Binding Specificity of Mutagenic Tryptophan Pyrolysates for DNA Conformation: Spectroscopic and Viscometric Studies

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The compounds, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), are major potent mutacarcinogens isolated from tryptophan pyrolysate. In order to investigate their interaction with DNA and effects on DNA conformation, studies involving circular dichroism, fluorescence and absorption spectroscopy and viscometric titration were performed. The results show that (a) Trp-P-1 and Trp-P-2 are potent intercalators of DNA with nearly the same specificity for the A-T and G-C (alternative purine–pyrimidine) base sequences, (b) the interaction of Trp-P-1 with the B-form of DNA is biphasic so that stiffening of the B-DNA conformation occurs over the range r ([Trp-P-1]/[DNA]) = 0—2.5, followed by transformation of B to the non-B conformation at $r > 2.5$, (c) the transformation to the non-B structure is not observed for Trp-P-2, although stiffening of the B-DNA conformation similarly occurs, and (d) both Trp-P-1 and Trp-P-2 promote unwinding of the salt-induced Z-DNA to give the B-form. These data indicate that the noncovalent interaction of Trp-P with DNA is mainly dependent on the B-form conformation.

Key words tryptophan pyrolysate; DNA; interaction; spectroscopy; viscometry

The 3-amino-5*H*-pyrido[4,3-*b*]indole (Trp-P) and 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole derivatives (Glu-P) are potent mutacarcinogens isolated from tryptophan and glutamic acid pyrolysates, respectively,[1,2] and belong to a class of genotoxic compounds that react with DNA to form covalent adducts. Since structural determinations showed that 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]-indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 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) (see Fig. 1) are the main components of Trp-P and Glu-P carcinogens, various studies have been performed to investigate their chemical, physicochemical and biological properties.[3,4] The reaction mechanism of these compounds has been established: metabolically activated *O*-acetylated *N*-hydroxy-Trp-P or *O*-acetylated *N*-hydroxy-Glu-P (the ultimate active form) covalently modifies DNA through adduct formation of 3-(8-guanyl)-amino-Trp-P or -Glu-P, respectively.[5,6]

Understanding how the mutacarcinogen interacts with DNA or recognizes the base sequence is an essential first step towards understanding the molecular basis of its biological activity. As far as the DNA-binding property of Glu-P is concerned, the following has been reported so far[7–9]: its interaction with DNA is intercalative and occurs preferentially in the G-C-rich region of the DNA double-helical structure. In contrast, studies of the interaction of Trp-P with DNA have been rather limited.[10] Although the overall pattern of the interaction of Trp-P with DNA is probably similar to that of Glu-P, details concerning the binding mode, such as base preference, would be different. This paper deals with investigations involving circular dichroism (CD), fluorescence and ultraviolet (UV) absorption spectroscopy and the viscometric titration to study the way Trp-P-1 and Trp-P-2 interact with DNA and the effect on DNA conformation; a preliminary part of CD spectral studies of the Trp-P-1–DNA interaction has already been published.[11]

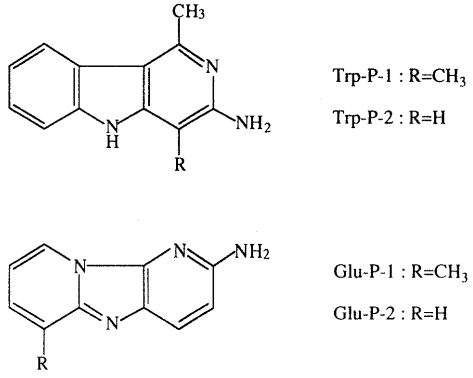

Fig. 1. Chemical Structures of Trp-P-1, Trp-P-2, Glu-P-1 and Glu-P-2

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Experimental

Materials Poly(dA-dT), poly(dG-dC), poly(dG-m5dC), poly(dA)·poly(dT), poly(dG)·poly(dC) and calf thymus (CT) DNA (deproteinized by phenol extraction and precipitated with ethanol) were purchased from Pharmacia P.L., and used without further purification. Oligonucleotides [$d(CGCGCG)_2$, $d(CGTACG)_2$, $d(ATGCAT)_2$ and $d(CGCGAATTCGCG)_2$] were synthesized by solid-phase phosphoramidite methods and purified by reverse-phase HPLC. Trp-P-1 and Trp-P-2 were purchased from Wako Pure Chemicals, distamycin A (Dist) and ethidium bromide (EtBr) from Sigma Chemical Co., and these were used without further purification.

