Volume 10 Number 24 1982 Nucleic Acids Research

A photoinduced cleavage of DNA useful for determining T residues

András Simoncsits[1]* and István Török[2]

[1]Institute of Genetics, and [2]Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, H-6701 Szeged, P.O.Box 521, Hungary

Received 26 October 1982; Accepted 17 November 1982

ABSTRACT

Irradiation of 5'-[^{32}P]-phosphate labeled DNA fragments with ultraviolet light in the presence of primary amines followed by piperidine treatment resulted in base-specific cleavage of the DNA chain at T residues, accompanied by a less intensive G reaction. This simple, T>G cleavage offers an alternative method for determining T residues in chemical DNA sequencing.

INTRODUCTION

The chemical DNA sequencing method described by Maxam and Gilbert (1, 2) is based on base-specific modification of end-labeled DNA followed by replacement of the modified bases and β-elimination to cleave the chain at the sites of the modified bases. The reactions, with various base selectivities, (G, A+G, A>G, A>C, C and C+T) include no specific T or T>X (where X stands for any of the other three bases) reaction. In the C reaction, incomplete supression of T reaction by NaCl, especially with "old" hydrazine (3, 4) may take place, which makes it difficult to distinguish C and T. To obtain a specific T reaction, potassium permanganate (4) and osmium tetraoxide (5) have already been tried and proved to be suitable, although uneven distribution of the radioactivity corresponding to cleavages at T residues was observed in both cases. This can be attributed to the secondary structure of the DNA and is probably the case as no strictly denaturing conditions were applied to ensure easy accessibility of these reagents to all regions of the DNA.

Recently, Saito et al. (6, 7) have shown that the photoexcited thymine residues in DNA can specifically be attacked

© IRL Press Limited, Oxford, England.

0305-1048/82/1024-7959$2.00/0

with primary amines resulting in free glycosyl units. This report describes the application of the above reaction for sequencing T residues in DNA.

MATERIALS AND METHODS

Cyclohexylamine, n-butylamine, t-butylamine (Ega-Chemie) and 2-aminoethanol (Fluka) were of reagent grade and used, without further purification as 2M stock solutions in distilled water. As an ultraviolet source a commercial germicidal lamp emitting primarily 254 nm light (Medicor, Budapest, Hungary, type BLF-12, 15 W) was used. Irradiation was performed at room temperature, at a distance of 25 cm for 2-10 min.

DNA preparation, labeling and sequencing. Plasmid DNA purification and DNA fragment isolation were performed as described elsewhere (8). The isolated DNA fragments were labeled at their 5'-ends and further cleaved with another restriction enzyme (1). The end-labeled fragments generated were separated on a 7.5% polyacrylamide gel and recovered from the gel slices by electroelution in the presence of 10 μg carrier yeast tRNA. The origin of the DNA fragments used as model compounds in this study is described elsewhere (9). The standard chemical sequencing reactions (1, 2) A>C, G, C and C+T were carried out in the presence of 10 μg sonicated calf thymus DNA.

T>G reaction. 5 μl of 5'-[^{32}P]-phosphate labeled DNA fragment containing less than 1 μg carrier nucleic acid was mixed with 5 μl of 2M cyclohexylamine on a microtiter plate (Greiner-Cooke microtest plate) at room temperature. The solution was exposed to ultraviolet light for 5 min and added to 100 μl of 0.3 M sodium acetate, pH 5.2 containing 100 μg/ml sonicated calf thymus DNA. 500 μl of 95% ethanol was then added, the mixture was chilled in a liquid nitrogen bath for 5 min and centrifuged at 15,000 g for 5 min. The pellet was washed with 1 ml of ethanol, dried and treated with 100 μl of 10% piperidine at 90°C for 30 min. After cooling, 35 μl of 3M sodium acetate, pH 5.2 was added and the DNA fragments were precipitated with 1 ml of ethanol as above. The pellet was

