Footprinting of DNA secondary structure by high-intensity (laser) ultraviolet irradiation

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The action of high-intensity ultraviolet pulse laser radiation on a 161 bp fragment of pBR 322 DNA (*Eco*RI-*Msp*I fragment) was studied. At doses up to 5×10^{18} photons/cm^2 the *N*-glycosidic bond splitting is negligible. The action of hot piperidine on irradiated DNA leads to chain splitting at the residues, modified via biphotonic processes. The modification and, hence, splitting efficiences depend on the type of base (G > T > A > C) and on its position in the sequence. Preferentially modified bases in the opposite strands of double-stranded DNA belong, mainly, to the same or adjacent base pairs. Residues in the Pribnow box are modified considerably less, than in the sequences, immediately upstream and downstream. This approach seems to be useful in footprinting of DNA secondary structure peculiarities and alterations, conjugated with the functional role and state of the respective fragment.

Footprinting *DNA secondary structure* *DNA splitting* *Laser ultraviolet irradiation* *Plasmid pBR 322*

1. INTRODUCTION

The secondary structure of double-stranded DNA is non-homogeneous along the chain and is determined by the primary structure of the respective regions [1]. Binding of low-M_r ligands and proteins leads to local alterations of the DNA secondary structure [2]. In turn, local peculiarities of the DNA secondary structure can determine the sites for specific binding of ligands, regulatory proteins, etc. [3]. These peculiarities should affect the mutual disposition of the neighbouring bases and, hence, rates of their modification by the action of chemical agents and ultraviolet (UV) radiation.

Recently, it has been shown that the efficiency of pyrimidine dimerisation by the action of low-intensity ($\sim 10^{15}$ photons/cm^2 per s) UV irradiation on DNA significantly depends on their location in the chain, but does not directly correlate with the nature of the neighbouring bases [4]. A similar approach was used for determination of the DNA residues involved in interactions with RNA polymerase (photofootprinting) [5]. The action of H_2O_2 and $NaBH_4$ also results in non-uniform modification of pyrimidines in double-stranded DNA [6].

The advantage of the action of low-intensity UV irradiation, and of most of the chemical agents specific to certain nucleic bases, is a disadvantage in the use of these actions for the investigation of higher structures of polynucleotides and nucleoproteins, since it only allows investigation of the peculiarities of the respective bases environment. More complete information can give the action of high-intensity (laser) UV-irradiation, which leads to modification of nucleic bases of any type [7], as well as to the splitting of *N*-glycosidic bonds of any nucleoside residue [8]. The efficiency of nucleic base modification by the action of either chemical agents or of low-intensity UV irradiation depends on the base accessibility and its reactivity in the ground and lower excited states, respectively. Laser-induced conversions of nucleic acid com-

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ponents take place via higher excited states, populated by consecutive absorption of 2 photons [7,9]. In this case the efficiency of the photoconversion also depends on the probability of absorption of the second photon, electronic structure and lifetime of the higher excited state. Therefore the efficiency of biphotonic conversions of nucleic acid residues should be more sensitive to the base environment (i.e. to the local peculiarities of polynucleotide higher structure), than that induced by the action of either chemical agents or low-intensity UV radiation. The powerful lasers available now allow one to obtain the extent of modification necessary for analysis with a single pulse, the duration of which, depending on the laser type, is as short as 10^{-8}–10^{-13} s. The lifetime of the higher excited state(s) of nucleic bases in liquid aqueous solution is less than that of the S_1 state (10^{-11} s). Therefore direct photoconversions of nucleic acid residues via higher excited state(s), induced by a single laser pulse, should be complete even before local DNA structure perturbations, caused by direct or indirect photomodification of residues, can take place.

Based on these considerations we have studied the relative efficiency of laser-induced nucleoside residue modification in the fragment of plasmid pBR 322 DNA.

2. MATERIALS AND METHODS

The double-stranded DNA fragment was obtained by the action of restriction endonucleases *Eco*RI and *Msp*I on the plasmid pBR 322 DNA. Fragment 1–161, 5′-^{32}P-labelled in the non-transcribed strand by means of polynucleotide kinase and [γ-^{32}P]ATP, or 3′-^{32}P-labelled in the transcribed strand by means of the Klenow DNA polymerase fragment and [α-^{32}P]TTP (5′-P* and 3′-P*, respectively), was irradiated in 0.1 M NaCl solution. The absorbance of the irradiated layer at 266 nm was less than 0.1. As UV radiation sources we used a low-pressure mercury lamp (254 nm, incident light intensity $\sim 10^{15}$ photons/cm^2 per s) and an Nd: YAG laser (fourth harmonic, 266 nm, pulse duration 10 ns, light intensities given in the legend to fig.1).

