Enzymatic quantification of strand breaks of DNA induced by vacuum-UV radiation

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Received 26 June 1986; revised version received 28 July 1986

*Hind*III-digested plasmid DNA dried on an aluminum plate was irradiated by vacuum-UV at 160 and 195 nm using a synchrotron irradiation system. A change induced in the DNA, presumably a single strand break, was quantified by the aid of the strand break-derived stimulation of poly(ADP-ribose) synthetase activity. The end group of strand breaks so induced was recognized by the enzyme as effectively as that by DNase I treatment, suggesting a nicking as the major lesion inflicted on the DNA. The fluence (UV) dependent stimulation of poly(ADP-ribose) synthetase activity was much higher upon 160 nm irradiation than upon 195 nm irradiation.

Vacuum-UV radiation *Plasmid pBR322* *Poly (ADP-ribose) synthetase* *DNA strand break* *Synchrotron radiation* *160 nm photon*

1. INTRODUCTION

A synchrotron system for monochromatic UV irradiation was constructed [1] at one of the beam ports of the electron storage ring (0.4 GeV) (Synchrotron Radiation Laboratory, University of Tokyo), and monochromatic radiation in the vacuum-UV range from 140 to 200 nm is now available for studies of UV damage in biological substances. Several lines of evidence obtained with this system have shown that single strand breaks are formed in DNA in the solid phase at varying degrees depending on the wavelength [2], even in the range where the photon energy is not sufficient to cause ionization. Several types of structural changes responsible for the X-ray-induced strand breaks have been examined by utilizing specific DNA-modifying enzymes such as polynucleotide kinase, terminal transferase, DNA polymerase I, etc. [3]. Poly(ADP-ribose) synthetase recognizes the strand breaks of DNA induced by a variety of DNA-digestive enzymes, and the structures of end groups that stimulate the synthetase activity have been well characterized [4]. Here, we attempted to utilize this unique property of poly(ADP-ribose) synthetase to quantify the strand breaks of DNA induced by vacuum-UV radiation at two wavelengths critically different in their absorption mode with DNA, namely, 160 nm with the sugar-phosphate backbone and 195 nm with the base moiety of DNA.

2. EXPERIMENTAL

2.1. *Materials*

A plasmid DNA (pBR322) was prepared according to Maniatis et al. [5]. Poly(ADP-ribose) synthetase was prepared by the procedures described in [6]. [*adenine*-U-^{14}C]NAD was obtained from Amersham. *Hind*III restriction enzyme, DNase I and diaminobenzoic acid were purchased from Takara Shuzo, Worthington and Nakarai Chemicals, respectively. Calf thymus DNA and histone H1 were products of Sigma.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2.2. *Vacuum-UV irradiation*

A *Hin*dIII-digested linear form of plasmid DNA (pBR322) on an aluminum plate was irradiated in vacuum at 160 and 195 nm by a synchrotron irradiation system (bandwidth, 8.7 nm) as described [1,7]. In brief, synchrotron radiation from the electron storage ring operated at 0.4 GeV was dispersed with a normal incidence monochromator and led to a vacuum irradiation chamber that holds samples so that the monochromatized radiation impinges on the samples for the desired time. The fluence (photons per unit area of the sample) was controlled by a computer-assisted system using spectral intensity and ring electron current.

2.3. *Measurements of strand breaks of DNA*

Strand breaks of DNA were measured as the strand break-derived stimulative activity of poly(ADP-ribose) synthetase as follows. The irradiated DNA on an aluminum plate was dissolved in 40 μl distilled water, the concentration of which was determined by the method of Kissane and Robins [8]. For the assay of poly(ADP-ribose) synthetase activity, aliquots (10 μl) of the DNA solution were added to the reaction mixture stock (40 μl). The final reaction mixture contained 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol, 100 μg/ml histone H1, 6.3 μg/ml poly(ADP-ribose) synthetase, 100 μM [*adenine*-U-^{14}C]NAD (11.6 dpm/pmol) and 10 μg/ml DNA. Incubation was carried out at 25°C for 10 min. The reaction was terminated by the addition of trichloroacetic acid at a final concentration of 10%. The radioactivity incorporated into the acid-insoluble materials was determined in a Searle Analytic mark III liquid scintillation spectrometer. When DNase I-digested calf thymus DNA was used at the same concentration of 10 μg/ml, poly(ADP-ribose) synthetase expressed the maximum activity (13000 dpm) [9]. We take it as 100% in the present experiments. When intact DNA was used at the same concentration approx. 20% of the maximum activity was obtained under the present conditions.

