MOLECULAR AND CELLULAR BIOLOGY, Feb. 1986, p. 707–709
0270-7306/86/020707-03$02.00/0

Vol. 6, No. 2

Detection of UV Purine Photoproducts in a Defined Sequence of Human DNA

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Received 25 June 1985/Accepted 28 October 1985

The UV-irradiated, 3′-end-labeled, 92-base-pair terminus of the human alphoid sequence was incubated with purified endonuclease v. Previously unreported photoproducts were incised at purine loci. These were not pyrimidine photodimers, 6-4′-(pyrimidin-2′-one)-pyrimidines, base loss sites, or ring-opened purines. Therefore, purine-containing photoproducts, possibly dimers, were incised by the enzyme preparation.

UV irradiation produces a variety of lesions in DNA which, if unrepaired, are known to have lethal, mutagenic, or tumorigenic effects (8, 17). One major product is the cyclobutane dimer formed between adjacent pyrimidines (17). Endonuclease v (endo v), the *denV* gene product of bacteriophage T4-infected *Escherichia coli*, recognizes such dimers and initiates their excision via a two-step reaction. Step 1 is the cleavage of the *N*-glycosylic bond between the 5′ pyrimidine of the dimer and its sugar, and step 2 is hydrolysis of the phosphodiester bond on the 3′ side of the resultant apyrimidinic site (5, 10, 18, 20, 23). Lippke et al. have identified another photoadduct, the 6-4′-(pyrimidin-2′-one)-pyrimidine, with the use of a defined sequence of human alphoid DNA (14). This system permits the analysis of DNA damage and repair at the level of individual nucleotide sequences (15). We employed this method to investigate formation of base damage by broad-spectrum UV irradiation. Using purified endo v as a probe, we identified previously unreported photoproducts. These novel lesions were detected at purine loci, suggesting formation of a family of purine-containing photoproducts.

The alphoid segment, which was obtained by *Eco*RI digestion of a pBR322 plasmid containing the inserted sequence, was 3′ end labeled and recut as previously described (24). The 92-base-pair sequence was irradiated in 100 mM Tris hydrochloride–5 mM dithiothreitol–10 mM dipotassium EDTA (pH 7.5) (buffer A) with a broad-spectrum mercury lamp (250 to 400 nm, maximum intensity at 302 nm) for 10 min. Endo v was purified as previously described (6). Two micrograms of the phosphocellulose fraction IV of Friedberg et al. (9) was incubated with irradiated or nonirradiated 92-base-pair fragment in buffer A for 60 min at 37°C. The DNA was ethanol precipitated, and the pellet was redissolved in a gel-loading buffer (90% formamide, 10 mM sodium hydroxide, 1 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) or in 25 μl of 1.0 M piperidine as indicated. The piperidine-treated samples were incubated at 90°C for 30 min, redissolved twice in 10 μl of distilled water, and lyophilized before addition of the loading buffer. Standard DNA-sequencing reactions were performed according to Maxam and Gilbert (16), except that 25 μl of 1.0 M piperidine was used.

Prominent bands corresponded to all possible pyrimidine dimer sites in the sequence after treatment of the irradiated segment by endo v (Fig. 1, lanes 7 and 9). These fragments were detected in the absence of piperidine treatment, indicating that the endonuclease for DNA apurinic or apyrimidinic sites of endo v was active. The 6-4′-(pyrimidin-2′-one)-pyrimidine photoproducts were observed in the sequencing lanes containing UV-irradiated DNA treated with piperidine (lanes 9 and 10). Neither isolation nor irradiation of the DNA segment resulted in the formation of appreciable DNA strand breakage, base losses, or other alkali-labile sites (lanes 1, 2, 8, and 9). Four additional bands, as indicated by arrows (lanes 7 and 9), were observed after treatment of the irradiated 92-base-pair substrate with endo v. Three of these bands were found in a region lacking adjacent pyrimidines (G51 to G62), demonstrating that these are not cyclobutane pyrimidine dimers. Another band (C26) is directly under a C-C dimer band (A27). These photoproducts were not detected by treating UV- or non-UV-irradiated DNA with hot alkali in the absence of the enzyme (lanes 1, 2, and 10) and therefore are not 6-4′-(pyrimidin-2′-one)-pyrimidine photoproducts, base loss sites, openings of purine imidazole rings, or another variety of alkali-labile damage (14, 16). Minor bands at positions A30, A33, T40, and T42, which also do not occur in areas of adjacent pyrimidines, were observed. Overexposure of the gel showed several additional bands between C20 and A30 of the DNA sequence (data not shown). Therefore, the endo v preparation recognizes and incises purine-containing photoproducts formed by UV irradiation of the DNA. The novel bands were not seen between pyrimidine clusters (T42 to T50, T63 to T78); this raises the possibility of pyrimidine photodimer interference with the formation of these new bands.

