Jovin, T. M., Englund, P. T., & Bertsch, L. L. (1969) *J. Biol. Chem. 244*, 2996.
Joyce, C. M., & Grindley, N. D. F. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 1830.
Kornberg, A. (1980) *DNA Replication*, W. H. Freeman, San Francisco, CA.
Kunkel, T. A., Schaaper, R. M., Beckman, R. A., & Loeb, L. A. (1981) *J. Biol. Chem. 256*, 9883.
Lanzetta, P. A., Alvarez, L. J., Reinach, P. S., & Candia, O. A. (1979) *Anal. Biochem. 100*, 75.
McClure, W. R., & Jovin, T. M. (1975) *J. Biol. Chem. 250*, 4073.
Modrich, P., & Lehman, I. R. (1970) *J. Biol. Chem. 245*, 3226.
Seal, G., & Loeb, L. A. (1976) *J. Biol. Chem. 251*, 975.
Setlow, P., Brutlag, D., & Kornberg, A. (1972) *J. Biol. Chem. 247*, 224.
Sharp, T. R., & Benkovic, S. J. (1979) *Biochemistry 18*, 2910.
Walseth, T. F., Graff, G., Krick, T. P., & Goldberg, N. D. (1981) *J. Biol. Chem. 256*, 2176.

Thymine Lesions Produced by Ionizing Radiation in Double-Stranded DNA

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ABSTRACT: A DNA glycosylase which catalyzes the release of thymine residues damaged by ring saturation, fragmentation, or ring contraction from double-stranded DNA has been used to characterize such base derivatives in γ-irradiated DNA. It is shown by chromatographic analysis that irradiation of DNA in neutral solution generates the ring-saturated forms *cis*-thymine glycol, *trans*-thymine glycol, and a monohydroxydihydrothymine, probably 6-hydroxy-5,6-dihydrothymine. The latter compound is only observed after irradiation under hypoxic conditions. The ring-contracted thymine derivative 5-hydroxy-5-methylhydantoin is also formed, and it is the major lesion after irradiation of DNA under O_2. Ring-fragmented products such as methyltartronylurea were only generated in small quantities. Isolation and analysis of the DNA from γ-irradiated human cells also revealed the formation of ring-saturated thymine derivatives, but 5-hydroxy-5-methylhydantoin was not found in this case.

An important component of cellular injury following ionizing radiation consists of base damage in DNA (Cerutti, 1976). This type of radiation damage has not been characterized as extensively as DNA strand breaks or large-scale chromosomal alterations, at least in part because of the technical difficulties involved in the detection and analysis of the lesions. Studies with prokaryotes and lower eukaryotes which permit detailed genetic analysis have indicated that base substitutions are the most common type of mutational event caused by ionizing radiation; both transitions and transversions occur, at A·T as well as at G·C base pairs (Glickman et al., 1980). In general, A·T pairs appear more susceptible than G·C pairs, as judged from the ability of various tester strains employed in the "Ames test" to detect mutations caused by oxidative damage and ionizing radiation (Levin et al., 1982). DNA pyrimidine residues seem more sensitive to radiation than purines, and major pathways of their degradation involve the saturation of the 5,6 double bond and fragmentation of the pyrimidine ring (Scholes, 1976; Teoule et al., 1977).

Quantitative assessments of the spectrum of base lesions produced in DNA by ionizing radiation face the problem that several products are acid labile and decompose into secondary derivatives during chemical hydrolysis. To circumvent this difficulty, we have used a reagent enzyme of broad specificity which releasese altered thymine residues in free form from double-stranded γ-irradiated DNA under mild conditions. The released material has been characterized by chromatographic analysis. The enzyme employed is *Escherichia coli* endonuclease III (also called thymine glycol–DNA glycosylase, urea–DNA glycosylase, and X-ray endonuclease), which is the product of the *nth*$^+$ gene (Cunningham & Weiss, 1985). This enzyme is specific for double-stranded DNA and catalyzes the release of thymine residues damaged by ring saturation, fragmentation, or ring contraction by cleavage of the appropriate glycosyl bonds; in addition, the enzyme has an associated endonuclease activity for apyrimidinic and apurinic sites (Demple & Linn, 1980; Breimer & Lindahl, 1980, 1984; Katcher & Wallace, 1983).