Sample Preparation The solution of the B-form of DNA was adjusted with 10 mM sodium cacodylate buffer (pH 7.0). For preparation of the Z-form of DNA, 50 mM NaCl and 1.25 mM $MgCl_2$ were added to the B-form DNA solution. All measurements were carried out in triplicate and each spectrum reported is an average of these. Polynucleotide concentrations were determined using the standard molar extinction values ($M^{-1}\,cm^{-1}$) reported in the commercial documentation from P-L Biochemicals or in the literature[12,13] and were expressed in terms of moles of phosphate per liter. The molar extinction coefficients per base of the oligomers were calculated according to the method of Cantor and Warshaw.[14] The concentrations of Dist and EtBr were determined spectrophotometrically using the reported molar extinction coefficients.[13,15] In addition, the stock solutions of 10 mM Trp-P-1 and Trp-P-2, which were dissolved in 5% aqueous methanol, were prepared gravimetrically and added to the DNA solution for the titration experiment. Measurements covering a wide range of [Trp-P]/[DNA] ratios ($r = 0$—10) were performed at either constant nucleotide or constant drug concentrations, where r was defined as the molar ratio of

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the total amount of drug to the DNA base pair.

CD Measurements CD spectra were recorded on a Jasco J-20C spectropolarimeter with a DP-500N data processor using 5-mm path-length cells. The sample temperature in the cuvette was regulated by a circulating water bath and the temperature was kept at 25 °C. A 10 μM (base pair) solution of DNA was used for the measurements and the CD spectral change was monitored as a function of r. The molar ellipticity $[\theta]$ (deg·cm^2·dmol^{-1}) was calculated from the equation $[\theta]=100\times\theta$obsd/lC, where θ is the measured ellipticity in degrees, C is the DNA concentration in terms of phosphate, and l is the path length in centimeters. The measurement of each CD spectrum was repeated 8 times and checked for possible base-line shift.

Fluorescence Measurements In order to obtain the association constant (K_0) between Trp-P and DNA, the fluorescence intensity of Trp-P was measured as a function of DNA concentration using a JASCO FP-770F spectrometer (Nihon Bunko) equipped with Hg–Xe arc lamp, 10-mm path-length cells, and the temperature in the cuvette being maintained at 25 °C. The sample solution was prepared using 10 mM sodium cacodylate buffer (pH 7.0).

The association constant (K_0) for intercalative binding was evaluated by using the Scatchard plot[16]:

$$\frac{r}{C_f}=K_0(n-r)$$

where $r(=C_b/C_p)$ is the molar ratio of DNA-bound drug to DNA base pair, C_b=concentration of bound Trp-P, C_p=total concentration of DNA, C_f=concentration of unbound Trp-P, and n=binding stoichiometry in terms of bound Trp-P per nucleotide. The value of $C_f(=C_p-C_b)$ was estimated from the fluorescence quenching data. Since this Scatchard equation is valid under the condition $C_p>C_{Trp\text{-}P}$, the fluorescence titration was performed by adding suitable aliquots of DNA to a 3 ml solution of 2.5 μM Trp-P, to vary the concetration ratio of [DNA]/[Trp-P] from 2 to 9 (r=0.1—0.5). All fluorescence measurements were corrected for sample dilution during the course of the titration experiment. The intensities of the emission spectra excited at 315 nm were measured at the appropriate λ_{max} (400 nm for Trp-P-1 and 412 nm for Trp-P-2). The values of K_0 and n were determined by least-squares linear regression analysis of the plot of r/C_f *vs.* r, and are listed in Table 1. Since these data were all evaluated with correlation coefficients >0.95, side-effects such as electrostatic interaction were not taken into consideration.

Absorption Measurements The UV melting curves of poly(dA-dT) and poly(dA)·poly(dT) were measured on a Jasco UVIDEC-610 spectrometer using 5-mm path-length cells, with the DNA concentration being adjusted to 15 μM (base pair), and the drug concentration varied as a function of r. The temperature was controlled by a circulating water bath. The optical density (OD) at 260 nm was monitored as a function of temperature, with the heating rate of the sample solution being set to 0.5 °C/min. Under these conditions, it was impossible to measure the melting point of poly(dG-dC); the T_m was too high to be analyzed by this method.

Viscometric Measurements Viscometric measurements were conducted using a Cannon-Manning semi-microdilution viscometer (No. 75). The 100 μM DNA (base pair) solution was prepared in 10 mM sodium cacodylate buffer (pH 7.0) and the temperature was set at 35 °C. A 330 μl aliquot of the solution was placed in the viscometer and the titration conducted as a function of r.