An aliquot of endo v was heated at 100°C for 2 min before incubation with the substrate to determine if incision at the novel photoproducts was an enzymic reaction. As shown in Fig. 2 (lane 5), no incision by the heated enzyme preparation at these new photoproducts was observed. By contrast, it was necessary to heat endo v for 5 min to eliminate incision at pyrimidine dimers (data not shown). This demonstrates that the cleavage products at purine sites result from the activity of the protein preparation.

The deleterious biological effects caused by UV irradiation of living cells have been attributed to alterations of pyrimidines. It is thought that the formation of pyrimidine dimers can result in lethality, mutagenicity, or transformation (8, 12, 13), while 6-4′-(pyrimidin-2′-one)-pyrimidine photoproducts in DNA act as mutagenic lesions (4). It is generally assumed that DNA purines are relatively inert to photochemical modifications (7). However, there are de-

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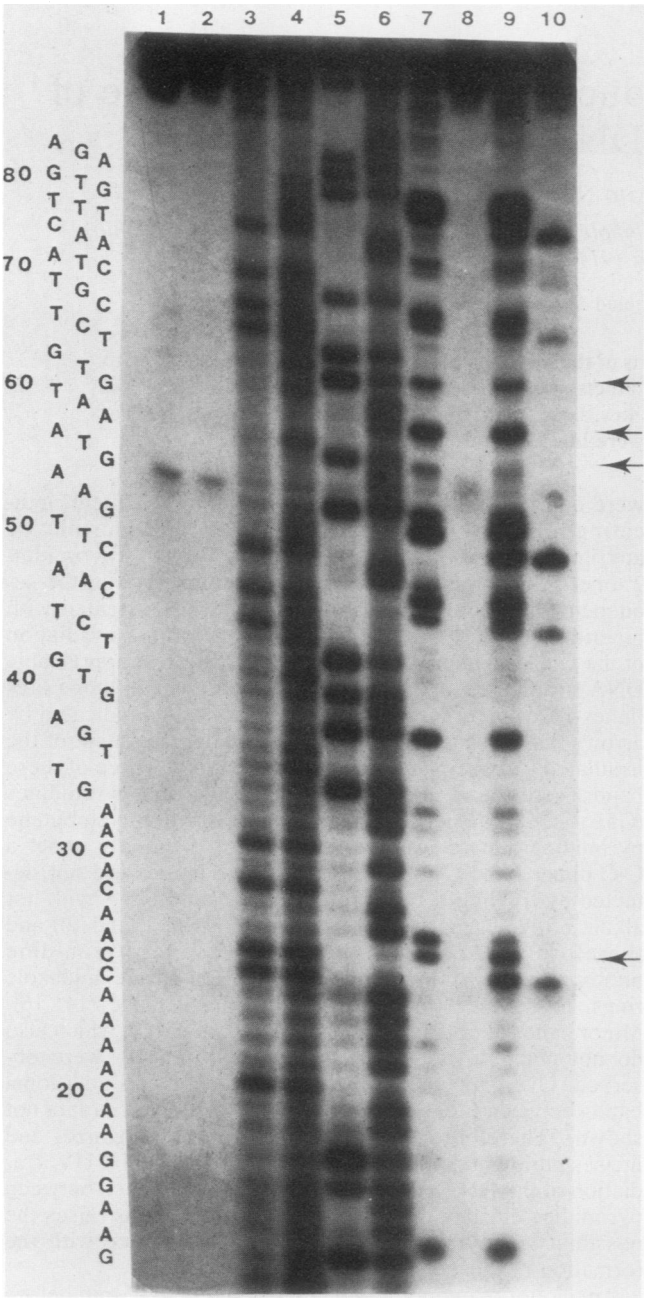

FIG. 1. Detection of UV-induced photoproducts in a 92-base-pair DNA sequence. Aliquots of 3′-end-labeled DNA were heated in piperidine (lane 1); incubated with endo v before piperidine treatment (lane 2); subjected to base-specific chemical cleavages: C (lane 3), C-T (lane 4), G (lane 5), G-A (lane 6); irradiated for 10 min, then incubated with endo v (lane 7); irradiated and lyophilized (lane 8); irradiated for 10 min, then incubated with endo v and heated in piperidine (lane 9); or irradiated and then heated in piperidine (lane 10). The arrows indicate loci of the novel purine photoproducts.

scriptions of purine dimers at A-A and A-T sites in UV-irradiated DNA (1–3, 19).

