Volume 258, number 2, 274–276 FEB 07894 December 1989

Irradiation of the template with high-intensity (pulse-laser) ultraviolet light results in DNA-polymerase termination events at deoxyguanosine residues

I.G. Panyutin, O.I. Kovalsky and E.I. Budowsky

N D Zelinsky Institute of Organic Chemistry, Academy of Sciences of the USSR, Leninsky Pr 47, Moscow 117913, USSR

Received 9 September 1989, revised version received 10 October 1989

During primer elongation by *Escherichia coli* DNA-polymerase I large fragments on the template were irradiated with UV laser pulses at an intensity $\geqslant 10^{10}$ W/m^2 In addition to the termination events at photoproducts typical of low-intensity UV irradiation, termination is observed before deoxyguanosine residues The effect of the UV light intensity on the ratio of termination efficiencies before dPy and dG suggests that the termination of polymerization before deoxyguanosine residues results from the formation of photoproducts yielded by two-quantum reactions The results obtained herein, together with data published previously, imply that photomodification of dG residues is the major two-quantum reaction under the action of high-intensity UV radiation on DNA

Pulse ultraviolet laser, DNA two-quantum lesion, DNA-polymerase termination

1. INTRODUCTION

The action of UV laser radiation of an intensity higher than 10^{10} W/m^2 on DNA components permits two-quantum excitation of bases [1]. Therefore, apart from the usual single-quantum photoproducts of low-intensity UV radiation (mainly pyrimidine dimers of different types [2–4]), modifications of nucleoside residues take place due to two-quantum excitations [5]. The known two-quantum modifications in DNAs include direct single-strand nicks [6,7] and alkali-sensitive lesions of dG residues [5,8], the latter occurring at higher efficiency than the former [8]. The question about two-quantum modification of other types of nucleoside residues in DNA still remains open, although monomeric components of nucleic acids of all kinds in aqueous solutions undergo two-quantum transformations with comparable efficiency [9].

Many modifications of the template DNA residues result in the termination of primer elongation by various polymerases exactly before the modified residue [10]. Therefore the enzymatic elongation of a primer provides a sensitive tool for investigation of modifications in DNA. In this work we have used this approach to study what type of residues are modified in DNA exposed to high-intensity UV radiation. Single-stranded DNA of phase M13 and the Klenow fragment of *E.coli* DNA polymerase I were used as a standard system.

Correspondence address. I G. Panyutin, N D Zelinsky Institute of Organic Chemistry, Academy of Sciences of the USSR, Leninsky Pr 47, Moscow 117913, USSR

2. MATERIALS AND METHODS

DNA polymerase I (Klenow fragment), phage M13mp10 DNA, 17-member primer complementary to the residue 6273-6257 of this DNA and dideoxynucleoside triphosphates were taken from the 'Gene I' sequencing kit (Vector Scientific Productions, USSR) DNA sequencing was performed according to the supplier's instructions Experiments on an irradiated template were done with primer labeled at the 5'-end with polynucleotide kinase (Ferment Scientific Productions, USSR) and [γ^{32}P]ATP (Isotope, USSR). Primer (approx. 1 pM) was annealed on phage M13 DNA (1 μg) by heating at 55°C for 1 min in 20 μl of a buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 10 mM $MgCl_2$, 0 2 mM EDTA and gradually cooled (approx. 30 min) down to room temperature

Irradiation of these samples was carried out in a quartz cell, the optical density of the irradiated layer at 266 nm being approx 0 2 optical unit The source of low-intensity UV radiation was a low-pressure mercury lamp, that of high-intensity was the fourth harmonic (266 nm) of YAG:Nd^{3+} lasers with a pulse duration of 10 ns (nanosecond irradiation) or 30 ps (picosecond irradiation)

Irradiated samples were supplemented with dithiotreitol up to 1 mM, with deoxynucleoside triphosphates (250 nM each), and with 5 units of the Klenow fragment of DNA polymerase I The reaction was carried out in a volume of 25 μl at 37°C for 30 min and quenched by adding the equal volume of phenol/chloroform (1.1) mixture The aqueous phase was desalted by gel filtration through microcolumns with Sephadex G-50 equilibrated against water [11] and, on lyophilization, dissolved in 5 μl of formamide with dyes Before being loaded on a gel, samples were heated at 100°C for 1 min Samples were analyzed on a denaturing polyacrylamide gel. Part of irradiated and mock samples were electrophoresed in a 1% agarose gel, whereupon gel was stained with ethidium bromide and photographed with a red filter

3. RESULTS AND DISCUSSION

Mapping of the polymerase termination sites on an irradiated template is illustrated by fig.1. Standard