Results and Discussion

CD Studies. (A) Interaction of Trp-P-1 with the B-Form of DNA Figure 2 shows CD spectra of the B-form of poly(dG-dC), poly(dA-dT), CT DNA, poly(dA)·poly(dT), and poly(dG)·poly(dC) in the presence and absence of Trp-P-1; the $[\theta]$ variation at 270 nm as a function of r is shown in Fig. 3. Although no markedly induced CD spectra were observed, the CD spectra of the B-form of poly(dG-dC) and poly(dA-dT) changed biphasically with increasing Trp-P-1 concentration, *i.e.*, (a) enlargement of the concomitant positive (*ca.* 270 nm) and negative (*ca.* 250 nm) ellipticities, which is typical of the conservative B-form of DNA, is Trp-P-1—dependent over the range r=0—3, and (b) transformation of the B to non-B structure, which is characterized by an intense, negative and long-wavelength Cotton band near 260 nm,[17,18] occurs at $r\geq 3$.

In the former phase, Trp-P-1 is thought to render the B-form structures of poly(dG-dC) and poly(dA-dT) rigid, because the strength of CD ellipticity is dependent on the extent of stacking of the DNA bases, in addition to the relative geometry of these bases (distance, twist, tilt, and so on); similar enlargement of the B-form of DNA has been observed for EtBr, a typical intercalator.[19] Generally, such a spectral change can be interpreted in terms of binding of the intercalating compound, which leads to stiffening of the DNA duplex structure as a result of DNA lengthening.[20] On the other hand, when r was greater than 3, both B-forms of the DNAs were rapidly transformed into non-B structures. It is not definite at present whether the CD spectrum of the non-B structure reflects the DNA structure itself, such as a ladder-like DNA structure fully saturated with intercalator,[21] or is due to the ligands "alone" resulting from a special ordered arrangement to a B-form of DNA. However, the degree of transition to the non-B structure was markedly different between the DNAs and was sequence-dependent: the CD ellipticities of the poly(dA-dT) and poly(dG-dC) saturated at r=8 were $[\theta]_{270nm}=-5.0\times10^4$ deg·cm^2·dmol^{-1} and -15.2×10^4 deg·cm^2·dmol^{-1}, respectively.

Concerning the biphasic interaction of Trp-P-1 with the B-form of DNA, the following should be noted. The increasing degree of the B-form of DNA at the initial interaction stage is in the order poly(dG-dC)≒poly(dA-dT)>CT DNA>poly(dG)·poly(dC)≒poly(dA)·poly(dT), and this indicates that the binding of Trp-P-1 is not necessarily specific only for the G-C sequence, contrary to our earlier expectations, and it has a preference for the alternative purine-pyrimidine sequence; the analysis of the Scatchard plots (Table 1) gave nearly the same binding site (n=0.28—0.29) and binding affinity ($K_0=2.5$—2.7×10^6 M^{-1}) of Trp-P-1 for poly(dA-dT) and poly(dG-dC). On the other hand, the degree of transformation to the non-B form follows the order poly(dG-dC)>poly(dA)·poly(dT)>poly(dA-dT)>CT DNA≒poly(dG)·poly(dC). From these results, it seems that Trp-P-1 interacts much more strongly with the alternative G-C sequence of DNA than the alternative A-T sequence at $r>6$. On the other hand, since poly(dA)·poly(dT), which shows no marked enlargement of the B-form at the initial interaction stage, is also transformed into the non-B structure ($[\theta]_{270nm}=-10.0\times10^4$ deg·cm^2·dmol^{-1} at r=6), this interaction is not selective only for alternative purine-pyrimidine sequences, and appears to be due to another factor which is not directly related to the B-form structure itself, because poly(dG)·poly(dC) and CT DNA structures do not exhibit any transformation to the non-B structure.

As for the interaction of Trp-P-1 with the B-form of the synthetic oligonucleotides, the variation of $[\theta]_{270nm}$ as a function of r is shown in Fig. 4. The transition of the B to the non-B conformation was observed only for d(CGCGAATTCGCG)$_2$ at $r>4.0$, but not for d(CGCGCG)$_2$ and d(CGTACG)$_2$; these hexamers caused a linear increase in the ellipticities of B-type CD spectra

