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Interaction of mutagenic tryptophan pyrolysate with DNA

CD spectral study on the binding specificity

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The interactions of DNA duplexes with 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), a potent mutacarcinogen isolated from tryptophan pyrolysate, have been studied using CD spectroscopy. The results are that (a) the spectral change of B-form DNA caused by the interaction with Trp-P-1 is biphasic, i.e. the enlargement of CD bands characteristic to the B-DNA conformation in the range of r ([Trp-P-1]/[DNA]) = 0–2.5, followed by the rapid transition to the non-B conformation at $r > 2.5$; (b) this transition degree of B to non-B conformation of DNA is not necessarily dependent on the G-C content; and (c) the salt-induced Z-DNA is transformed to B-DNA ($0 < r < 0.1$) and then to non-B-DNA ($r > 5$), depending on the concentration of Trp-P-1 added. These data indicate that the non-covalent interaction of Trp-P-1 with DNA is mainly dependent on the B-DNA conformation.

Trp-P-1; Tryptophan pyrolysate; DNA; Interaction; CD

1. INTRODUCTION

Trp-P and Glu-P are potent mutacarcinogens isolated from tryptophan and glutamic acid pyrolysates, respectively, and represent a class of genotoxic compounds that react with DNA and eventually form covalent adducts. Since the isolation of Trp-P-1, Trp-P-2, Glu-P-1 and Glu-P-2 (Fig. 1), various studies have been carried out with regard to their chemical, physicochemical and biological properties [1,2]. The reaction mechanism of these compounds has been established: metabolically activated *N-O*-acyl-Trp-P or *N-O*-acyl-Glu-P (the ultimate form) modifies DNA through the formation of Gua-Trp-P or Gua-Glu-P, respectively [3,4].

Concerning the DNA-binding characteristics of Glu-P, the following has been reported so far [5–7]: the interaction with DNA is intercalative and prefers the G-C-rich region of the DNA double-helical structure. In contrast, study of the interaction of Trp-P with DNA has been limited [2]. Although the overall interaction mechanism of Trp-P to DNA would be the same as that of Glu-P, detailed aspects may be different.

The elucidation of how the Trp-P interacts with DNA or how it recognizes the base sequence is an essential first step for the understanding of the molecular basis of its mutacarcinogenic activity. The present paper deals with CD spectral changes of the B- and Z-forms of DNA caused by the interaction with Trp-P-1. We have also obtained nearly the same results with regard to the DNA interaction with Trp-P-2.

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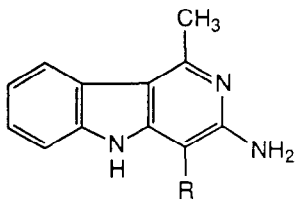
Abbreviations poly(dA-dT), alternating copolymer of poly(dA-dT)·poly(dA-dT); poly(dG-dC), alternating copolymer of poly(dG-dC)·poly(dG-dC); poly(dG-m5dC), alternating copolymer of poly(dG-m5dC)·poly(dG-m5dC); CT DNA, calf thymus deoxyribonucleic acid, CD, circular dichroism; Trp-P, 3-amino-5H-pyrido[4,3-b]indole derivative; Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole; Trp-P-2, 3-amino-1-methyl-5H-pyrido[4,3-b]indole; Glu-P, 2-aminodipyrido[1,2-a:3′,2′-d]imidazole derivative; Glu-P-1, 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole; Glu-P-2, 2-aminodipyrido[1,2-a:3′,2′-d]imidazole; *N-O*-acyl-Trp-P, *O*-acetylated *N*-hydroxy-Trp-P; *N-O*-acyl-Glu-P, *O*-acetylated *N*-hydroxy-Glu-P; Gua-Trp-P, 3-(8-guanyl)amino-Trp-P; Gua-Glu-P, 2-(8-guanyl)amino-Glu-P; HPLC, high-pressure liquid chromatography.

2. MATERIALS AND METHODS

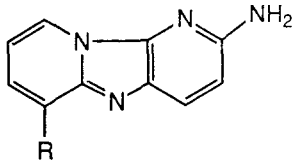
Trp-P-1 was purchased from Wako Pure Chemicals. Poly(dA-dT), poly(dG-dC), poly(dG-m5dC) and calf thymus (CT) DNA (deproteinized by phenol extraction and precipitated with ethanol) were purchased from Pharmacia and used without further purification. Oligonucleotides were synthesized according to solid-phase phosphoramidite methods and purified by reverse-phase HPLC.