3. RESULTS AND DISCUSSION

A linear form of plasmid DNA (pBR322) was irradiated at 160 and 195 nm. Fig.1 shows the relative activity of poly(ADP-ribose) synthetase with the irradiated DNA as a function of UV fluence. The relative activity of poly(ADP-ribose) synthetase with the unirradiated plasmid DNA was 20%. Fig.1 shows that a remarkable increase of the activity occurred as a result of irradiation at 160 nm while the activity was only slightly increased at 195 nm in the fluence range below 1.8×10^{20} photons $\cdot m^{-2}$. The final level of the activity with 160-nm-irradiated DNA reached 80% that of DNase I-digested DNA. The results indicate that the vacuum-UV radiation at 160 nm could induce strand breaks which are recognized by poly(ADP-ribose) synthetase.

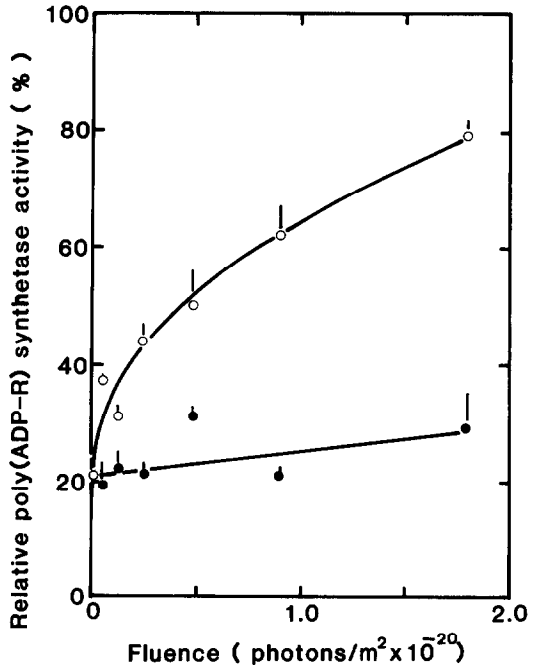

Fig.1. Stimulation of poly(ADP-ribose) synthetase activity with a linear form of plasmid DNA irradiated at 160 (○) and 195 nm (●) at various fluences. The activity obtained with DNase I-digested calf thymus DNA at a concentration of 10 μg/ml is represented as 100% (13000 dpm). (For details of assay see text.)

It is known that DNase I-digested DNA under mild conditions especially stimulates the poly(ADP-ribose) strand breaks [9]. Benjamin and Gill [4] have found that the stimulated activity is proportional to the number of single strand breaks per plasmid. This conforms well with the present

fluence-response relationship. As to the efficient recognition of the vacuum-UV-induced damage by poly(ADP-ribose) synthetase, it is interesting to note other work by Benjamin and Gill [10] where X-ray-induced damage was recognized less efficiently than that by DNase treatment.

The wavelength dependence of induced strand breaks as revealed by the present enzymatic quantification paralleled the finding by Hieda et al. [2] with quantification by agarose gel electrophoresis based on the vacuum-UV-induced damage in Col E1 DNA from its superhelical to circular form under the same irradiation conditions. The latter is supposed to be the relaxation from the twisted state by the formation of a single strand break. It was noted there that the rate of induction of strand breaks sharply decreases from 150 nm towards the longer wavelength region where the more generally known far-UV radiation is located.

Since that the absorption of 160-nm photons by DNA occurs in the sugar-phosphate group and since that the absorption of 195-nm photons is attributable to the base moiety [11], a suggestion can be made from the present results that the excitation of the sugar-phosphate group by 160-nm photons is the primary cause of the strand breaks. This would mean that the ionization event such as that produced by X-rays may not be unique in producing strand breaks by radiation in DNA. The incompetence of 195-nm photons can be explained on the basis of their absorption mode with DNA bases as mentioned above. Incidentally, it may be pointed out here that the photons with still longer wavelengths, in the so-called far-UV region (e.g. 260 nm), are also absorbed by the DNA bases, as generally known, but the excited state attained is different from that by 195-nm photons.

Taking the foregoing discussion into account, it would be reasonable to surmise that the vacuum-UV photon at 160 nm excites an electron associated with one of the backbones of the duplex structure of DNA and, as a consequence, a chemical change confined, in principle, to the specified strand, a single strand break, would result.

ACKNOWLEDGEMENTS

We acknowledge the use of synchrotron radiation facilities at the Synchrotron Radiation Laboratory, University of Tokyo. Also we thank Drs A. Ito, K. Hieda, H. Maezawa and other members of the cooperative project for assisting in the experiments. This work was supported by a Grant-in-Aid for Cooperative Research from the Ministry of Education, Science, and Culture, Japan.

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