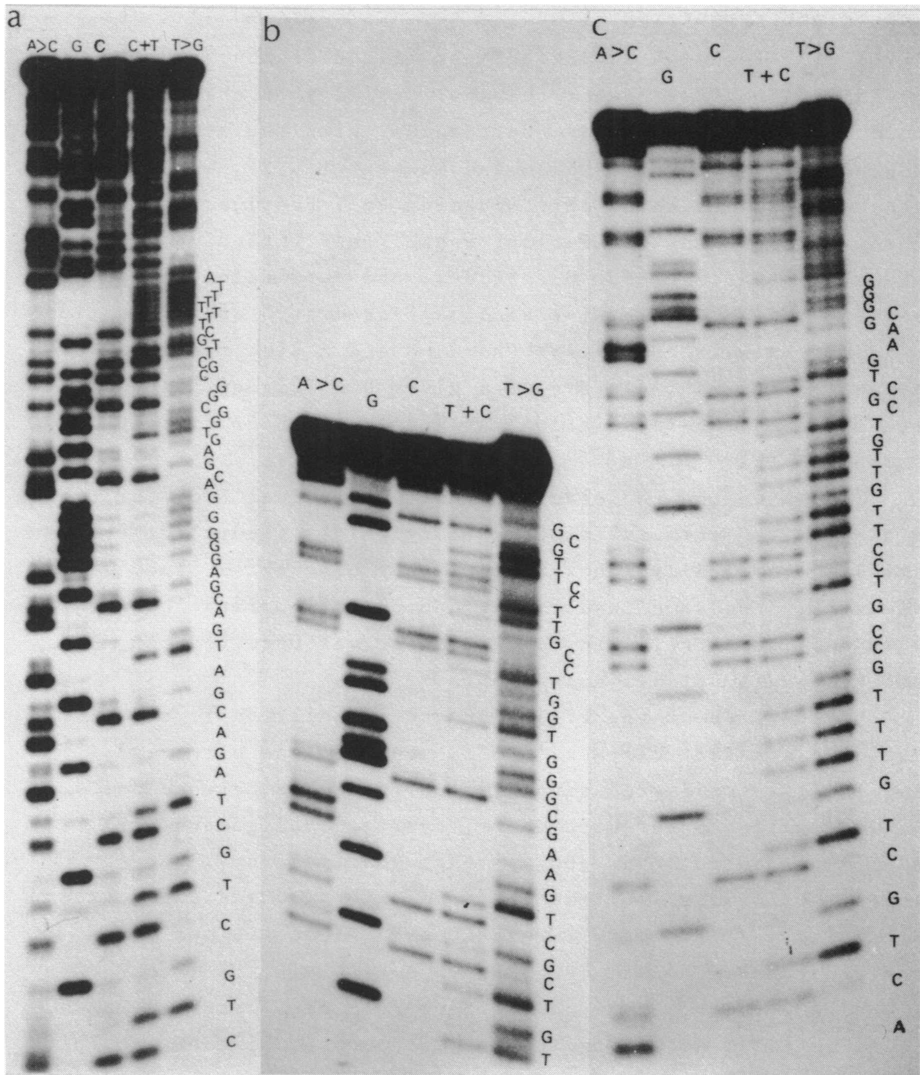

Figure 1

Autoradiographs of sequencing gels of two DNA fragments showing both the Maxam-Gilbert reactions and the photoinduced T>G reaction. A short and a longer running of the same fragment are shown in gels c and b, respectively.

washed with ethanol, dried and dissolved in formamide-dye mixture (10). Gel electrophoresis of the sample was performed as described (1, 2) on 12% polyacrylamide-7M urea gel (0.35 mm thin).

RESULTS AND DISCUSSION

Figure 1 shows autoradiographs of the sequencing gels of two DNA fragments. To test the applicability of the photoinduced T>G reaction, it was run together with the standard sequencing reactions of Maxam and Gilbert (1, 2). The strongest bands were always those corresponding to T residues and all T residues gave bands of equal strength. Less intensive bands were always obtained for G residues, and very faint, although somewhat variable intensive bands appeared at C and A positions. We do not know the nature of the G, C and A side reactions. The characteristic band pattern of a given DNA fragment was not altered, when the irradiation was performed under nitrogen atmosphere.

To remove the photoexcited thymine residues from the ribose moiety, a few primary amines (n-butylamine, t-butylamine, 2--aminoethanol and cyclohexylamine) have been tried. They gave essentially the same result in a concentration range between 0.2 - 2M. Therefore, 1M cyclohexylamine was used for convenience in almost all experiments.

Figure 2 shows the effect of the irradiation time. Keeping a constant distance (25 cm) between the ultraviolet lamp and the irradiated sample, time intervals between 2-10 min were suitable for both short, synthetic oligomers (11) and longer (up to about 200 bp long) DNA fragments. The longer the irradiation time the more extensive was the degradation. The most specific T reactions in Figure 2, and in many other experiments, were obtained at 5 min.

