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Excision of Damaged Thymine Residues from Gamma-Irradiated Poly(dA-dT) by Crude Extracts of *Escherichia coli*

(excision repair/DNA base damage/gamma rays)

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ABSTRACT **Crude extracts of *E. coli* endo I$^-$ and *E. coli* endo I$^-$*uvrA6*$^-$ possess the ability to remove thymine products of the 5,6-dihydroxy-dihydrothymine type from γ-irradiated or osmium tetroxide-oxidized poly(dA-dT). It is shown that the uvrA-gene product, which is responsible for incision close to photodimers in prereplication ultraviolet repair in *E. coli*, is not required for, but may aid in, the excision of γ-ray products of the 5,6-dihydroxy-dihydrothymine type. Ring-damaged thymine products are also removed by *E. coli* extracts from osmium tetroxide-oxidized poly(dA-dT), which contains only 5,6-dihydroxy-dihydrothymine but no strand breakage, indicating that product excision occurs in the absence of radiation-induced breaks. On the average, 8 to 16 nucleotides are removed from the polymer per ring-damaged thymine residue excised by extracts from both strains and for γ-irradiated and osmium tetroxide-oxidized polymer.**

Irradiation of cells by ionizing radiation damages the heterocyclic bases of DNA and causes DNA strand breakage. Resealing of strand breaks and the removal from the DNA of damaged thymine residues during incubation after irradiation occurs in bacterial (1) and mammalian cells (2). The results obtained have not yielded much information concerning the molecular mechanism leading to the release of γ-ray-damaged thymine, however. In particular, the question remained unanswered whether the removal of damaged thymine residues from DNA is accomplished by a *selective* excision similar to that effecting the removal of cyclobutane-type photodimers, or whether it is a reflection of DNA degradation after irradiation. Recently indirect evidence was presented that favors the first alternative. Bacterial (3, 4) and mammalian cells (5) possess endonucleolytic activities that recognize x-ray- or γ-ray-induced DNA lesions which may involve the heterocyclic bases. For *E. coli*, it was found that such "endonuclease-sensitive sites" were rapidly removed from the DNA during incubation of the culture after irradiation (3, 6). The experiments reported here directly demonstrate the selective removal of thymine products of the 5,6-dihydroxy-dihydrothymine type from γ-irradiated or osmium tetroxide-oxidized poly(dA-dT) by crude extracts of *E. coli* endo I$^-$ and *E. coli* endo I$^-$*uvrA6*$^-$. Ring-saturated products of the 5,6-dihydroxy-dihydrothymine type, especially the corresponding hydroxy-hydroperoxy derivatives, represent a major class of γ-ray-induced DNA lesions formed under conditions *in vitro* (7, 8) and *in vivo* (9).

EXPERIMENTAL PROCEDURES

Determination of Products of the 5,6-Dihydroxy-dihydrothymine Type (t′) in OsO_4-Oxidized or γ-Irradiated [methyl-^{3}H]-Poly(dA-dT). The principle of the assay is similar to that used in the reductive assay of Hariharan and Cerutti for thymine radiation products (1, 13), but the new procedure proved to be simpler and more sensitive. The irradiated or OsO_4-oxidized [*methyl*-^{3}H]poly(dA-dT) preparations (specific activity 26.5 μCi/μmole of phosphate) were treated with 2 ml of 0.2 M KOH. In experiments where the acid-precipitable material was analyzed for t′, the 7% trichloroacetic acid precipitates were directly dissolved in 2 ml of 0.2 M KOH. After 1.5 hr at room temperature, 0.66 ml of 2 M HCl was added, and the incubation was continued at 70° for 15 min. The samples were then centrifuged at 15,000 rpm for 10 min (Sorvall RC2-B, Rotor type SS-34); the supernatants were neutralized with KOH and analyzed for their content of acetol-*methyl*-^{3}H by ion-exchange chromatography. The formation of acetol from 5,6-dihydroxy-dihydrothymine by the base-acid treatment has been shown by Burton and Riley (11). The ion-exchange columns contained 1.6 ml of DEAE-Sephadex A25 on top and, separated by a layer of sand, 4 ml of Biorad AGl-X10 (OH^-) in the middle and, separated by another layer of sand, 4 ml of Dowex 50WX8 (H^+) on the bottom. The columns were eluted with decarbonated water, and 1-ml fractions were collected directly into scintillation vials containing Aquasol. One major radioactivity peak was obtained in fractions 13–16. The yield of the radioactive fragment produced per thymine ring destroyed was estimated to be about 20% for OsO_4-oxidized [*methyl*-^{3}H] poly(dA-dT). Total thymine ring destruction in OsO_4-oxidized poly(dA-dT) was determined by measuring the loss in absorbance at 267 nm in 0.2 M KOH. The corresponding yield for γ-irradiated poly-(dA-dT) is also estimated to be about 20%, although this value is less reliable than that for the OsO_4-oxidized polymer.