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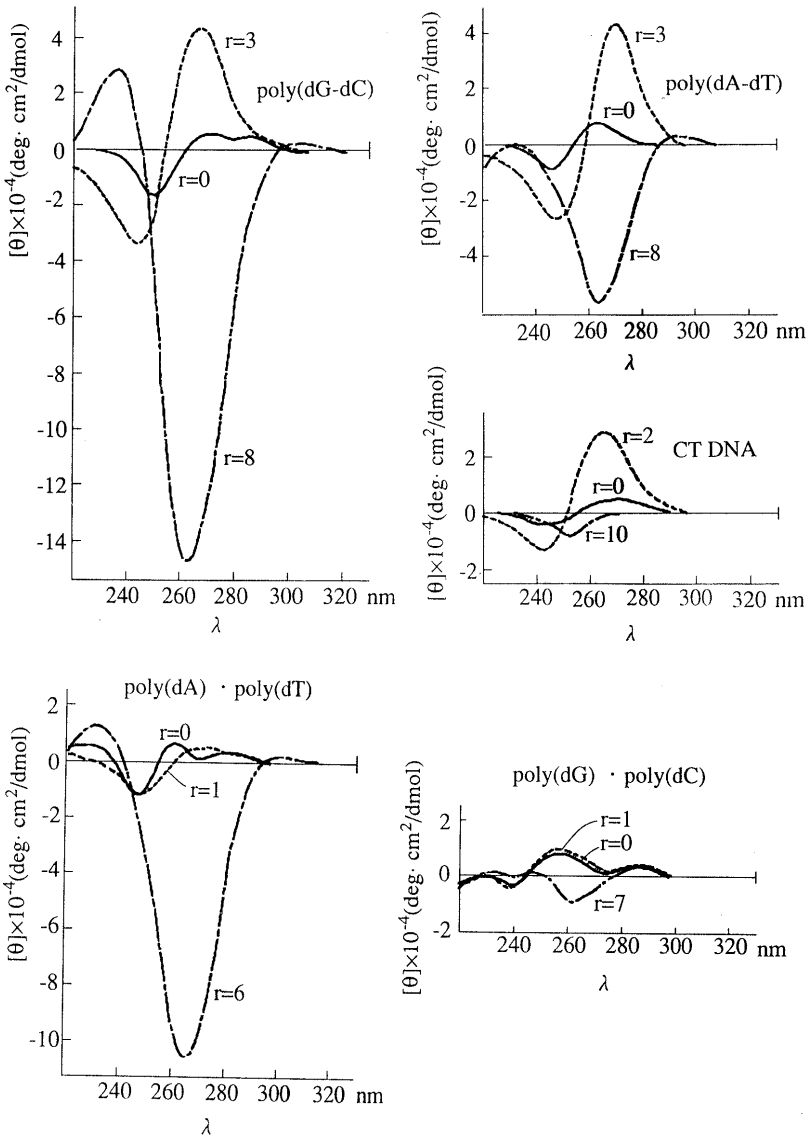

Fig. 2. CD Spectra of the B-Form of Poly(dG-dC), Poly(dA-dT), CT DNA, Poly(dA)·Poly(dT) and Poly(dG)·Poly(dC) in the Presence and Absence of Trp-P-1

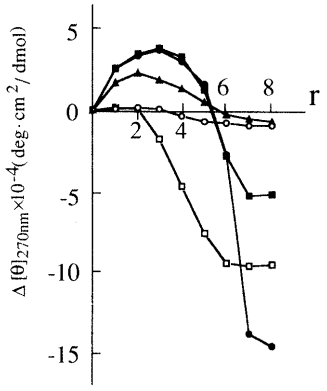

Fig. 3. Variation in $[\theta]$, as a Function of r at 270 nm, of the B-Form of Poly(dA-dT) (—■—), Poly(dG-dC) (—●—), Poly(dA)·Poly(dT) (—□—), Poly(dG)·Poly(dC) (—○—) and CT DNA (—▲—) in the Presence of Trp-P-1

Table 1. Binding Constants for the Interaction of Trp-P-1 and Trp-P-2 with the B-Form of DNA[a]

	n	$K_o \times 10^{-6}$ (M^{-1})	Correlation[b]
Trp-P-1			
Poly(dA-dT)	0.28	2.7	0.991
Poly(dG-dC)	0.29	2.5	0.988
CT DNA	0.24	1.5	0.958
Trp-P-2			
Poly(dA-dT)	0.26	2.1	0.988
Poly(dG-dC)	0.27	2.3	0.994
CT DNA	0.27	1.0	0.961

a) The values were obtained using 8 data points in the range $0.1 \leq r$ (=[Trp-P]/[DNA]) ≤ 0.3. The values for poly(dA)·poly(dT) and poly(dG)·poly(dC) were not accurate because of large fluctuations in the data (correlation coefficient < 0.7). *b*) This represents the correlation coefficient.

with increasing Trp-P-1 concentration and reached their saturated states at $r=8$. This may be interpreted as indicating that a DNA length of more than one helical turn is necessary for such a conformational transformation to the non-B structure. It is interesting to note that no conformational transition was observed for the B-type $(ATGCAT)_2$. Although further detailed analysis is necessary to account for this behavior, the sequential preference of Trp-P-1 is suggested for binding with oligonucle-