Experimental Procedures

Materials and Reference Compounds. [*methyl*-^{3}H]TTP (45 Ci·mmol^{-1}), [2-^{14}C]TTP (50 mCi·mmol^{-1}), [*methyl*-^{3}H]thymidine (90 Ci·mmol^{-1}), and [*methyl*-^{14}C]thymine (58 mCi·mmol^{-1}) were obtained from Amersham. *E. coli* DNA polymerase I, pancreatic DNase I, and S1 nuclease were purchased from Boehringer. *E. coli* endonuclease III was purified as described (Breimer & Lindahl, 1984). *cis*-Thymine glycol was prepared from [*methyl*-^{14}C]thymine by OsO_4 treatment (Burton & Riley, 1966). To generate the trans form, this compound was isomerized by heating in aqueous solution at 95 °C for 4 h (Barszcz et al., 1963). 6-Hydroxy-5,6-dihydrothymine was observed (by chromatography) as one of the major products of [^{14}C]thymine γ-irradiated under N_2, in agreement with the results of Nofre & Cier (1966), and this compound was purified by reverse-phase high-pressure liquid chromatography (HPLC) on a Micro-Pac MCH-10 column with water as eluant. 5-Hydroxy-5-methylhydantoin was obtained by condensation of [^{14}C]urea with pyruvic acid

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(Murahashi et al., 1966). Methyltartronylurea was prepared according to Iida & Hayatsu (1970). Dihydrothymine and 5-(hydroxymethyl)uracil were purchased from Sigma.

Nucleic Acids. Double-stranded, covalently closed circular DNA of plasmid pAT 153 (a derivative of pBR322) was nick translated in the presence of [^{3}H]TTP according to Rigby et al. (1977). After purification by phenol treatment, ethanol precipitation, and gel filtration, the DNA had a specific radioactivity of 3.8×10^6 cpm·μg^{-1}. This DNA was used within 2 days of preparation to avoid the accumulation of thymine derivatives generated by ^{3}H decay. Poly(dA), poly([^{3}H]dT), and poly([^{14}C]dT) were made with terminal deoxynucleotidyltransferase (Kato et al., 1967). Poly(dT) was annealed with an equimolar amount of poly(dA) to generate a double-stranded structure.

Irradiation of DNA. Radioactively labeled DNA was dialyzed against 1 mM potassium phosphate, pH 7.4 (prepared with doubly distilled H_2O), and transferred to microreaction V glass vials (Wheaton Scientific, Millville, NJ), each containing 8 μg of DNA in 200 μL. For hypoxic irradiation, the solutions were saturated with N_2 and the vials sealed. To generate oxic conditions, gaseous O_2 was bubbled continuously through the solutions. Irradiation was at 10 °C from a ^{60}Co source at a dose rate of 240 Gy·min^{-1}. Each DNA sample was precipitated with 2 volumes of ethanol at −20 °C in the presence of 0.5 M NaCl, centrifuged, redissolved in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl) and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5, and dialyzed against the same buffer at 4 °C for 14 h prior to incubation with the reagent enzyme.

Irradiation of Cells. The human B-cell lymphoma line Raji, growing in suspension culture in 200 mL of RPMI 1640 medium supplemented with 10% dialyzed fetal calf serum, was labeled with 1 mCi of [^{3}H]thymidine for 24 h. The cells were collected by centrifugation, washed 3 times in Dulbecco's phosphate-buffered saline (containing Mg^{2+} and Ca^{2+}), and immediately irradiated with a dose of 2000 Gy under N_2 at 0 °C, at a dose rate of 966 Gy·min^{-1}. Within 30 s of the dose having been delivered, the suspensions were supplemented with EDTA (to 10 mM) and Sarkosyl (to 2%) and agitated vigorously. These procedures were employed to minimize intracellular DNA repair during and after the radiation treatment. DNA was prepared by treatments with proteinase K and phenol, followed by ethanol precipitation, dialysis, and immediate analysis. The isolated DNA had a specific radioactivity of 6×10^4 cpm·μg^{-1}.