Selective Oxidation of Thymine in Poly(dA-dT) with OsO_4 and γ-Irradiation of Poly(dA-dT). The conditions of Beer *et al.* (12) were modified for the selective oxidation of thymine in poly(dA-dT) to *cis*-5,6-dihydroxy-dihydrothymine. Poly-(dA-dT) was denatured by heating to 80° for 15 min, followed by quick cooling. An equal volume of a 0.8% OsO_4 solution in 1.25×10^{-4} M cacodylate buffer, pH 7.2, was then added to the polymer solution, and the reaction mixture was incubated for 10 min at 55°. Excess OsO_4 was then removed by three extractions with two volumes of cold ether. An equal volume of ethanol was added to the final aqueous layer, and the samples were stored at −20° until use. This procedure yielded 1.7% of t′, as determined in the total sample by the alkali-acid degradation assay described above. Since the assay yield is about 20% (see above) total thymine ring destruction is estimated at 8.5%. Analysis of the oxidized material by DEAE–Sephadex chromatography according to

Abbreviation: t′, γ-ray products of the 5,6-dihydroxy-dihydrothymine type.

Mattern *et al.* (2) showed that about $^3/_5$ of t′ was contained in the polymer and $^2/_5$ was present as uncharged acid-soluble material. A slightly higher fraction of t′ remained soluble if the oxidized polymer was precipitated with 7% trichloroacetic acid. The high-molecular-weight polymeric materials, therefore, contained about 5.1% t′ remaining attached to the polymer and 2.4% apyrimidinic sites*. Total acid solubilization of the polymer by the OsO_4 treatment was only 4.6%, and no significant change was observed, upon oxidation of the polymer, in its sedimentation properties on 5–20% alkaline sucrose gradients. A series of polymer preparations was produced with higher and lower levels of thymine modification by varying the length of the incubation with OsO_4.

Poly(dA-dT) was irradiated in nonprotective aqueous solution in the presence of air with a ^{137}Cs γ-irradiator. A dose of 33 krads was used in the experiments. The yield of t′ determined in the total samples was 0.72 and 1.40% in two representative experiments. The variation in the levels of t′ is attributed to differences in the radiation-chemical purity of the poly(dA-dT) samples. Analysis of the irradiated samples by DEAE–Sephadex chromatography yielded results similar to those described above for OsO_4 oxidation. Only half of t′ (i.e., 0.36 and 0.70%) was attached to the polymer. Total level of ring-damaged thymine in the polymer is estimated at 1.8 and 3.5% for the two preparations, using a value of 20% for the yield in the alkali-acid degradation assay (see above). The polymers are expected to contain additionally about 1.8 and 3.5 apyrimidinic sites*. Total radiation-induced solubilization of the polymer was 7 and 11%, respectively (7% trichloroacetic acid). Irradiated poly(dA-dT) is expected to contain t′, thymine damage that is not measurable by the alkali-acid degradation assay, adenine damage, and apurinic and apyrimidinic sites, as well as radiation-induced strand breaks.

Bacterial Strains, Cultivation, and Preparation of Crude Extracts. *E. coli* MRW25 endo I^-, HfrH was obtained from Dr. R. McMacken and *E. coli* RB1001 endo I^- *uvrA6*$^-$ was obtained from Dr. R. P. Boyce. The procedures for bacterial growth and preparation of crude extracts were essentially those outlined by Wickner *et al.* (14).

