-P-2 ) are very potent mutagens found in pyrolysates of L-glutamic acid ( 2 ) and casein ( 3 ), and also in charred dried spuid ( 4 ). *In vitro* transformation of embryo cells by these chemicals has been demonstrated ( 5 ) and tests on their carcinogenicities are in progress at the National Cancer Research Institute of Japan. We recently reported the covalent binding of Glu-P-1 with DNA and determined the chemical structure of the modified base ( 6, 7 ) In the

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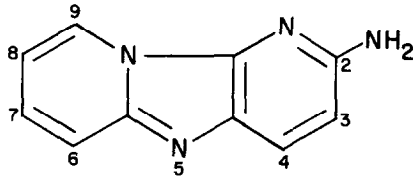
present study, we examined the physical interaction of these compounds with DNA, because many chemical carcinogens and mutagens not only bind to DNA covalently but also intercalate between DNA base pairs ( 8 ). The compounds studied are shown in the text figure.

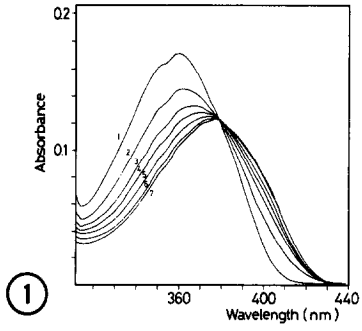



MATERIALS AND METHODS:

Calf thymus DNA was from Sigma. The compounds tested were prepared as hydrobromides by the reported method ( 9 ) with some modifications. Calf thymus DNA was dissolved in 1 mM sodium phosphate ( pH 7.4 )-1 mM $Na_2EDTA$. Absorption spectra were recorded at 20°C in the absence and presence of various concentrations of DNA using a Shimadzu UV 20 spectrometer. The concentrations of chemicals were about $1 \sim 2 \times 10^{-5}$ M and the concentration of DNA was $0 \sim 1.42 \times 10^{-3}$ MP. Scatchard plots for the chemicals were obtained from optical titration ( 10 ); the absorptions at 360 nm ( Glu-P-1 and 4-Me-Glu-P-2 ), 363 nm ( 7-Me-Glu-P-2 and 9-Me-Glu-P-2 ) and 365 nm ( Glu-P-2, 8-Me-Glu-P-2 and 3-Me-Glu-P-2 ) were used for calculations. Since these compounds have a strong fluorescence with peak at about 435 nm, solutions were excited at 360 nm at 20°C and emission spectra were recorded from 380 nm to 600 nm in the absence and presence of various concentrations of the DNA in a Hitachi MPF-4 fluorescence spectrophotometer. The concentrations of chemicals were $2 \sim 10 \times 10^{-7}$ M and the concentrations of DNA were $0 \sim 3 \times 10^{-3}$ MP. The apparatus and procedures used for recording flow dichroism were described by Wada ( 11 ). Dichroism, $\Delta\varepsilon$, is defined as the difference in the absorption coefficient or optical density parallel and perpendicular to the flow line. Data are usually expressed as $\Delta\varepsilon/\varepsilon$, where $\varepsilon$ is the optical density in the non-flowing state. The concentration of chemicals were $2.5 \sim 6.8 \times 10^{-3}$ M and that of DNA was $2.7 \times 10^{-3}$ MP, in 1 mM sodium phosphate-1 mM $Na_2EDTA$ ( pH 7.4 ). Spectra were obtained at room temperature.

RESULTS AND DISCUSSION:

Ultraviolet absorption spectra and fluorescence spectra were measured in the presence and absence of DNA; shifts of absorption spectra were recorded for all compounds tested with an isosbestic point. Fluorescence quenching in the presence of DNA was also



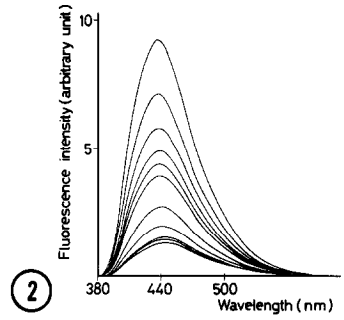




Fig.1 Absorption spectrum of 4-Me-Glu-P-2.
The concentration of 4-Me-Glu-P-2 was $1.659 \times 10^{-5}$M and the concentrations of DNA were 0(1), 0.017(2), 0.142(3), 0.213(4), 0.355(5), 0.710(6), and 1.42(7) mMP.

Fig.2 Fluorescence spectrum of Glu-P-1.
The concentration of Glu-P-1 was $2.472 \times 10^{-7}$M and the concentrations of DNA were 0, 0.0303, 0.0607, 0.0910, 0.121, 0.152, 0.303, 0.607, 1.21, 1.52, and 3.03 mMP from top to bottom.

observed with each compounds. Examples of spectra are shown in Figs. 1 and 2. Scatchard plots for 6-Me-Glu-P-2 ( Glu-P-1 ) is shown in Fig. 3. Values for the association constant, K, and the association constant per binding site ( r ), k, are summarized in Table 1. The association constants were also obtained by fluorescence quenching and the values are shown in parentheses in Table 1. Values obtained from absorption spectra were more reliable and reproducible than those obtained from fluorescence. The k values of seven compounds tested ranged from $2 \times 10^4$ $M^{-1}$ to $9 \times 10^4$ $M^{-1}$. Those values are not large but they are comparable with

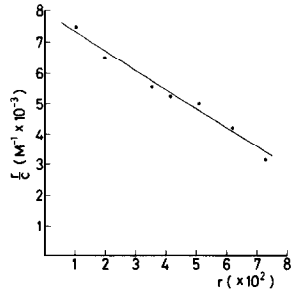



Fig.3 Scatchard plots for Glu-P-1.