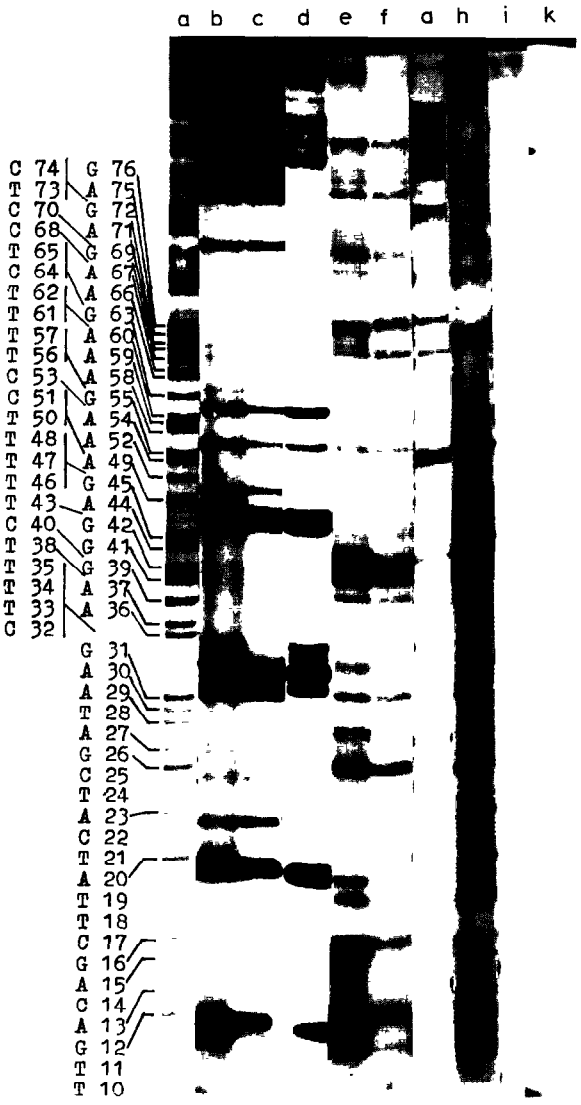
The irradiated fragment was incubated either in hot 1 M piperidine (90°C, 30 min [10] or with T4-endonuclease V, kindly donated by M.L. Becker (Leningrad Institute of Nuclear Physics), under standard conditions [11]. The obtained oligonucleotide mixtures were separated by electrophoresis in a 10% sequencing polyacrylamide gel, according to [10]. The location of the split residues in the fragment was performed by comparison of the respective oligonucleotide position with A + G and C + T 'ladders'.

3. RESULTS AND DISCUSSION

The action of low-intensity UV radiation on DNA results mainly in formation of pyrimidine dimers [12,13]. T4-endonuclease V splits the DNA chain at the pyrimidine dimers and apurinic-apyrimidinic sites [11]. The action of this enzyme on the 5′-P* fragment after low-intensity UV irradiation results in splitting of internucleotide linkages only at the sites where pyrimidine dimers can be formed (fig.1d). The extent of enzymatic splitting and, hence, of pyrimidine dimerization in this fragment is non-uniform along the chain (cf. [4].

The location and relative intensities of the bands, formed by electrophoresis of the UV–endonuclease-treated 5′-P* fragment, are similar after either low-intensity or laser irradiation (the same is true for the 3′-P* fragment) and are not changed by the 50-fold increase in laser radiation dose (fig.1b–d). Transition from low-intensity to laser UV radiation leads only to an increase of the relative intensity of the bands, corresponding to 3 TC sequences (21–22, 24–25 and 50–51) within the studied region of the 5′-P* fragment. The absence of bands, coinciding with purines and Pu-Py-Pu sequences in the 5′-P* fragment (fig.1b,c), as well as in the 3′-P* fragment (not shown), indicates, that the efficiency of *N*-glycoside bond splitting in DNA, caused by laser irradiation, is much less than that of thymine dimerisation.

UV irradiation of DNA can result in the appearance of 3 types of lesions, leading to splitting of internucleotide linkages by treatment with alkali: (a) non-cyclobutane TC dimer [4]; (b) apurinic-apyrimidinic sites, due to direct UV-induced splitting of an *N*-glycoside bond [8]; (c) modified nucleoside residues with an alkali-labile *N*-glycoside bond. Among these types of lesions only the first appears on low-intensity irradiation



of DNA. The quantum yield of non-cyclobutane dimer formation is much less than that of cyclobutane dimers [4,12]. Therefore even at the incident light dose 10^{19} photons/cm^2 of low-intensity radiation the following treatment with hot piperidine does not practically induce the chain splitting (fig.1g).

As shown by the action of UV-endonuclease (see above), either low-intensity or laser irradiation did not lead to the appearance of considerable amounts of apurinic-apyrimidinic sites in the DNA fragment. But heating with 1 M piperidine of the laser-irradiated fragments leads to the appearance of a limited number of discrete bands after electrophoretic separation (fig. 1e,f). This demonstrates specific splitting of the polynucleotide chain at definite sites, most of which do not coincide with the TC sequences. It is obvious that such splitting of DNA after laser irradiation is determined by the formation (most probably, via higher excited states) of considerable amounts of lesions of the third type.