Extracts were prepared by the method *A* of Wickner *et al.* (14), with the exception that the lysate was made 1mM in dithiothreitol and that the centrifugation was carried out at 4° for an hour at 1000,000 × *g*. The protein concentration in the extracts was about 10 mg/ml, as determined by the biuret method. Fresh extracts were prepared immediately before use for each experiment.

Excision of Products of the 5,6-Dihydroxy-dihydrothymine Type (t′) from OsO_4-Oxidized or γ-Irradiated Poly(dA-dT) by E. coli Extracts. To a solution of modified [*methyl*-^{3}H]poly(dA-dT) in 350 μl of 50 mM phosphate buffer (pH 7.0) containing about 1 μg of the polymer and 5 to 10 × 10^4 cpm was added 200 μl of the freshly prepared crude extract of *E. coli* described above. The samples were incubated at 37°, and the reaction was terminated at various time intervals up to 30 min by the addition of 2.5 ml of cold 7% trichloroacetic acid. After 30 min of incubation in ice, the precipitates were collected by centrifugation at 15,000 rpm for 10 min. Both the precipitates and the acid-soluble fractions were analyzed for their content of t′ by the alkali-acid degradation assay described above. Controls with nonmodified [*methyl*-^{3}H]poly(dA-dT) were carried out under identical conditions; in controls with inactivated extract, preparations were used that had been heated to 70° for 10 min.

Chromatographic Analysis of the Material Excised from OsO_4-Oxidized or γ-Irradiated Poly(dA-dT) by the E. coli Extracts. Samples of the neutralized, trichloroacetic acid-soluble material released from OsO_4-oxidized poly(dA–dT) by extracts of *E. coli* endo I^- within 5 min of incubation were chromatographed on DEAE–Sephadex A25 (1-cm diameter glass column of 8-cm height). A separate series of samples was treated with alkaline phosphatase before application to the column. The column was eluted with a linear salt gradient of 0.1 M NaCl–20 mM Tris·HCl (pH 7.5) to 0.5 M NaCl 20 mM Tris·HCl (pH 7.5). The following authentic markers were cochromatographed for comparison: thymine, thymidine, thymidine-5′-monophosphate, thymidine-5′-diphosphate, thymidine-5′-triphosphate, and the oligodeoxynucleotides $(pT)_3$ and $(pT)_9$.

RESULTS AND DISCUSSION

Choice of DNA Substrates and Assay for Thymine Products of the 5,6-Dihydroxy-dihydrothymine Type. The excision by crude *E. coli* extracts of products of the 5,6-dihydroxy-dihydrothymine type from an exogeneous polydeoxynucleotide substrate was studied in our experiments, the alternating synthetic polydeoxynucleotide, poly(dA-dT), being used instead of DNA for the following reasons: (*i*) For an alternating polymer containing only one type of base pair, the formation of mismatched, distorted, or looped-out regions during renaturation is minimized. Upon initiation of helix formation, all base pairs are in automatic register. Minimal unspecific disruption of the helix is important for an assessment of the specificity of damage recognition and excision. (*ii*) Unlike ultraviolet light, ionizing radiation produces a large variety of lesions involving the heterocyclic bases of DNA. For γ-irradiated alternating poly(dA-dT), the level of complexity in this regard is considerably reduced, and products of the 5,6-dihydroxy-dihydrothymine type, in particular 5-hydroxy-6-hydroperoxy-dihydrothymine, undoubtedly represent a major class of lesions. (*iii*) Selective oxidation of poly(dA-dT) with OsO_4 allowed the preparation of a substrate containing mainly 5,6-dihydroxy-dihydrothymine and apyrimidinic sites*, but no adenine damage and no strand breaks (11, 12).

