Inhibitory Effect of Irradiation of DNA with Near Ultraviolet Light in the Presence of 8-Methoxypsoralen on Cleavage by Restriction Endonucleases

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The pBR322 DNA was irradiated with black light in the presence of 8-methoxypsoralen and digested by various restriction endonucleases including HindIII and Rsa I. It was found that for every restriction enzymes examined digestion products specific for pretreated DNA appeared as expected from the inhibition of cleavage presumably due to the formation of crosslinks at the cutting site or within the recognition sequence. Cleavage by HindIII was affected sensitively by pretreatment of DNA with irradiation in the presence of 8-MOP. The results were discussed in terms of stringency of enzymes to the structural changes of DNA and/or of the unique base sequence favorable to form crosslink.

Restriction endonucleases are characterized by their property to distinguish specific base sequence in DNA and cut the sequence at a definite site.1) The specificity of such recognition is so strict that a minute structural change in or near the recognition sequence would affect the cleavage of DNA strands by these enzymes. For this reason, homogeneous small DNA having a limited number of recognition sequence and corresponding restriction endonuclease provides a good system to detect structural changes in the known sequence. Based on this idea, studies have been performed with plasmid or viral DNA such as pBR322 or SV40 on the effect of ultraviolet light2) or psoralen plus near ultraviolet light.3) The present study was performed in order to know whether there is a sensitive base sequence favorable to produce crosslink in pBR322 DNA by irradiation with near ultraviolet light in the presence of 8-methoxypsoralen (8-MOP).

The pBR322 DNA was prepared from Escherichia coli HB101 harboring the plasmid according to the lysis by alkali method.1) A stock solution of 8-MOP (Taisho Pharmaceutical Co.) in ethanol, 0.6 mg/ml, was diluted 2 to 20-fold with Tris-HCl buffer (10 mM, pH 7.2) before use. Restriction endonucleases were purchased as follows: HindIII, Eco RI and Pst I from Takara Biochemical Co., Rsa I from Wako Pure Chemical Industrial Co. and Sph I

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and *Pvu* II from Boehringer Mannheim.

Typical procedure for treatment of pBR322 was as follows: Ten μl of pBR322 (1 μg/μl in 10 mM Tris-HCl buffer pH 8.0 containing 1 mM EDTA) was mixed with 20 μl of diluted 8-MOP solution. The mixture was sealed in a glass capillary and irradiated with near ultraviolet light (NUV) under a black light lamp (Toshiba, FL 20S BLB, 20 W) at a fluence rate of 10 J/m²·sec. After irradiation, 10 or more units of restriction enzyme was added to 1 μg of DNA and incubated at 37°C for 1 to 2 h to attain complete digestion in an appropriate buffer indicated to each enzyme specimen. The digested DNA was subjected to electrophoresis on horizontal 0.8% agarose gel in TBE buffer (0.089 M Tris-borate buffer pH 8.0 containing 2 mM EDTA) at 7 mA.

Intact pBR322 DNA gave a major band for closed circular form with a faint band for contaminating open circular form (Figs. 1 and 2, lane a). The electrophoretic profile of intact DNA did not change by irradiation with a large fluence of NUV (108 kJ/m²) (Fig. 1, lane c). Intact DNA digested by enzymes having one cutting site gave a unique band for the linear form of 4362 base pairs (Fig. 1, lane b for *Hin* dIII; data not shown for other enzymes). The produc-

![HindIII Diagram](image)

**Fig. 1.** Agarose gel electrophoresis of *Hin* dIII digested pBR322 DNA preirradiated with NUV in the presence of 8-MOP.
Lane a: intact DNA without digestion
Lane b: intact DNA digested by *Hin* dIII
Lane c: DNA irradiated with NUV (108 kJ/m²)
Lane d: DNA irradiated with NUV (108 kJ/m²) and digested by *Hin* dIII
Lane e – i: DNA irradiated with NUV in the presence of 8-MOP (0.02 μg/μl) and digested by *Hin* dIII. The fluence of NUV: 1, 10, 36, 72 and 108 kJ/m² for lanes e, f, g, h and i, respectively.

OC, L and CC with arrows show the position of open circular form, linear 4362 base pairs and closed circular form, respectively.
Fig. 3. Agarose gel electrophoresis of pBR322 DNA digested by restriction endonucleases after irradiation with NUV in the presence of 8-MOP (0.2 µg/µl).

Lane a: intact circular pBR322 DNA. Marker for closed and open circular forms.
Lane b: Same as lane a. Irradiated with NUV (208 kJ/m²).
Lanes c – j: Results obtained by using enzymes indicated over the lanes. For every enzyme, the left lane shows the digestion product of DNA irradiated with NUV (108 kJ/m²) alone, and the right lane shows the product of DNA irradiated with NUV (108 kJ/m²) in the presence of 8-MOP (0.2 µg/µl).

OC, L, and CC represent as explained in Fig. 1.
bands appeared as expected from the mode of blockage of enzymatic cleavage: bands for linear fragments of 3682 (2117 plus 1565), 2797 (2117 plus 680) and 2245 (1565 plus 680) base pairs, respectively, which must have been produced by inhibition at either one of three cutting sites; linear molecule of 4362 base pairs which must have been produced by inhibition at either two cutting sites; open and closed circular forms which must have been produced by inhibition at three cutting sites (Fig. 2, lanes d to f). It is seen that increasing fluences increased the frequency of block at two or three sites.

As far as has been examined, Hin dIII was influenced more sensitively by preirradiation with NUV in the presence of 8-MOP than other enzymes, Sph I, Eco RI, Pvu II and Pst I (Fig. 3). Appearance of a detectable amount of open- or circular-molecule which can be taken as a criterion of cleavage block was observed at a relatively large fluence (108 J/m²) and at a concentrated amount of 8-MOP (0.2 µg/µl) for enzymes shown in Fig. 3 as compared with the conditions for Hin dIII shown in Fig. 1.

The present results show that irradiation of DNA with NUV in the presence of 8-MOP prevents strand cleavage by restriction enzymes. Presumably the inhibition was due to the formation of crosslinks between two pyrimidines in opposite strands.⁴ Therefore the sequence (5')purine-p(3')pyrimidine or vice versa at the cutting site or within the recognition sequence can be considered as the favorable site of formation of crosslinks.⁵ In this connection, it appears that T-p-A sequence is a favorable site, since both Hin dIII and Rsa I have commonly the sequence, but others do not. However, recent studies of Saffran and Cantor showed that T-p-A and A-p-T sequences are not always hot spots for mutation induced by NUV and psoralen.⁶

There remains a possibility that the sensitivity of responses may be due to the stringency of recognition of enzymes. The study of Woodbury et al. showed that the specificity of site recognition of Eco RI is reduced by certain environmental conditions.⁷ In addition, the involvement of various forms of psoralen-pyrimidine monoadducts⁸ should be considered as a factor for making structural modification of DNA.

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REFERENCES