The solution of 10 μM B-form DNA was adjusted in 10 mM sodium cacodylate buffer (pH 7.0). For preparation of the Z-form DNA, 50 mM NaCl and 1.25 mM $MgCl_2$ were further added to the B-form DNA solution. The DNA concentration in moles per liter was determined using the molar extinction coefficients ($M^{-1}\cdot cm^{-1}$) of ε_{260}[poly(dA-dT)] = 6700, ε_{254}[poly(dG-dC)] = 8400, ε_{260}[poly(dG-m5dC)] = 6900 and ε_{260}[CT DNA] = 6600 [8]. The molar extinction coefficients of the oligomers were calculated according to the method of Cantor and Warshaw [9]. The stock solution of 10 mM Trp-P-1 was gravimetrically prepared and added to the DNA solution for the titration experiment, so as to vary the ratio (r) of [Trp-P-1]/[DNA] from 0 to 10. CD spectra were measured with a Jasco J-20C spectropolarimeter, during which the temperature was kept at 25°C. The



Trp-P-1: R = CH_3

Trp-P-2: R = H



Glu-P-1: R = CH_3

Glu-P-2: R = H

Fig. 1. Chemical structures of Trp-P and Glu-P compounds

circular dichroic value was expressed as molar ellipticity (θ). Each CD spectrum was run at least twice and checked for possible baseline shift

The association constant (K_o) between Trp-P-1 and DNA was evaluated using the Scatchard plot [10]:

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

where C_f = concentration of unbound Trp-P-1 and n = binding stoichiometry in terms of bound Trp-P-1 per nucleotide. The value of C_f, given by the difference between the total and bound Trp-P-1 concentrations, was estimated from the fluorescence quenching data. The values of K_o and n were determined by least-squares linear regression analysis of the plot of r/C_f vs. r.

3. RESULTS AND DISCUSSION

3.1. *Binding with B-form DNA*

Fig. 2 shows CD spectra of B-form poly(dA-dT), poly(dG-dC) and CT DNA, in both the absence and presence of Trp-P-1. The θ variation at 270 nm as a function of r is shown in Fig. 3. The CD spectra of DNA changed biphasically with the concentration of Trp-P-1, i.e. the increase of the positive (~ 280 nm) and negative (~ 250 nm) ellipticities in the conservative B-DNA was Trp-P-1-dependent in the range of r = 0–2, and the transformation from B to non-B structure took place at $r > 2.5$. In the former phase, it could be interpreted that Trp-P-1 increases the rigidity of the B-conformation, because the strength of CD ellipticity is directly dependent on the extent of stacking among DNA bases. The analysis of Scatchard plots gave $n = 0.37$ and $K_o = 4.5 \times 10^6$ M^{-1} for poly(dA-dT), $n = 0.31$ and $K_o = 4.5 \times 10^6$ M^{-1} for poly(dG-dC), and $n = 0.35$ and $K_o = 3.3 \times 10^6$ M^{-1} for CT DNA. Generally, such a spectral change could be interpreted in terms of the binding of intercalation-type compounds, which leads to stiffening of the DNA duplex structure as a result of DNA lengthening [11]. When r was beyond 2.5, however, the B-form DNA was rapidly transformed into the non-B conformation, which is characterized by an intense, negative and long-wavelength Cotton band [12]. The binding mode of Trp-P-1 during this conformational transition appears to be the same in both of poly(dA-dT) and poly(dG-dC), as could be estimated from Fig. 3A. However, the degree of transition between them is significantly different. While the conformational change of poly(dA-dT) reached a saturated