A more sensitive method was developed for the determination of products of the 5,6-dihydroxy-dihydrothymine type, following the principle of our earlier assay for saturated thymine radiation products, and is described under *Experimental Procedures.* (13). Gamma-irradiated poly(dA-dT) is expected to contain, besides products of the 5,6-dihydroxy-dihydrothymine type, other types of thymine damage that are not measurable by our assay, adenine damage, apurinic and apyrimidinic* sites, and radiation-induced strand breaks, while OsO_4-oxidized poly(dA-dT) mainly contains 5,6-dihydroxy-dihydrothymine and apyrimidinic sites*. The symbol t′ is used in the following for thymine products of the 5,6-dihydroxy-dihydrothymine type.

Excision of γ-Ray-Damaged Thymine by Crude Extracts of E. coli Endo I^- and E. coli Endo I^- uvrA6$^-$. The removal of t′ from the acid-precipitable fraction of γ-irradiated poly-

* The term "apyrimidinic site" is used in a broad sense. We cannot exclude the possibility that the urea portion of the fragmented thymine ring remains attached to the deoxyribose moiety.

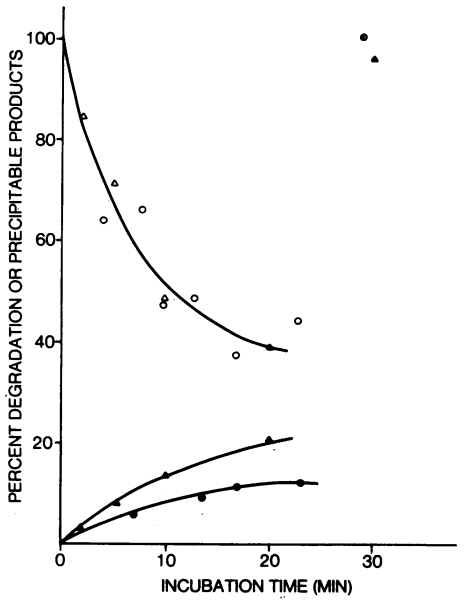

FIG. 1. Removal of products of the 5,6-dihydroxy-dihydrothymine type (t′) and of undamaged thymine from γ-irradiated poly(dA-dT) by *E. coli* extracts. Experimental conditions are given in *Experimental Procedures*. ○, Removal of t′ from acid-precipitable, γ-irradiated poly(dA-dT) by crude extracts of *E. coli* endo I⁻; △, removal of t′ from acid-precipitable, γ-irradiated poly(dA-dT) by crude extracts of *E. coli* endo I⁻*uvrA6*⁻; ◐ and ▲, same as ○ and △, respectively, except that heat-inactivated extracts were used in the incubation mixture. ●, Acid-solubilization of thymine radioactivity by crude extracts of *E. coli* endo I⁻; ▲ acid-solubilization of thymine radioactivity by crude extracts of *E. coli* endo I⁻*uvrA6*⁻.

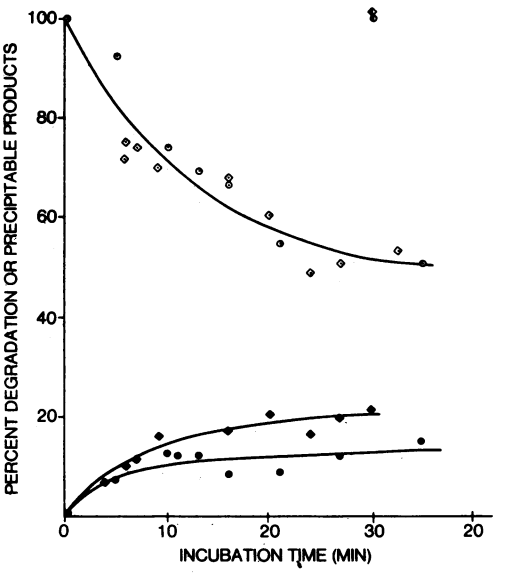

FIG. 2. Removal of 5,6-dihydroxy-dihydrothymine (t′) and undamaged thymine from OsO_4-oxidized poly(dA-dT). Experimental conditions are given in *Experimental Procedures*. ⊙, Removal of t′ from acid-precipitable, OsO_4-oxidized poly(dA-dT) by crude extracts of *E. coli* endo I⁻; ◈, removal of t′ from acid-precipitable, OsO_4-oxidized poly(dA-dT) by crude extracts of *E. coli* endo I⁻*uvrA6*⁻; ◐ and ◈, same as ⊙ and ◈, respectively, except that heat-inactivated extracts were used in the incubation mixture. ●, Acid-solubilization of thymine radioactivity by crude extracts of *E. coli* endo I⁻; ◆, acid-solubilization of thymine radioactivity by crude extracts of *E. coli* endo I⁻*uvrA6*⁻.

