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New reagent for discrimination of single- and double-stranded regions in DNA

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It was found that DNA alkylation at the N-7 guanine with the bulky alkylating reagent, *N,N,N'*-tri-(β-chloroethyl)-*N'*-(*p*-formylphenyl)propylene diamine-1,3 (TFP) is much diminished when DNA is double-stranded We report here an application of this reaction for probing the hairpin structure in the palindrome-containing single-stranded (ss) DNA fragment of 377 bases prepared from the *Eco*RI-*Bam*HI fragment of plasmid pBR322 5'-Labeled ss fragment was modified with TFP and cleaved by piperidine hydrolysis at the alkylated guanine residues according to the Maxam-Gilbert procedure Guanines in the hairpin formed by palindrome of 9 bp were protected from TFP action, while dimethyl sulfate modified all guanines

Bulky alkylating reagent, Guanine alkylation, N-7, Structure probing, hairpin

1. INTRODUCTION

Several chemical methods have been developed recently for probing the sequence-dependent structural variations in the local conformation of B-DNA [1], non-B-DNA structures [2,3], and unpaired regions in the DNA duplex [4,5]. These methods are based on employing a number of specific reagents.

Earlier, for probing unpaired regions in DNA at the sequence level, dimethyl sulfate was used [6]. The single-stranded specificity of methylation at the N-3 cytosine has been exploited. However, this method has some disadvantages. (i) Alkylating agents (including DMS) react preferentially with the N-7 guanine; the N-3 cytosine is only a minor site of methylation [7]. (ii) Additional hydrazine treatment of methylated DNA is necessary.

In this paper, to detect single-stranded regions in duplex DNA, we utilized the methylation at the N-7 guanine with the bulky alkylating reagent, *N,N,N'*-tri-(β-chloroethyl)-*N'*-(*p*-formylphenyl)propylene diamine-1,3 (TFP) [8] (fig.1A).

Because the N-7 guanine is not involved in hydrogen bond formation in a Watson-Crick duplex structure, methylation at this site by small alkylating reagent (e.g. DMS) does not depend on DNA structure. However, we have found that alkylation at the N-7 guanine by bulky alkylating reagents is diminished in double-stranded form. Here we report the application of this reaction for detection of hairpin structures in single-stranded DNA.

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2. MATERIALS AND METHODS

All the enzymes were supplied by the Nikti Bav (Berdsk, USSR) or Ferment (Vilnjus, USSR) and used as specified by the manufacturers The alkylating reagent, *N,N,N'*-tri-(β-chloroethyl)-*N'*-(*p*-formylphenyl)propylene diamine-1,3 (TFP) was synthesized in the Institute of Bioorganic Chemistry (Novosibirsk, USSR)

2.1 *DNA*

T7 DNA prepared as in [9] was provided by Dr Maksimova The plasmid pBR322 was purified as described in [10] and subjected to CsCl-ethidium bromide density centrifugation To isolate the DNA fragment containing the palindromic sequence, pBR322 DNA was digested with the restriction endonucleases, *Eco*RI and *Bam*HI, and the resulting restriction fragments were separated by electrophoresis on 5% polyacrylamide gel A 375 bp *Eco*RI-*Bam*HI fragment was recovered from the gel by electroelution

DNA strand separation and labeling of the 5'-ends of the DNA fragment with T4 polynucleotide kinase and [γ-^{32}P]ATP were performed according to [11]

2.2 *Alkylation of DNA with the bulky alkylating agent, TFP*

The alkylation was carried out in 50% methanol, 5 mM Tris-HCl, pH 7 5, at 21°C Concentration of TFP in the reaction mixture was 0 5 mg/ml To terminate the reaction, DNA was precipitated with ethanol To remove the unbound reagent, precipitation was repeated twice and the pellet was washed with ethanol The extent of reaction was determined spectrophotometrically according to [12]

3. RESULTS AND DISCUSSION

For the modification of DNA, polyfunctional nitrogen mustard TFP was used. The aliphatic (β-chloroethyl)amino group R_1-R_2 of the reagent (fig.1A) employed for the reaction is highly reactive, while reactivity of the R_3 group is strongly inhibited by the formyl residue [8,13].

Fig.1B illustrates the rates of alkylation of native and heat-denatured T7 DNA with TFP. It was found that

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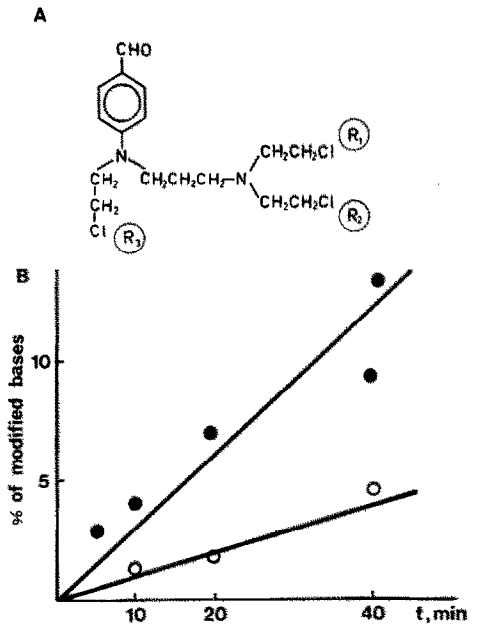

Fig.1. (A) The chemical structure of TFP. (B) The kinetics of alkylation reaction of native (○) and heat-denatured (●) T7 DNA with TFP. DNA concentration was 40–50 μg/ml.