Unlike in the Maxam and Gilbert reactions, carrier DNA should be added only after the reaction to facilitate precipitation, otherwise it impairs the base specificity. Figure 2 (lane _e_) also shows the effect of added carrier DNA (1 µg/µl) on the irradiation reaction. To avoid the possible side reactions caused by the carrier DNA, its concentration was kept below 0.1 µg/µl during the irradiation.

The T>G reaction described here is simple and easy to perform. It has given reproducible results on many DNA fragments in our laboratory and it may be especially useful together with the methylene blue sensitized photooxidation of G residues (5)

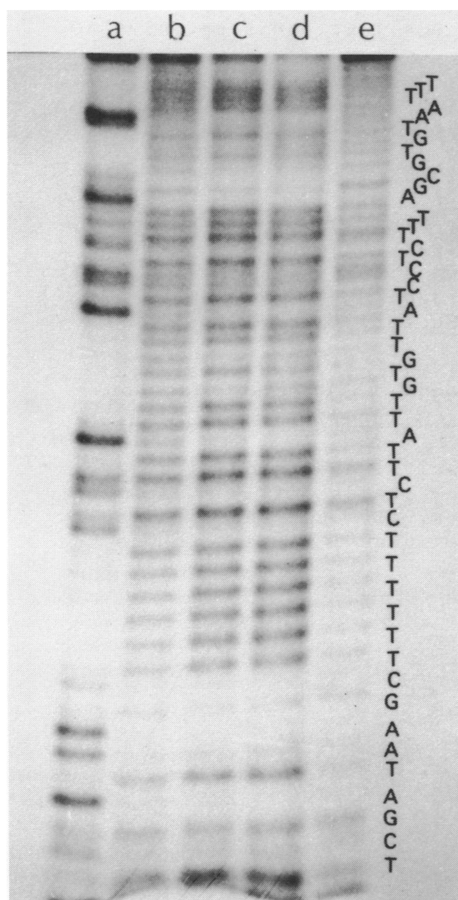

Figure 2
Effects of the irradiation time and of the added carrier DNA on the DNA degradation. To identify the sequence, only the A>C reaction (lane _a_) of a previously sequenced DNA fragment is shown. In lanes _b_, _c_ and _d_, the irradiation was performed for 2, 5 and 10 min, respectively. In lane _e_, 5 min irradiation was performed in the presence of 1 µg/µl carrier DNA.

as no hazardous chemicals are used in the photochemical reactions.

ACKNOWLEDGEMENTS

Thank is due to Dr. D.M. Brown for the careful and critical reading of the manuscript.

*Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH ENGLAND

REFERENCES

1. Maxam,A.M. and Gilbert,W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564
2. Maxam,A.M. and Gilbert,W. (1980) in Methods in Enzymology

(Grossman,L. and Moldave,K., eds.) Vol. 65, pp. 499-560, Academic Press, New York
3. Catterall,J.F., O'Malley,B.W., Robertson,M.A., Staden,R., Tanaka,Y. and Brownlee,G.G. (1978) Nature 275, 510-513
4. Rubin,C.M. and Schmid,C.W. (1980) Nucleic Acids Res. 8, 4613-4619
5. Friedmann,T. and Brown,D.M. (1978) Nucleic Acids Res. 5, 615-622
6. Saito,I., Sugiyama,H., Ito,S., Furukawa,N. and Matsuura, T. (1981) J. Am. Chem. Soc. 103, 1598-1600
7. Saito,I., Sugiyama,H., Furukawa,N. and Matsuura,T. (1981) Tetrahedron Letters 22, 3265-3268
8. Török,I. and Karch,F. (1980) Nucleic Acids Res. 8, 3105-3123
9. Mason,J.P., Török,I., Kiss,I., Karch,F. and Udvardy,A. (1982) J. Mol. Biol. 156, 21-35
10. Brownlee,G.G. and Cartwright,E.M. (1977) J. Mol. Biol. 114, 93-117
11. Simoncsits,A., unpublished results. Short, chemically synthesized oligodeoxyribonucleotides (12-14 nucleotide long) are also good substrates, although the specificity is less pronounced (T+G rather than T>G reaction was observed).