(dA-dT) by crude extracts of *E. coli* endo I⁻ and *E. coli* endo I⁻ *uvrA6*⁻ prepared according to Wickner *et al.* (14) is shown in Fig. 1. Poly(dA-dT) was used which had been irradiated under nonprotective conditions with 33 krads of ^{137}Cs γ-rays in the presence of air. The polymer used with extracts of *E. coli* endo I⁻ contained 0.36% t′, and the preparation used with extracts of *E. coli* endo I⁻ *uvrA6*⁻, 0.70% t′. Since the assay yield is about 20% (see *Experimental Procedures*), the total content of ring-damaged thymine residues remaining attached to the polymer backbone was estimated at 1.8% and 3.5%, respectively, in the two preparations. Within about 20 min of incubation at 37°, 60% of t′ was removed by the extracts of both *E. coli* strains. The kinetics of the product removal and the final level of t′ remaining precipitable after 20 min of incubation were the same regardless of the presence or absence of the uvrA-gene product. Excision of t′ by the extracts of both strains remains incomplete. Release of undamaged thymine is shown by the curves in the lower part of Fig. 1. Nonirradiated poly(dA-dT) was degraded to 4–5% by the extracts of both strains under the same conditions, and it follows, therefore, that irradiation leads to a substantial increase in polymer degradation by the extracts. Neither excision of t′ nor polymer degradation was observed when the extracts were heated to 70° for 10 min. It is evident that degradation of the irradiated polymer occurred at a lower rate and to a smaller degree than excision of t′. At the time when 60% of t′ had been solubilized, only 13% of the total thymine had been removed from the polymer by extracts of *E. coli* endo I⁻ and 20% by extracts of *E. coli* endo I⁻ *uvrA6*⁻. Stoichiometry was obtained for the disappearance of t′ from acid-precipitable material and the appearance of products in acid-soluble form. Experiments with other preparations of irradiated poly(dA-dT) containing comparable levels of t′ yielded similar results.

Our experiments demonstrate that the uvrA-gene product† is not required for the excision of t′. Wilkins (6) found that the *Micrococcus luteus* "endonuclease-sensitive sites" in the DNA of γ-irradiated wild-type *E. coli* and *E. coli uvrA*⁻ disappeared at equal rates during incubation after irradiation. The chemical identity of the radiation lesions recognized by the *Micrococcus luteus* extracts is not known. The uvrA-gene product, on the other hand, was shown to participate in the excision of arylalkylated residues from DNA (15). Our results, of course, raise the question concerning the identity of the endonuclease recognizing ring-saturated thymine products of the t′ type. Possible candidates are endonuclease II (16), the endonuclease recognizing depurinated DNA (17) of *E. coli*. An endonuclease specific for γ-ray-induced DNA damage has been purified from *Micrococcus luteus* (18).

It follows from our results with extracts of *E. coli* endo I⁻ *uvrA6*⁻ that the incision step differs for the removal of γ-ray-damaged thymine and pyrimidine photodimers. Later steps in the pathways of γ-ray and UV repair, however, could be the same. It should be noted in this context that partial synergism has been observed for the lethal effects of ultraviolet light and x-rays (19, 20).

† Braun and Grossman have recently shown that partially purified extracts of *uvrA* and *uvrB* mutants of *E. coli* lack endonucleolytic activity specific for uv-irradiated DNA [Braun, A. & Grossman, L. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1838–1842].