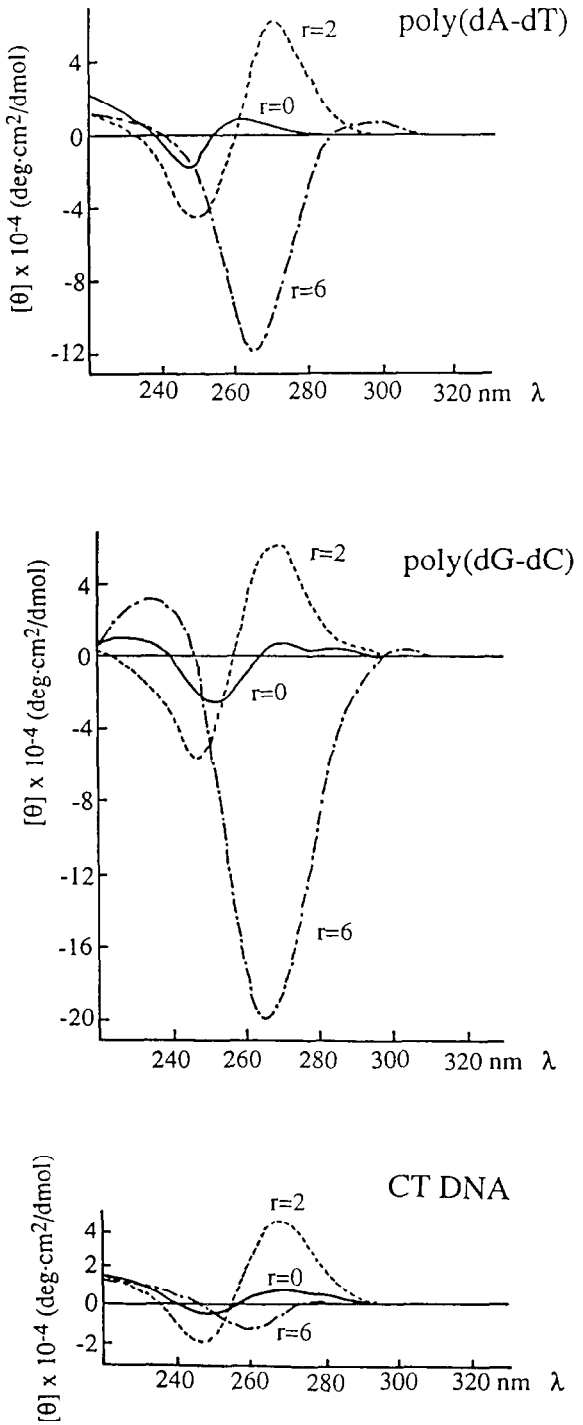


Fig. 2. CD spectra of B-form poly(dA-dT), poly(dG-dC) and CT DNA in the absence (r = 0) and presence (r = 2 and 6) of Trp-P-1.

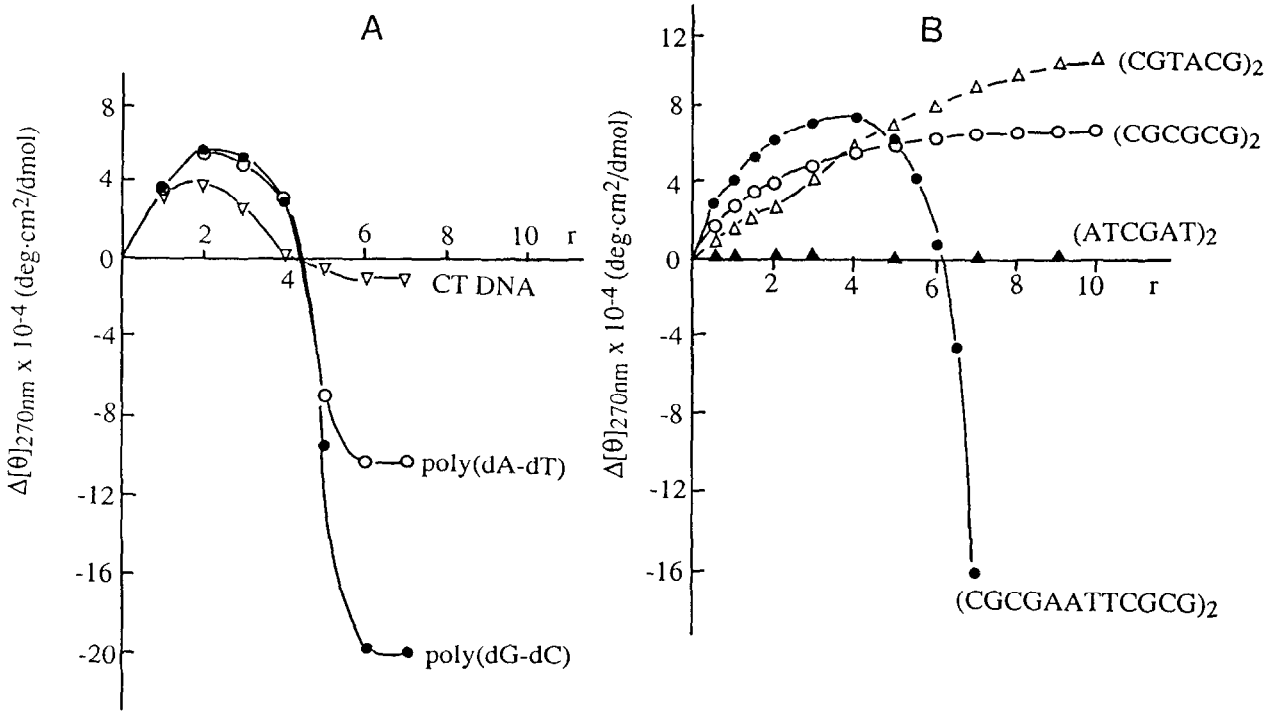


Fig. 3. Variation profiles of $[\theta]$ as a function of r at 270 nm of poly(dA-dT), poly(dG-dC) and CT DNA (A) and of $(CGCGCG)_2$, $(CGTACG)_2$, $(ATGCAT)_2$ and $(CGCGAATTCGCG)_2$ (B).