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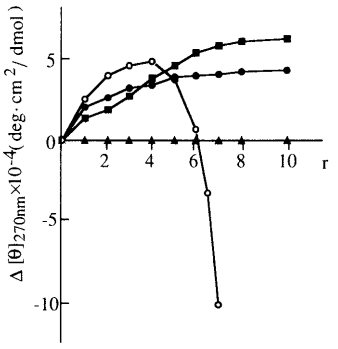

Fig. 4. Variation in $[\theta]$, as a Function of r at 270 nm, of $(CGCGCG)_2$ (—●—), $(CGTACG)_2$ (—■—), $(ATGCAT)_2$ (—▲—) and (CGCGAATTCGCG)$_2$ (—○—)

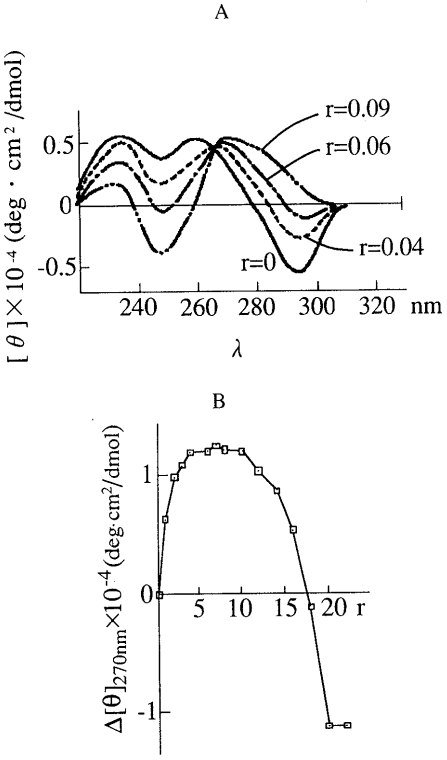

Fig. 5. CD Spectra of the Z-Form of Poly(dG-m5dC) over the Range of $0 \leq r \leq 0.09$ (A) and Variation in $[\theta]$ at 270 nm over the Range $r > 0.09$ (B)

otides.

(B) Interaction of Trp-P-1 with the Z-Form of DNA The CD spectral change in the salt-induced Z-conformation of poly(dG-m5dC) by interaction with Trp-P-1 ($0 < r \leq 0.09$) is shown in Fig. 5A, and the $[\theta]_{270nm}$ variation as a function of r($r > 0.09$) is shown in Fig. 5B. Upon addition of Trp-P-1 to the DNA solution, the CD spectrum characteristic of the Z-form of DNA was transformed into the B-form spectrum over the range $0.03 \leq r \leq 0.09$, indicating that Trp-P-1 promotes structural reversion from the Z-DNA to the B-structure. A similar conversion from the Z- to the B-form of poly(dG-m5dC) has been observed in the interaction with the intercalative EtBr[19] and benzo[*a*]phenazine derivatives.[22] After the completion of the Z→B conversion at about $r = 0.09$, the structure followed a biphasic conformational transition, *i.e.*, stiffening of the B-structure ($1 < r < 6$) and transformation

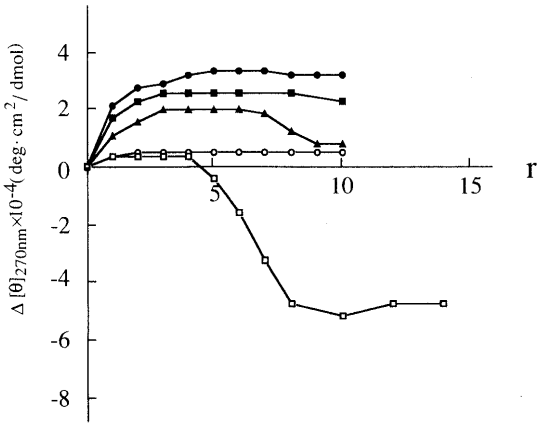

Fig. 6. Variation in $[\theta]$, as a Function of r at 270 nm, of the B-Form of Poly(dA-dT) (—■—), Poly(dG-dC) (—●—), Poly(dA)·Poly(dT) (—□—), Poly(dG)·Poly(dC) (—○—) and CT DNA (—▲—) in the Presence of Trp-P-2

to the non-B structure ($r > 10$), in a similar manner to the B-form of poly(dG-dC) and poly(dA-dT) shown in Fig. 3, and this conformational change was saturated at about $r = 20$.