TABLE 1. *Extent of polymer degradation accompanying excision of ring-damaged thymine*

	% Ring-damaged thymine*	Incubation time (min)	Total residues solubilized/ 10^6 daltons†	Ring-damaged thymine excised/ 10^6 daltons‡	Total residues solubilized/ ring-damaged thymine excised§
E. coli endo I$^-$	1.8 (by γ-rays)	5	118	8.8	13.4
		20	266	16.5	16.1
E. coli endo I$^-$*uvrA6*$^-$	3.5 (by γ-rays)	5	207	17.0	12.2
		20	503	35.2	14.2
E. coli endo I$^-$	5.0 (by OsO_4)	5	118	13.0	9.1
		20	266	31.5	8.5
E. coli endo I$^-$*uvrA6*$^-$	5.0 (by OsO_4)	5	207	13.0	16.0
		20	444	31.5	14.1

* Total ring-damaged thymine in the modified polymer was computed by multiplying the amount of t′ in the trichloroacetic acid-precipitable material by a factor of 5.

† The fraction of thymine radioactivity rendered acid-soluble multiplied by 2, to account for the release of unlabeled adenine residues, and by 1480, the number of thymine residues in 10^6 daltons of poly (dA-dT).

‡ The fraction of ring-damaged thymine removed from the acid-precipitable material multiplied by the number of ring-damaged thymine residues originally present in 10^6 daltons of modified poly(dA-dT).

§ Ratios of the values in the third column over those in the fourth column.

Excision of t′ from OsO_4-Oxidized Poly(dA-dT) by Crude Extracts of E. coli Endo I$^-$ and E. coli Endo I$^-$uvrA6$^-$. An analogous series of experiments to those described in the preceding section was carried out with OsO_4-oxidized poly-(dA-dT), which contained 5,6-dihydroxy-dihydrothymine and apyrimidinic sites but no adenine damage or strand breakage. The formation of a small amount of saturated thymine products other than 5,6-dihydroxy-dihydrothymine by the OsO_4 treatment cannot be excluded. The level of ring-saturated thymine residues in the polymer preparation used in these experiments was about 5%. As shown in Fig. 2, ring-saturated products t′ were selectively removed from acid-precipitable material both by extracts of *E. coli* endo I$^-$ and *E. coli* endo I$^-$*uvrA6*$^-$. As for γ-irradiated poly(dA-dT), the excision process was incomplete. The time course of product removal was identical for both strains, and after 30 min of incubation about 50% of the products were rendered acid-soluble. Similar results were obtained with poly(dA-dT) containing a lower level of modified thymine. As observed for γ-irradiated poly-(dA-dT), polymer hydrolysis leveled off at a much lower percentage than product excision, and hydrolysis by extracts of *E. coli* endo I$^-$*uvrA6*$^-$ exceeded that by extracts of *E. coli* endo I$^-$ by a few percent. As discussed below, the absence of an active uvrA-gene product did not, therefore, prevent the excision of t′, but the excision process was almost twice as selective in its presence. Stoichiometry was obtained for the disappearance of 5,6-dihydroxy-dihydrothymine from acid-precipitable material and the appearance of the products in acid-soluble form. A comparison of Figs. 1 and 2 shows that product removal was more efficient and more complete from γ-irradiated poly(dA-dT) than from the OsO_4-oxidized polymer.

Number of Nucleotides Removed from the Polymer per Damaged Thymine Residue. In Table 1 the total number of undamaged and the number of ring-damaged thymine residues solubilized per 10^6 daltons of poly(dA-dT) are listed for 5 min and 20 min of incubation with extracts of *E. coli* endo I$^-$ and of *E. coli* endo I$^-$ *uvrA6*$^-$. As shown in the last column, which contains the ratio of the two values, 8–16 nucleotides are removed on the average from the polymer per ring-damaged thymine residue under all conditions. Our estimates for the number of nucleotides removed per damaged residue for OsO_4-oxidized poly(dA-dT) are comparable to those obtained by Kushner *et al.* (21) with purified UV-endonuclease and UV-exonuclease from *M. luteus*. These authors found that 7 nucleotides were, on the average, released per incision event from UV-irradiated *E. coli* DNA. Since incision and subsequent exonucleolytic degradation may also occur at apyrimidinic sites in OsO_4-oxidized poly(dA-dT), our values are expected to be on the high side, i.e., fewer undamaged residues may be removed from the polymer to accomplish the excision of a ring-damaged thymine residue alone. This should be even more pronounced for γ-irradiated poly(dA-dT), which contains a variety of additional repairable lesions besides t′ that cannot be quantitated with our present procedures.