The sites of internucleotide linkage splitting by treatment with hot piperidine of laser-irradiated 5′-P* fragment, coincide with the positions of all 4 bases in the primary structure (fig.1e,f). This reveals that all 4 types of bases undergo biphotonic modifications, leading to a decrease of *N*-glycoside bond stability in hot alkali, but the efficiency of such modifications depends on the type of base; G>T>A> C (fig.1e,f). The modification efficiency depends not only on the base type, but also, on its position in the 5′-P* fragment (fig.1e,g, cf. G41 and G42; G69 and G72; A15 and A20; A23

Fig.1. Electrophoretic separation in 10% polyacrylamide gel of oligonucleotide mixture, formed after UV irradiation of 5-labelled 1–161 fragment (*Eco*RI-*Msp*I) of plasmid pBR 322 by the following treatment with UV-endonuclease or with hot 1 M piperidine; a, distribution of purines in the fragment (A + G 'ladder').

Treatments	'Ladders'								
	b	c	d	e	f	g	h	i	k
Piperidine	–	–	–	+	+	+	–	+	–
UV-endonuclease	+	+	+	–	–	–	–	–	+
Incident light intensity ($\times 10^{25}$, photons/cm^2 per s)	1.0	1.0	10^{-10}	1.0	0.1	10^{-10}	1.0	–	–
Incident dose ($\times 10^{17}$, photons/cm^2)	50	1.0	100	50	40	100	100	–	–

and A27; T33 and T34; C17 and C22, etc.). By the intensity decrease from 10^{25} to 3×10^{24} photons/cm^2 per s the general patterns of the 5′-P* fragment splitting remain similar, although splitting at T28 and T33 significantly decreases (cf. fig. 1e and f). The presence of β-mercaptoethanol (final concentration up to 10 mM) does not influence the splitting pattern (not shown).

As shown by the action of low-intensity UV radiation [4] and hydrogen peroxide or sodium borohydride [6], the modification efficiency does not directly correlate with the type (nature) of the neighbouring bases on laser irradiation. Hence, such non-uniformity in modification efficiency reflects, most probably, some local peculiarities in the DNA secondary structure. It is worth mentioning that laser irradiation of fragment 1–161 modifies the Pribnow box much less than the adjoining sites. In this case only the 5′-terminal thymine of the Pribnow box is modified (T33, fig.1e), in contrast to the lac UV-5 promoter, in which the central thymine is mainly modified by the action of H_2O_2 and $NaBH_4$ [6].

In the opposite (transcribed) strand, as revealed by using the 3′-P* fragment, efficient splitting occurs at Gs 5,17,22,25,32,40,51,64 and 71 and Cs 12 and 26 (the numbering reflects base pair numbers in the non-transcribed strand). At several other Gs (53,68 and 70), C41 and all Ts (20,23,29,36,37 and 58) splitting occurs with much lower efficiency (not shown). It should be stressed that splitting in both strands of the laser-irradiated double-stranded fragment takes place preferentially at the residues belonging to the same or adjacent base pairs (cf. above data with fig.1f).

Thus, the modification efficiency of the bases by laser irradiation depends on the local peculiarities of the DNA secondary structure. This opens a new approach to study polynucleotide secondary structure and its alteration by variation of the conditions and by binding of ligands. Such an approach offers significant advantages in comparison with the use of low-intensity radiation and chemical agents for investigation of the higher structure of polynucleotides and nucleoproteins.

REFERENCES

[1] Dickerson, R.E. and Drew, H.R. (1981) J. Mol. Biol. 149, 761–786.
[2] Helene, C. and Maurizot, J.C. (1981) CRC Crit. Rev. Biochem. 213–258.
[3] Hawley, D.K. and McClure, W.R. (1983) Nucleic Acids Res. 11, 2237–2255.
[4] Brash, D.E. and Haseltine, W.A. (1982) Nature 298, 189–192.
[5] Becker, M.M. and Wang, J.C. (1984) Nature 309, 682–687.
[6] Sverdlov, E.D. and Kalinina, N.F. (1984) Dokl. Akad. Nauk SSSR 274, 1508–1512.
[7] Kryukov, P.G., Letokhov, V.S., Nikogosyan, D.N., Borodavkin, A.V., Budowsky, E.I. and Simukova, N.A. (1979) Chem. Phys. Lett. 61, 375–379.
[8] Menshonkova, T.N., Simukova, N.A., Budowsky, E.I. and Rubin, L.B. (1980) FEBS Lett. 112, 299–301.
[9] Rubin, L.B., Menshonkova, T.N., Simukova, N.A. and Budowsky, E.I. (1981) Photochem. Photobiol. 34, 339–344.
[10] Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
[11] Nakabeppu, X. and Sekiguchi, M. (1981) Proc. Natl. Acad. Sci. USA 78, 2742–2746.
[12] Mitchell, D.L. and Clarkson, J.M. (1984) Photochem. Photobiol. 40, 735–741.