state ($\theta_{270\,nm} = -9.8 \times 10^4$ deg·cm^2·dmol^{-1}) at about $r = 6$, the poly(dG-dC) was transformed to the non-B conformation having the $\theta_{270\,nm}$ value of -18.8×10^4 deg·cm^{-2}·dmol^{-1} at $r = 6$. The degree of interaction of Trp-P-1 follows the order poly(dG-dC) > poly(dA-dT) > CT DNA. This indicates that although Trp-P-1 interacts much more strongly with the alternative G-C region of DNA than with the alternative A-T region, its binding is not necessarily specific for the G-C sequence, contrary to our earlier expectation.

Concerning the interactions of Trp-P-1 with the B-type oligonucleotides synthesized, the transition from B to non-B conformation was observed only for $d(CGCGAATTCGCG)_2$ at $r > 4.0$ (Fig. 3B), and not for $d(CGCGCG)_2$ and $d(CGTACG)_2$; the latter two hexamers caused a linear increase in the ellipticities of B-type CD spectra with increasing Trp-P-1 concentration and reached their saturated states at $r = 7$–10. This may be interpreted in terms of the fact that a DNA length of more than one helical turn is necessary for such a conformational transition. 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 for binding with DNA is suggested, because similar results were also obtained from the fluorescence experiment.

3.2. *Binding with left-handed Z-DNA*

The CD spectral changes in the salt-induced Z-con-

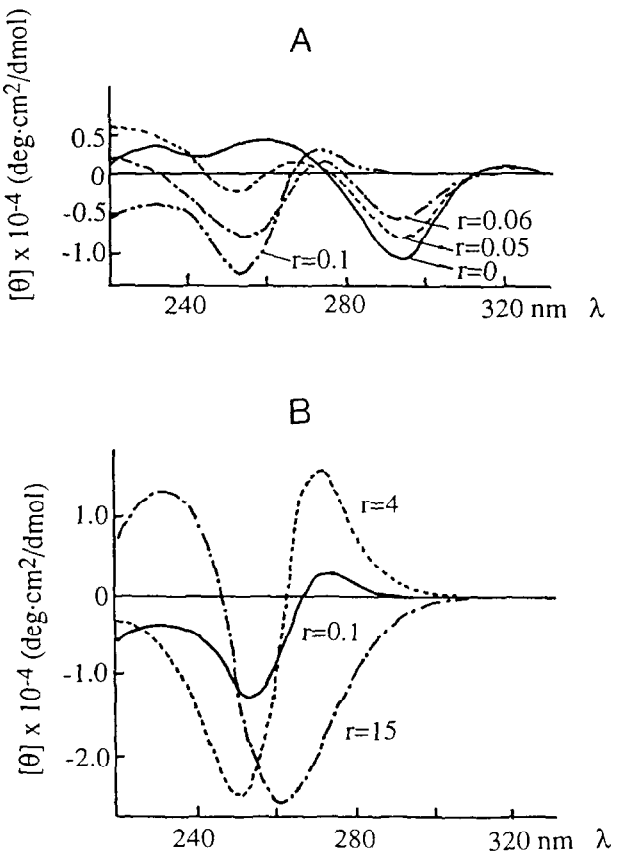


Fig. 4 CD spectra of Z-form poly(dG-m5dC) in the range of $0 \leq r \leq 0.1$ (A) and of $r \geq 0.1$ (B).