Chromatographic Analysis of the Excised Material. The acid-soluble material produced within 5 min by a crude extract of *E. coli* endo I$^-$ from OsO_4-oxidized poly(dA-dT) was chromatographed together with authentic markers on DEAE–Sephadex, using a linear salt gradient. The major radioactive component in the material released within 5 min was identified as TMP; smaller amounts of radioactivity were eluted later at higher salt concentration, and these peaks chromatographed with oligonucleotides of variable chain length. The major radioactivity peak moved to the position of thymidine if the sample had been pretreated with bacterial alkaline phosphatase. Pooled fractions from different regions of the chromatograms were analyzed for their t′ content by the alkali-acid degradation assay. Without treatment with alkaline phosphatase, 46% of t′ was present in the form of the mononucleotide, 28% eluted at the position of thymine or thymidine, and 22% was eluted at high salt. After treatment with alkaline phosphatase, most of t′ eluting originally at the position of the mononucleotide had moved to that of the nucleoside or free base. When the acid-soluble material formed within 20 min of incubation with the extracts was analyzed by the same procedure, 73% of the total radioactivity and 88% of t′ was eluted at the position of thymidine and thymine

without pretreatment with alkaline phosphatase. It appears likely from these results that t′ is removed from the polymer in the form of the mononucleotide or as part of oligodeoxynucleotides. The oligodeoxynucleotides are then rapidly degraded and the mononucleotides dephosphorylated upon further incubation with the extracts. Analogous analysis of the acid-soluble material released by *E. coli* endo I^- extracts from γ-irradiated poly(dA-dT) did not yield conclusive results.

Radiation-Induced Strand Breaks Are Not Required for the Excision of Ring-Damaged Thymine Residues. Evidence has been obtained by workers in several laboratories that suggests that DNA strand breaks induced by ionizing radiation may play a role in the removal of base-damaged residues by serving as starting points for exonucleolytic degradation (1, 10). Our results with OsO_4-oxidized poly(dA-dT) show that strand breakage is not required for the excision of ring-damaged thymine by *E. coli* extracts. This result does not exclude some participation of strand breakage in the excision of products from γ-irradiated substrate. From a comparison of the data shown in Figs. 1 and 2, it is evident that removal of t′ is more rapid and more complete from γ-irradiated than from OsO_4-oxidized poly(dA-dT). The differences in these results could be due to the additional presence of strand breaks in the γ-irradiated substrate. Alternatively, it is conceivable that despite their close structural relatedness the major radiation products of thymine, i.e., 5-hydroxy-6-hydroperoxy-dihydrothymine and its isomers, and the OsO_4-oxidation product, *cis*-5,6-dihydroxy-dihydrothymine, are excised from the polymer with different efficiencies by the repair enzymes of the *E. coli* extracts.

CONCLUDING REMARKS

It should be clearly recognized that the excision of one class of thymine radiation products from a polydeoxynucleotide was studied in our work. However, a large variety of structurally different products is formed from the nucleic acid bases by ionizing radiation *in vitro* (8, 22) and most probably also *in situ* in the living cell (9, 23). Results concerning the repair and biological effects of one class of products are not necessarily representative of other types of base damage. In particular, damage recognition and incision in prereplication excision repair could involve different enzymes for different types of base damage. Gamma-irradiated DNA may contain a considerable amount of sugar residues that lack the heterocyclic base. Depurination and depyrimidination without consecutive strand breakage may occur by radiation-induced cleavage of the *N*-glycosidic bond or as a secondary reaction after radiation-induced saturation or fragmentation of the heterocyclic moiety. Evidence for the second mechanism has been given under *Experimental Procedures* and will be discussed in detail elsewhere (Dunlap, Hariharan, and Cerutti, unpublished results). Apurinic and apyrimidinic sites* may therefore represent an important class of γ-ray-induced DNA lesions. It is obvious from these considerations that only a start has been made in the elucidation of excision repair of nucleic acid base damage induced by ionizing radiation.

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