Table 1. Binding characteristics and mutagenicities of Glu-P-2 and its derivatives.

| Compound | K, $\times 10^3 M^{-1}$ | k, $\times 10^4 M^{-1}$ | Binding site per base | mutagenicity* rev./ μg |
|---|---|---|---|---|
| Glu-P-2 | 2.73 ( 2.70 )** | 2.00 | 0.137 | 1,030 |
| 3-Me- | 5.15 ( 9.98 ) | 5.54 | 0.093 | 7 |
| 4-Me- | 5.57 ( 12.6 ) | 4.67 | 0.123 | 12,900 |
| 6-Me- | 7.85 ( 10.1 ) | 6.33 | 0.124 | 18,600 |
| 7-Me- | 5.53 ( 5.36 ) | 3.92 | 0.141 | 2,720 |
| 8-Me- | 8.40 ( 9.14 ) | 7.70 | 0.109 | 3,610 |
| 9-Me- | 16.9 ( 30.9 ) | 9.15 | 0.185 | 1,350 |

* Cited from ref.15.

** Values in parentheses were obtained from fluorescence quenching.

those of harman and norharman ( 12 ), and 3-amino-1-methyl-5H-pyrido[4,3-b]indoles ( Trp-P-1 and Trp-P-2 ) ( 13, 14 ),indicating a strong interaction of these mutagens with DNA. 9-Me-Glu-P-2 had the highest association constant. The values for Glu-P-1 and Glu-P-2 were $6.33 \times 10^4 M^{-1}$ and $2.00 \times 10^4 M^{-1}$, respectively, indicating that the stronger mutagen Glu-P-1 has stronger affinity to DNA than Glu-P-2. The range, however, seems rather too small to explain the wide range of mutagenicities determined using the frame shift mutation prove, Salmonella typhymurium TA 98, which are shown in the last column of Table 1 ( 15 ). The order of increasing physicochemical affinity to DNA of the closely related compounds is not correlated with the order of their mutagenic activity. Recently it was reported that there is a correlation between the DNA association constants and mutagenicities of 3-amino-5H-pyrido[4,3-b]indoles ( Trp-P-1 and Trp-P-2 ) ( 14 ). The present

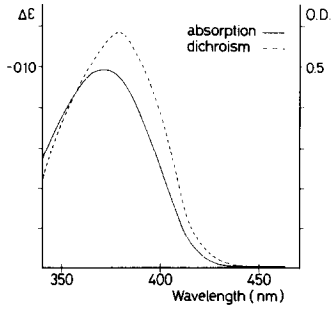



Fig.4 Dichroism spectrum of Glu-P-1.

results, however, suggest that the order of mutagenicity are discussed in relation to the association constants of the activated forms of these mutagens, but not of the mutagens themselves, with DNA.

Flow dichroism is useful for demonstrating a direct interaction between DNA and small molecules with absorption in the visible region. As discussed earlier ( 16 ),this method provides information on whether small molecules are oriented parallel to the base planes through intercalation or perpendicular to base planes: if the sign of $\Delta\varepsilon$ is negative, the molecule is oriented parallel to the base planes of DNA. An example of the flow dichroism of Glu-P-1 is shown in Fig. 4. Numerical values are summarized in Table 2. The negative values indicate that these mutagens intercalate between DNA bases.

The values of $\Delta\varepsilon/\varepsilon$ shown in Table 2 were found to be closely and significantly correlated with their association constants, K, obtained by absorption spectral titration. This suggest that change of $\Delta\varepsilon/\varepsilon$ mainly reflects the amount of physical complexes formed between DNA and these chemicals.

In conclusion, the present work shows that the heterocyclic mutagens, Glu-P-2 and methyl substituted Glu-P-2 interact with DNA and that their mode of interaction is intercalation.

Table 2. Flow dichroism measurement of Glu-P-2 and its derivatives.

| Compound | O.D. ( 360 nm ) | $-\Delta\varepsilon$ ( 360 nm ) | $-\Delta\varepsilon/\varepsilon$ |
|---|---|---|---|
| Glu-P-2 | 0.423 | 0.046 | 0.109 |
| 3-Me- | 0.268 | 0.035 | 0.131 |
| 4-Me- | 0.335 | 0.056 | 0.161 |
| 6-Me- | 0.452 | 0.092 | 0.204 |
| 7-Me- | 0.467 | 0.085 | 0.182 |
| 8-Me- | 0.475 | 0.087 | 0.183 |
| 9-Me- | 0.390 | 0.104 | 0.267 |

Abbreviations.

The unsubstituted compound, 2-amino-dipyrido[1,2-a:3',2'-d]-imidazole was named as Glu-P-2 (3), and methyl substituted compounds were named accordingly. For instance, 2-amino-3-methyldipyrido[1,2-a:3',2'-d]imidazole was named 3-Me-Glu-P-2.

This type of interaction is believed to be a prerequisite for mutagenicity, but mutagenicity of these compounds does not directly reflect their association constants. The importance of intercalation of activated muta-carcinogens is shown by the fact that activated Glu-P-1 ( N-acetoxy-Glu-P-1 ) ( 7 ) modifies a complimentary dinucleotide, guanylcytidine, but not guanylic acid. Intercalation into the space of a dimeric $G_pC$ base pair is essential for covalent binding of the activated mutagen with the guanyl moiety.

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