(C) Comparison of Trp-P-2 with Trp-P-1 in Their Interaction with DNA The CD spectral changes of the B-form of DNA by Trp-P-2 differed from that of Trp-P-1. The $[\theta]$ variation at 270 nm, as a function of r, is shown in Fig. 6. Since Trp-P-2 showed similar stiffening of the B-form of poly(dG-dC), poly(dA-dT) and CT DNA to that of Trp-P-1 in the range $0 < r < 3$, the binding mode at the initial interaction stage is the same. However, the most significant difference between them is that Trp-P-2 does not cause transformation of the B-form of DNA to the non-B structure, while such a transformation could be characterized as behavior particular to Trp-P-1. A similar tendency was also observed for the synthetic oligonucleotides; no significant transformation to the non-B structure occurred in the interaction of Trp-P-2 with d(CGCGAATTCGCG)$_2$ duplex, while the stiffening of its B-form, as well as those of d(CGTACG)$_2$ and d(CGCGCG)$_2$, was induced. The CD spectral change of the Z-form of poly(dG-m5dC) duplex by Trp-P-2 is shown in Fig. 7. As judged from the comparison with Fig. 5, the Z→B conversion ability demonstrated by Trp-P-2 is weaker than that of Trp-P-1; a concentration of Trp-P-2 ($r = 0.15$), about twice that of Trp-P-1 ($r = 0.09$), is required for complete reversion to the B-form. After completion of the Z→B conversion, the B-form was strengthened with increasing Trp-P-2 concentration and was saturated at $r = 8$. This pattern differs from that of Trp-P-1, where transformation to the non-B structure is a continuous one.

It is interesting to note that the above-mentioned spectral difference is primarily due to the methyl group of Trp-P-1, which is lacking in Trp-P-2 (R=H in Fig. 1). Obviously, this methyl group plays an important role in the transformation of B-DNA to the non-B structure. Since there is no notable difference between Trp-P-1 and Trp-P-2, as far as the stiffening of the B-DNA structure by the intercalation at the early binding stage is concerned, the steric interaction of the methyl group of Trp-P-1 with DNA could become a trigger for transformation into the

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non-B structure, although further detailed analysis at an atomic level is necessary to account for this behavior.

It has been reported that the mutagenic activity of Trp-P-2 towards *Salmonella typhimurium* TA98, in the presence of rat liver microsomes, is about 2.5 times greater than that of Trp-P-1.[23] Although the minimum concentration of Trp-P for exhibiting activity probably corresponds to $r<1$ in the present study, a local situation involving a high r value would be possible. Provided that the adduct formation with the guanine base of DNA, Gua-Trp-P, is the main factor accounting for the mutagenic activity, the present spectral results indicate that (i) intercalation of Trp-P into a DNA base pair is a suitable environment for formation of the DNA adduct, where the C8 atom of the guanine base, which is the most reactive carbon atom among the four different bases, is consequently attacked by *O*-acetylated *N*-hydroxy-Trp-P (active form of Trp-P) to form a covalent bond, and (ii) transformation of the B-form of DNA to the non-B structure, probably caused by the steric hindrance between the Trp-P-1 methyl group and DNA in the intercalation state, is not suitable for such adduct formation, consequently leading to the weaker mutagenic activity of Trp-P-1 than Trp-P-2; in the case of Trp-P-2, the intercalation state is stably maintained regardless of the increase in concentration.

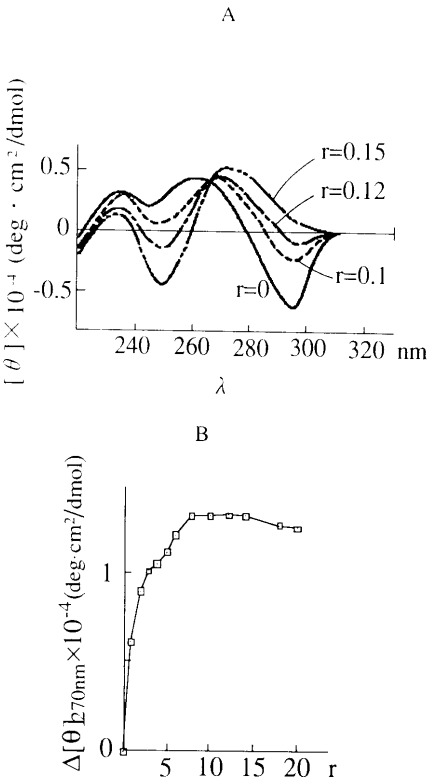

Fig. 7. CD Spectra of the Z-Form of Poly(dG-m5dC) over the Range $0\leq r\leq 0.15$ (A) and Variation in $[\theta]$ at 270 nm over the Range $r\geq 0.15$ (B)