Published by Elsevier Science Publishers B V (Biomedical Division)
00145793/89/$3 50

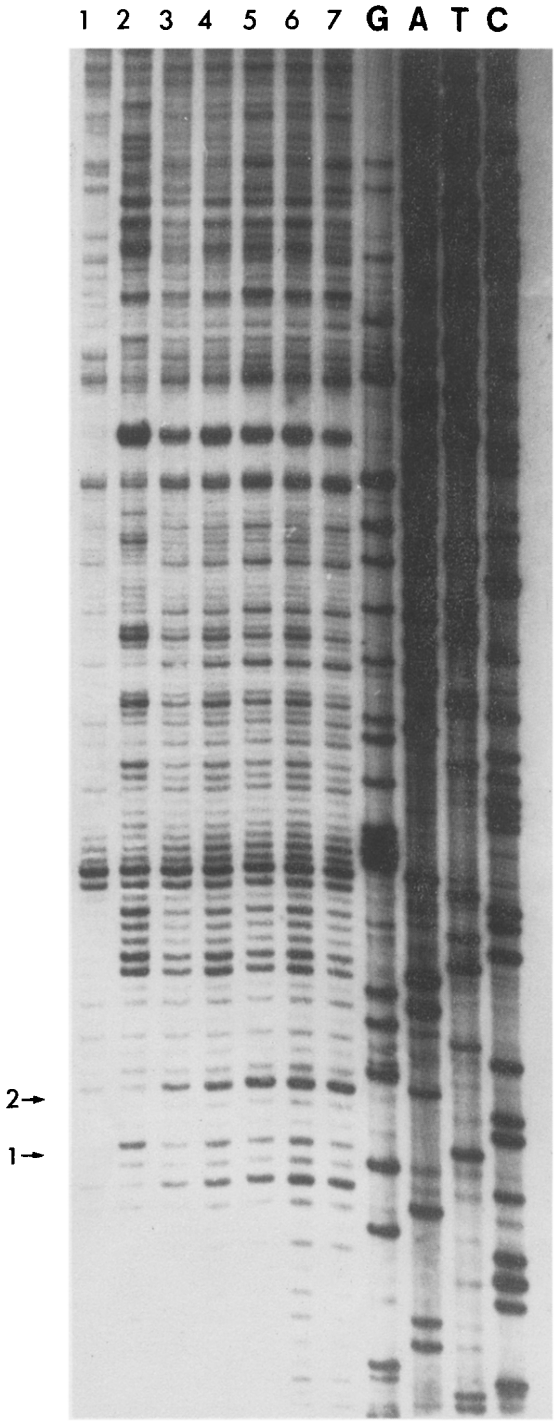

Fig 1 DNA polymerase termination events on an M13mp10 template (1) Unirradiated template (2–7) Template irradiated with intensity (I) and dose (D), (2) low-intensity UV, I = 33 W/m^2, D = 1000 J/m^2, (3) 1 laser pulse, I = 10^{10} W/m^2, D = 50 J/m^2, (4) 4 laser pulses, I = 10^{10} W/m^2, D = 200 J/m^2, (5) 1 laser pulse I = 10^{12} W/m^2, D = 50 J/m^2; (6) 7 laser pulses, I = 10^{12} W/m^2, D = 350 J/m^2; (7) 1 laser pulse, I = 2 10^{12} W/m^2, D = 100 J/m^2. (G,A,T,C) Dideoxy sequencing reactions

lanes G, A, T, and C, obtained by the dideoxy sequencing [12] of this DNA fragment, were used as reference. In the unirradiated sample (lane 1), pronounced termination is observed at the stretch of four Gs, and minor (background) terminations are observed at the sites of accidental template damage. Interestingly, these sites often coincide with deoxyguanosine residues.

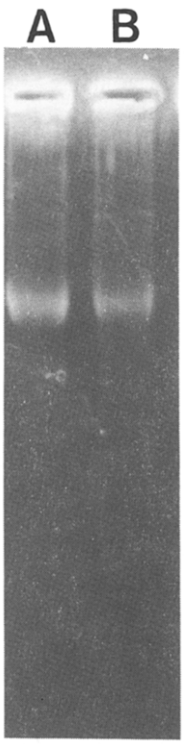

Fig 2 Electrophoresis M13mp10 in 1% agarose gel (A) Unirradiated; (B) irradiated with 10 laser pulses, I = 10^{12} W/m^2, D = 500 J/m^2.