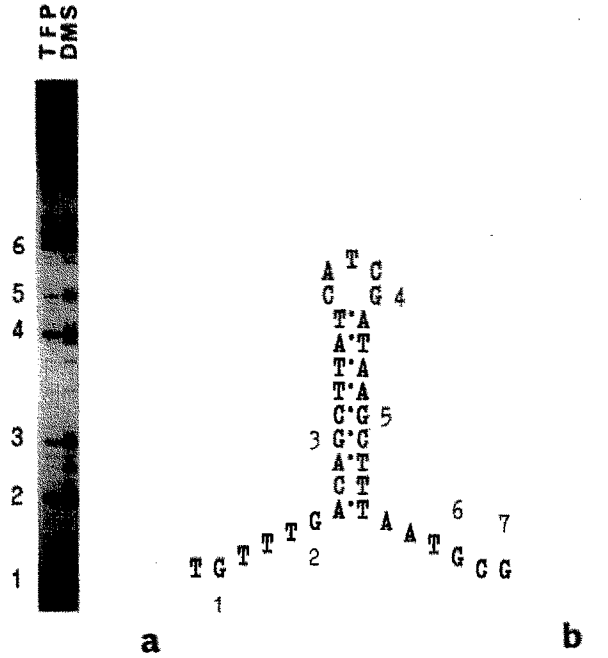

Fig.2. (a) Electrophoretic patterns of the 5′-end-labeled DNA fragments resulting from alkylation of *Eco*RI-*Bam*HI ss fragment with TFP and DMS followed by piperidine hydrolysis. DNA alkylation with TFP was carried out as described in section 2 in a reaction mixture of 50 μl for 10 min. The reaction mixture contained 0.1–0.5 μg of ss DNA. Dimethyl sulfate treatment was accomplished as in [11]. Both reactions were followed by ethanol precipitations and piperidine hydrolysis as described in [11]. (b) The secondary structure of DNA fragment is represented.

the rate of alkylation of heat-denatured DNA was considerably greater (2–2.5-fold). Therefore the reaction of alkylation of DNA was diminished when DNA was in double-stranded form. Because the major product of the DNA alkylation was N-7 alkylguanine, we proposed that this phenomenon was due to the decrease in susceptibility of the N-7 guanine to bulky reagents.

The difference in the ability of TFP to modify single- and double-stranded DNA was utilized for detection of hairpin structures in the single-stranded DNA at the sequence level. The *Eco*RI-*Bam*HI fragment of 375 bp obtained from the promoter region of pBR322 *tet* gene was analyzed. This fragment contains a quasipalindromic sequence of 9 bp close to the 5′-*Eco*RI-end. The 5′-labeled *Eco*RI-*Bam*HI fragment was heat-denatured and subjected to strand separation by electrophoresis as described [11]. The 5′-labeled single-stranded chain was alkylated with TFP. In control experiments, the DNA was modified with DMS according to Maxam and Gilbert [11]. Modified DNA was precipitated twice, and treated with piperidine to cause DNA chain cleavage at guanine residues. The resulting DNA fragments were separated on 15% polyacrylamide gel containing 8 M urea and revealed by autoradiography [11].

Fig.2a represents fragmentation patterns of *Eco*RI-*Bam*HI restriction fragment containing the palindromic sequence. As can be seen, like DMS, TFP alkylates guanine residues in DNA. However, the 3rd and 5th guanines numbered from the 5′-*Eco*RI-end were very poorly modified by TFP. Upon examination of the nucleotide sequence we found that these guanines are located within the region of quasipalindromic sequence (fig.2b). Therefore, the bulky alkylating reagent, TFP, can be used for probing of hairpin structures in single-stranded DNA. The difference in the rates of alkylation of guanines in double- and single-stranded DNA is, apparently, due to the helix geometry of double-stranded DNA. In double-stranded DNA, N-7 guanines exposed in the major groove are susceptible to alkylation with small but not bulky reagents.

We suggest that alkylation with TFP may be applied for probing at the sequence level of unpaired regions in double-stranded DNA and some other structural variations in the local conformation of DNA.

Finally, the entire procedure is simple and very similar to conventional sequencing technique.

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REFERENCES

[1] Sverdlov, E.D. and Kalinina, N.F. (1984) Dokl. Acad. Nauk. SSSR (in Russian) 274, 1508–1510.
[2] Johnston, B.H. and Rich, A. (1985) Cell 42, 713–724.

[3] Herr, W. (1985) Proc. Natl. Acad. Sci USA 82, 8009–8013
[4] Gough, G W , Sullivan, K.M and Lilley, D.M J. (1985) EMBO J 5, 191–196.
[5] Kohwi-Shigematsu, T , Manes, T and Kohwi, Y (1987) Proc Natl. Acad Sci USA 84, 2223–2227
[6] Kirkegaard, K , Buc, H., Spassky, A. and Wang, J.C. (1983) Proc Natl Acad. Sci USA 80, 2544–2547.
[7] Hoffmann, G R (1980) Mut. Res. 75, 63–109
[8] Salganik, R I , Dianov, G L., Ovchinnikova, L P , Voronina, E B , Kokoza, E B. and Mazin, A.V. (1981) Proc Natl. Acad Sci. USA 77, 2796–2800.
[9] Thomas, C.A. and Abesson, J (1966) in: Procedures in Nucleic Acids Research (Cantoni, G.L and Davis, D.R eds) pp.553–561, Academic Press, New York.
[10] Birnboim, H C and Doly, J (1979) Nucleic Acids Res. 7, 1513–1523
[11] Maxam, A and Gilbert, W (1980) Methods Enzymol 65, 499–560
[12] Dianov, G.L , Bondar, T S. and Salganik, R I (1979) Mol. Biol. (USSR) 13, 383–387.
[13] Gall, A A., Kurbatov, V A., Mustaev, A A. and Shishkin, G V (1979) Izv Sib. Otd AN SSSR, Ser Khim. Nauk 2, 99–104.