Thermal Stability of DNA due to Trp-P Figure 8 shows the UV melting curves (OD variations at 260 nm as a function of temperature) of poly(dA-dT) and poly(dA)·poly(dT) in the presence ($r=0.2$) and absence of Trp-P-1 or Trp-P-2. Respective melting temperatures (T_m) are listed in Table 2. The T_m of poly(dA-dT) increased with the concentration of Trp-P-1 and Trp-P-2 over the range $0<r<0.2$, and was saturated at $r\geq 0.2$. This saturation value of $r=0.2$ is in agreement with $n=0.28$ (per base pair) given in Table 1. There is a noteworthy difference

Table 2. Melting Temperatures (T_m) of Poly(dA-dT) and Poly(dA)·poly(dT) in the Presence and Absence of Trp-P-1 and Trp-P-2 ($r=0.2$)

	T_m (°C)	T_m (°C) +Trp-P-1	ΔT_m (°C)	T_m (°C) +Trp-P-2	ΔT_m (°C)
Poly(dA-dT)	45.4	52.2	+6.8	51.5	+6.1
Poly(dA)·poly(dT)	52.0	53.8	+1.8	52.9	+0.9

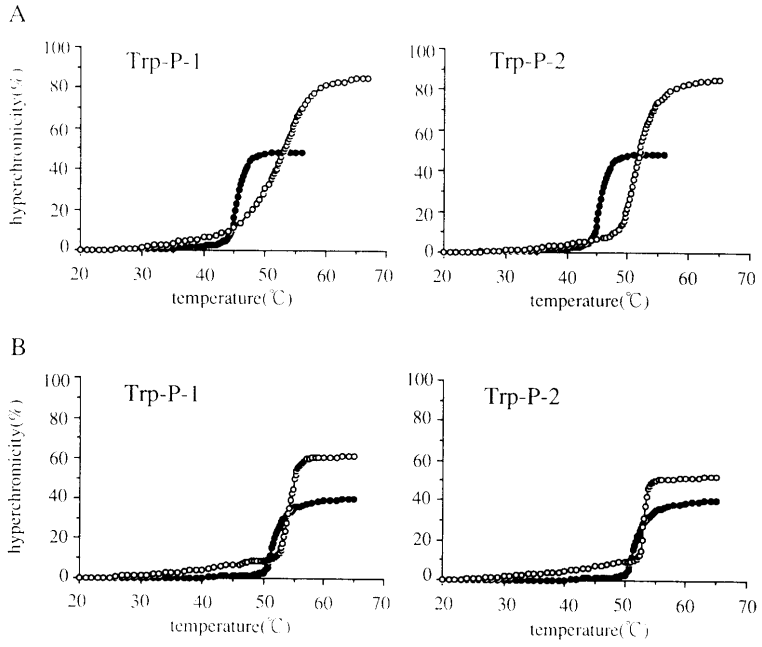

Fig. 8. UV Melting Curves of Poly(dA-dT) (A) and Poly(dA)·Poly(dT) (B) in the Presence ($r=0.2$, Open Circles) and Absence (Filled Circles) of Trp-P-1 and Trp-P-2

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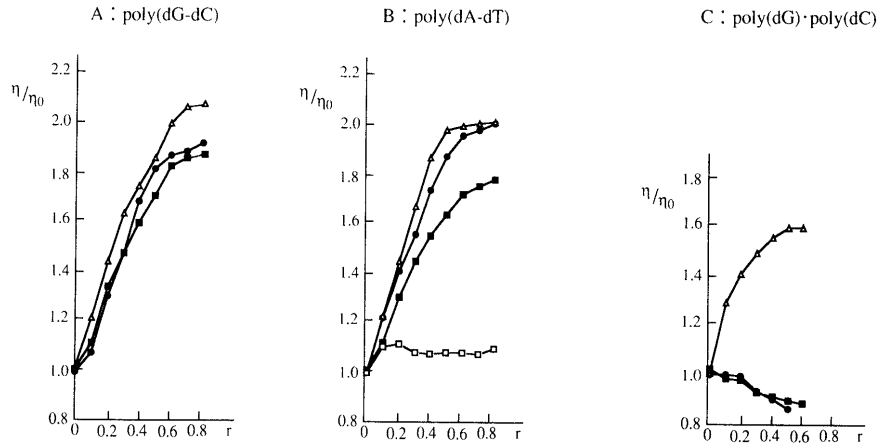