Our data demonstrate that endo v incises irradiated DNA at purines or purine-containing moieties. One possible substrate is a purine-containing photodimer. The four non-pyrimidine dimer bands (G60, A56, A53, and C26) would thus correspond to purine-containing dimer lesions A-T, G-T, G-A, and A-C (3′ to 5′), respectively, if the enzyme reaction proceeds by cleaving the purine-containing dimer on the 5′ side. Alternatively, if the incision occurs on the 3′ side of the altered moiety, the observed bands would correspond to photodimers G-T, A-A, A-G, and C-A (3′ to 5′). Either possibility is consistent with a purine-containing dimer. On the other hand, the substrates may be modified monoadducts incised on either the 3′ or the 5′ side. A coupled glycosylase-endonuclease mechanism is not excluded. Identification of the damaged purines and elucida-

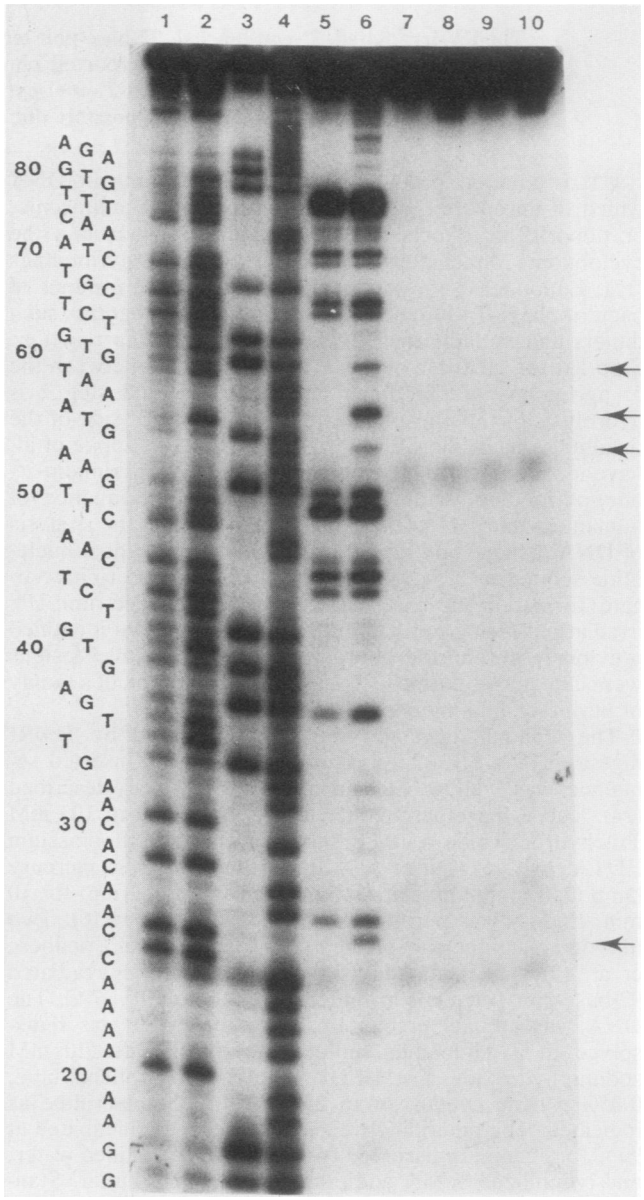

FIG. 2. Heat inactivation of endo v. Aliquots of 3′-end-labeled 92-base-pair DNA were subjected to base-specific chemical cleavages: C (lane 1), C-T (lane 2), G (lane 3), G-A (lane 4); irradiated for 10 min and then incubated with endo v that had been heated for 2 min (lane 5); irradiated for 10 min and then incubated with endo v (lane 6); irradiated for 10 min (lane 7); incubated with endo v that had been heated for 2 min (lane 8); incubated with endo v (lane 9); or lyophilized (lane 10). The arrows indicate loci of the novel purine photoproducts.

tion of the mechanism of their enzymic incision are in progress.

It has not yet been determined if these purine photoproducts are incised by an activity on the *denV* gene product or by another enzyme. The differing heat labilities of the pyrimidine dimer and the purine photoproduct-incising activities suggest that separate proteins are present. We have found that irradiated DNA is incised by a crude *Micrococcus luteus* extract at purine photoproduct loci (N. J. Duker and P. E. Gallagher, unpublished data). Preliminary experiments indicate that the *M. luteus* activity incising purine photoproducts can be separated from the pyrimidine dimer-DNA glycosylase (data not shown).

Most studies of UV-induced DNA damage have used 254-nm irradiation. However, the carcinogenic effects of actinic radiation are caused by wavelengths between 280 and 300 nm (11). Shorter wavelengths are filtered by atmospheric ozone, and it has been shown that longer wavelengths have little biological effect (11, 21, 22). A broad-spectrum light source (250 to 400 nm) was therefore used in these experiments. Gordon and Haseltine examined UV-irradiated DNA treated with endo v by sequencing techniques and observed bands exclusively at pyrimidine dimer sites (10). It is possible that purine photoproducts were not detected in those studies because of differences in light source outputs. The distribution and yield of purine photoproducts at biologically significant wavelengths are under investigation. These studies may indicate which, if any, of these moieties are of consequence in actinic carcinogenesis.

This work was supported by U.S. Public Health Service grant AG-00378 from the National Institute of Aging and Research Career Development Award CA-00796 from the National Cancer Institute.

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