In the sample exposed to low-intensity UV radiation (lane 2), terminations additional to those in mock experiments are observed at pyrimidines, chiefly before TT, TC and CC sequences, which is associated with the formation of cyclobutane and non-cyclobutane pyrimidine dimers [13]. In the samples exposed to UV laser radiation (lanes 3 through 7), additional intense bands arise which correspond to terminations before all deoxyguanosine residues. The intensity of these bands varies according to the primary-structure context, but it is in all cases much higher than that of the corresponding bands in the unirradiated and low-intensity UV-irradiated samples.

The intensities of the bands corresponding to terminations before single-quantum products and before deoxyguanosine residues are approximately equal at a radiation intensity of 10^{10} W/m^2 (lanes 3,4). When the radiation intensity is raised up to 10^{12} W/m^2, the probability of termination before dG becomes much higher than that before TC, especially when irradiation is with a single pulse (lanes 4 and 7, shown by arrows). This reflects the two-quantum mechanism of dG modifications causing polymerase termination at deoxyguanosines.

The reason for polymerase termination may be either modification of the residue or a nick within the DNA template. To rule out the latter possibility, we electrophoresed irradiated and unirradiated phage M13 DNA through a 1% agarose gel after preheating at 100°C for 1 min (fig.2). It is seen that the intensity of the native-molecule band has decreased insignificantly after irradiation. This means that there is much less than one nick per DNA molecule. On the other hand, it is easy to reckon [2] that irradiation of phage M13 DNA (7250 bases long) with UV light at a dose of 300 J/m^2 produces about 20 cyclobutane thymine dimers per molecule. Since the band intensities, proportional to the extent of the respective residue damages, are at least comparable for TT dimers and deoxyguanosines, it follows that the overwhelming majority of termination before dGs are due to these residues' modification rather than nicking.

Thus, the main irreversible two-quantum reaction within DNA exposed to high-intensity UV laser radiation, which causes DNA polymerase I termination, is modification of deoxyguanosine residues. On the other hand, two-quantum processes induced by the action of UV laser radiation on DNA contribute to hot piperidine cleavage of the polynucleotide chain at deoxyguanosine residues, too. Since both approaches, based on the different properties of modified residues, yield similar results, one can suppose that the most efficient two-quantum reaction accompanying the action of UV laser radiation on DNA is modification of a deoxyguanosine residue.

Any DNA modification which causes termination of replication may entail mutations. Therefore, it is obvious that the distribution of mutations over the genome after the action of laser radiation must be different from that caused by the action of usual UV radiation, when the major DNA damages are pyrimidine lesions.

REFERENCES

[1] Rubin, L.B., Menshonkova, T N., Simukova, N A and Budowsky, E.I. (1981) Photochem. Photobiol. 34, 339-344
[2] Patrick, M.H and Rahn, R.O. (1976) in: Photochemistry and photobiology of Nucleic Acids, vol. 2 (Wang, S Y ed.) pp 35-95, Academic Press, New York.
[3] Lipke, J A , Gordon, L.K., Brash, D.E. and Haseltine, W A (1981) Proc Natl Acad. Sci. USA 78, 3388-3392
[4] Gordon, L.K. and Haseltine, W.A. (1982) Rad Res 89, 99-112.
[5] Budowsky, E.I., Kovalsky, O.I., Yakovlev, D Yu , Simukova, N A. and Rubin, L.B. (1985) FEBS Lett 188, 155-158.
[6] Zavilgelsky, G.B., Gurzadyan, G.G. and Nikogosyan, N.D (1984) Photobiochem. Photobiophys. 8, 175-187
[7] Croke, D.T., Blau, W., Oh Uigin, C., Kelly, J M and McConnel, D.J. (1988) Photochem. Photobiol 47, 527-536
[8] Kovalsky, O.I., Panyutin, I G. and Budowsky, E I , Photochem. and Photobiol., in press.
[9] Nikogosyan, D.N. and Letokhov, V S. (1983) Riv. Nuovo Cimento, 6, ser 3, no. 8.
[10] Strauss, B , Larson, K., Rabkin, S., Sagner, D. and Sahm, J (1986) in: Role of Cyclic Nucleic Acid Adducts in Carcinogenesis and Mutagenesis, Proc. Meet., Lyon, 17-19 Sept , 1984; pp. 387-392.
[11] Maniatis, T., Fritsch, E.E. and Sambrook, J. (1982) Molecular Cloning A Laboratory Manual, Cold Spring Harbor Lab
[12] Sanger, R., Nicklen, S. and Coulson, A.R (1977) Proc Natl Acad Sci. USA 74, 5463-5467
[13] Chen, G.L., Doetch, P W. and Haseltine, W A (1985) Biochemistry 24, 5723-5728