Fig. 9. Viscometric Titrations of the B-Form of Poly(dG-dC) (A), Poly(dA-dT) (B) and Poly(dG)·Poly(dC) (C) with Trp-P-1 (—●—), Trp-P-2 (—■—), EtBr (—△—) and Dist (—□—)

between Trp-P-1 and Trp-P-2 concerning the melting profile of poly(dA-dT). The melting curve of Trp-P-1 shows a transition profile (45—58 °C) with a broad and gentle slope, in contrast to the steep profile of Trp-P-2 (49—54 °C). The former phenomenon could be interpreted by different conformations between drug-bound and -unbound DNAs, which reflects the unsaturated DNA binding of the drug, as has been frequently observed in T_m experiments involving DNA.[24,25] On the other hand, the binding of Trp-P-2 to DNA could be stoichiometric without different DNA conformations. This result appears to reflect a slightly different method of intercalation for Trp-P-1 and Trp-P-2.

As far as the binding to poly(dA)·poly(dT) is concerned, no marked change was seen in the T_m value and this is in agreement with the CD results, where neither Trp-P-1 nor Trp-P-2 strengthens the B-form of poly(dA)·poly(dT). This result indicates that no intercalation occurs in these cases.

Viscometric Studies In order to clarify the mode of interaction of Trp-P with DNA, the viscosity of the B-form of DNA was studied in the presence of Trp-P, EtBr (an intercalator) and Dist (a minor groove binder specific for the A-T sequence). The viscometric titrations of these drugs for the B-form of poly(dG-dC), poly(dA-dT) and poly(dG)·poly(dC) are shown in Fig. 9. The viscosity of DNA was significantly increased by EtBr, but not by Dist. The increase in DNA viscosity by EtBr is primarily due to lengthening of the DNA structure by intercalation of the drug.[26] Generally, it is known that the increment of η/η_0 by drug binding is most significant for the B-form of DNA and is within the range of 1.9 ± 0.3 for a typical intercalator.[27-29] From the η/η_0 profiles measured as a function of r, the binding of Trp-P-1 and Trp-P-2 is clearly intercalative for poly(dA-dT) and poly(dG-dC). However, Trp-P-2 appears to interact with poly(dG-dC) preferentially rather than with poly(dA-dT), while Trp-P-1 exhibits nearly the same affinity for both DNAs. In contrast to EtBr, a nonspecific intercalator, no notable change in η/η_0 was observed for poly(dG)·poly(dC), and this is in agreement with the CD spectral findings, *i.e.*, intercalative binding of Trp-P-1 and Trp-P-2 prefers the B-form of DNA with alternating purine–pyrimidine sequences.

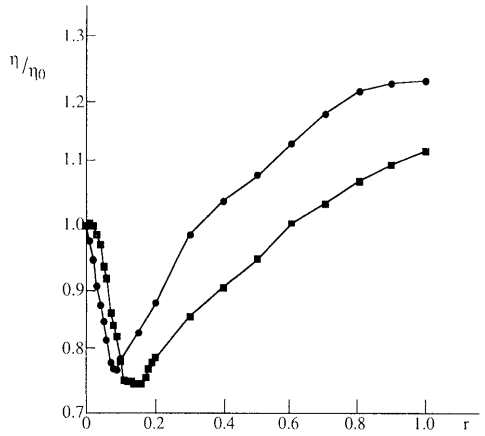

Fig. 10. Viscometric Titrations of the Z-Form of Poly(dG-m5dC) with Trp-P-1 (—●—) and Trp-P-2 (—■—)

Viscometric titrations of Trp-P-1 and Trp-P-2 for the Z-form of the poly(dG-m5dC) duplex are shown in Fig. 10. The η/η_0 value decreased with drug concentration over the range $0<r<0.09$ for Trp-P-1 and $0<r<0.15$ for Trp-P-2. From the CD spectra of the Z-form of poly(dG-m5dC) as a function of r (Figs. 5 and 7), it is reasonable to assume that this decrease corresponds to a transformation of the initial Z-form to the B-form. Beyond this concentration range, η/η_0 began to increase, and this could be interpreted as intercalation of the drug into the B-form of DNA. Viscometric observations of the interaction state of Trp-P-1 with the non-B structure was impossible because of the poor solubility of the drug.

The production of genotoxic compounds from the pyrolysates of food components has been a significant health problem. Within this context, it is important to understand the molecular basis for the alteration in gene expression. The present results have clarified the behavior of Trp-P at the initial stage in its interaction with DNA. Although Trp-P-1 and Trp-P-2 both interact intercalatively with the B-form of DNA exhibiting nearly the same preference for alternating A-T and G-T (purine-pyrimidine) sequences, there is a characteristic difference between Trp-P-1 and Trp-P-2 in terms of their ability to change from the B-form of DNA to the non-B form.

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