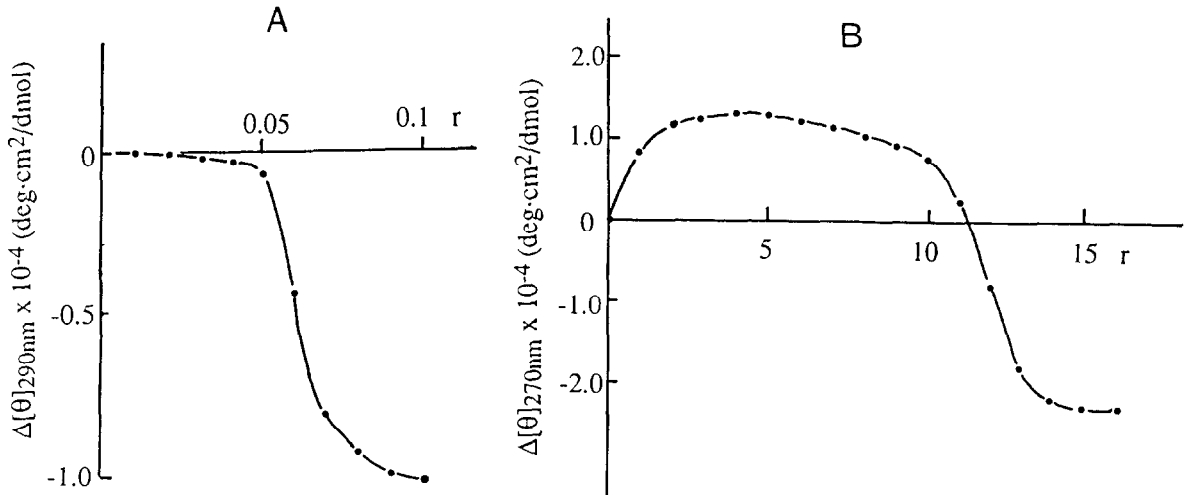


Fig. 5 Variation profiles of $[\theta]$ of poly(dG-m5dC) at 290 nm in the range of $0 \leq r \leq 0.1$ (A) and at 270 nm in the range of $r \geq 0.1$ (B).

formation of poly(dG-m5dC) by interaction with Trp-P-1 are shown in Fig. 4, and the θ variation profiles as a function of r at 290 nm (r = 0–0.1) and at 270 nm (r = 0.1–15) are shown in Fig. 5. Upon the addition of Trp-P-1 to the DNA solution, the CD spectrum characteristic of Z-form DNA was transformed into the B-form spectrum in the range of $0.03 \leq r \leq 0.1$, indicating that the Trp-P-1 promotes the reversion of the structure of Z-DNA to B-DNA. The binding constant of this B-form was 3.3×10^6 M^{-1} ($= K_0$) with $n = 0.37$. A similar conversion form Z- to B-form of poly(dG-m5dC) has been observed in the interaction with ethidium [13]. After the completion of Z $\rightarrow$ B conversion at about $r = 0.1$, the B-DNA structure was further transformed into the non-B structure at $r > 5$ in a similar manner, as shown in Fig. 2, and this conformational change was saturated at about $r = 15$.

The production of genotoxic compounds from the pyrolysates of food components has been a significant problem for the maintenance of health. Within this context, it is important to understand their molecular basis for the alteration of gene expression. The present results clarify the reaction mechanism of Trp-P at the early stage of interaction with DNA. A binding analysis of Trp-P-2 with oligonucleotides at the atomic level is now under way.

REFERENCES

[1] Kosuge, T. and Tsuji, K (1980) Kagaku no Ryoiki Zokan 128, Tennenbustu Kagaku 80 A, pp. 71–92, Nankodo, Tokyo.
[2] Hashimoto, Y and Shudo, K. (1985) Eisei Kagaku 31, 298–311.
[3] Hashimoto, Y., Shudo, K and Okamoto, T. (1980) Biochem. Biophys. Res. Commun 96, 355 362.
[4] Hashimoto, Y., Shudo, K and Okamoto, T (1982) J Am Chem. Soc. 104, 7636-7640.
[5] Imanura, M., Takeda, K , Shudo, K., Okamoto, T , Nagata, C. and Kodama, M. (1980) Biochem. Biophys. Res. Commun. 96, 611–617.
[6] Imamura, M , Shudo, K., Okamoto, T and Andoh, T (1980) Biochem Biophys Res. Commun 97, 968–972.
[7] Hashimoto, Y and Shudo, K (1983) Biochem Biophys. Res Commun 116, 1100–1106.
[8] Wells, R D , Larson, J E., Grant, R C , Shortle, B.E. and Cantor, C R. (1970) J. Mol Biol. 54, 465–497.
[9] Cantor, C R. and Warshaw, M.M (1970) Biopolymers 9, 1059-1077.
[10] Scatchard, G (1949) Ann. NY Acad Sci. 51, 660–672
[11] Lerman, L S. (1961) J Mol. Biol. 3, 18–30
[12] Zimmer, Ch , Tymen, S , Marck, Ch and Guschlbauer, W. (1982) Nucleic Acids Res. 10, 1081 1091.
[13] Walker, G.T., Stone, M P. and Krugh, T.R (1985) Biochemistry 24, 7462–7471