Analysis of Radiation Products. Altered forms of thymine were released as free bases from DNA by incubation with an excess of endonuclease III (Breimer & Lindahl, 1984). Irradiated nick-translated DNA (10^7 cpm) or DNA from irradiated cells (2×10^7 cpm) in 250 μL of 0.1 M KCl, 70 mM *N*-(2-hydroxyethyl)piperazine–*N'*-2-ethanesulfonic acid–potassium hydroxide (Hepes–KOH), 1 mM EDTA, and 1 mM dithiothreitol, pH 7.6, was incubated with 0.1 μg of endonuclease III at 37 °C for 20 min. The reaction mixtures were chilled to 0 °C, mixed with 25 μL of 2 M NaCl, 35 μL of 0.2% heat-denatured calf thymus DNA, and 640 μL of cold ethanol, and kept at −70 °C for 20 min. The DNA was removed by centrifugation, and the supernatant solutions were lyophilized, redissolved in 150 μL of cold H_2O, and divided into five aliquots for chromatographic analyses.

Reverse-phase HPLC analysis of radioactive thymine derivatives was performed on a Varian 5000 liquid chromatograph equipped with an MCH-10 column (4 mm × 30 cm, Micro-Pac). H_2O was used as eluant, at 0.2 mL·min^{-1} for 30 min and then at 2 mL·min^{-1}. Descending paper chromatography was performed with Whatman 3MM paper in the following systems: (I) 1-butanol/formic acid/H_2O (10:2:15); (II) ethyl acetate/1-propanol/H_2O (4:1:2); (III) ethyl acetate/acetic acid/H_2O (3:1:1); (IV) 1-butanol/ethanol/H_2O (4:1:5); (V) phenol/H_2O (9:1). Radioactive material and reference compounds were measured as described (Breimer & Lindahl, 1980).

Results

The DNA glycosylase activity of *E. coli* endonuclease III was employed to release oxidatively damaged thymine residues from double-stranded DNA by gentle enzymatic hydrolysis. Several preliminary experiments were performed to validate this approach. When poly([^{3}H]dT) containing 10 nmol of thymine residues (1.7×10^6 cpm) was irradiated (200 Gy under N_2) in either single-stranded or double-stranded form and [after annealing single-stranded poly(dT) with poly(dA)] incubated with the enzyme, 5–10 times more thymine glycol (50 vs. 5–10 pmol) was obtained in the former case. This is in good agreement with the results of Swinehart et al. (1974), who, in a comparison of the production of ring-saturated thymines by γ-irradiation in single-stranded *φX174* and double-stranded *E. coli* DNA, observed a 9–12-fold protection by the double-stranded structure. In another experiment, single-stranded poly(dT) substrate was challenged with endonuclease III either directly or after annealing with an equimolar amount of poly(dA). The rate of release of damaged base residues from the single-stranded polymer was less than 2% of that from the double-stranded material. This confirms the strong preference for double-stranded DNA as the enzyme substrate. A comparison of the product spectra obtained with poly(dA)·poly([*methyl*-^{3}H]dT) and poly(dA)·poly([2-^{14}C]dT) revealed no major differences at the low levels of thymine alteration investigated here, and ^{3}H-labeled material of high specific radioactivity was used in subsequent experiments. Variations of the amount of endonuclease III in reaction mixtures with irradiated substrate (2–6-fold enzyme excess) did not alter detectably either the total amount or the product spectrum of the enzymatically released material. This is in agreement with our previous observation (Breimer & Lindahl, 1984) that thymine glycol, 5-hydroxy-5-methylhydantoin, and ring-fragmented derivatives such as methyltartronylurea and urea are excised at similar rates.

Irradiation of Purified DNA. Freshly prepared, nick-translated [^{3}H]thymine-labeled DNA of high specific radioactivity was irradiated with a dose of 200 Gy under hypoxic conditions (in N_2-saturated buffer). This treatment generated <5% S1 nuclease sensitive material, indicating that the double-stranded DNA structure was largely preserved. After treatment of this DNA, and an unirradiated control sample, with an excess of endonuclease III to release altered thymines in free form, the DNA was removed by ethanol precipitation, and the low molecular weight material was analyzed by HPLC. Four distinct peaks of thymine products were present in the material enzymatically liberated from the irradiated DNA (Figure 1). These compounds were not present in supernatants from unirradiated, enzyme-treated DNA (data not shown). In addition, free thymine was observed in the supernatants after the irradiated DNA had been incubated either with or without enzyme; this probably reflects sugar damage leading to spontaneous breakage of labilized glycosyl bonds (Figure 1). The first two peaks (Figure 1) enzymatically released from the γ-irradiated DNA were identified as *trans*-thymine glycol (0.08 pmol) and *cis*-thymine glycol (0.11 pmol), respectively, by cochromatography with authentic

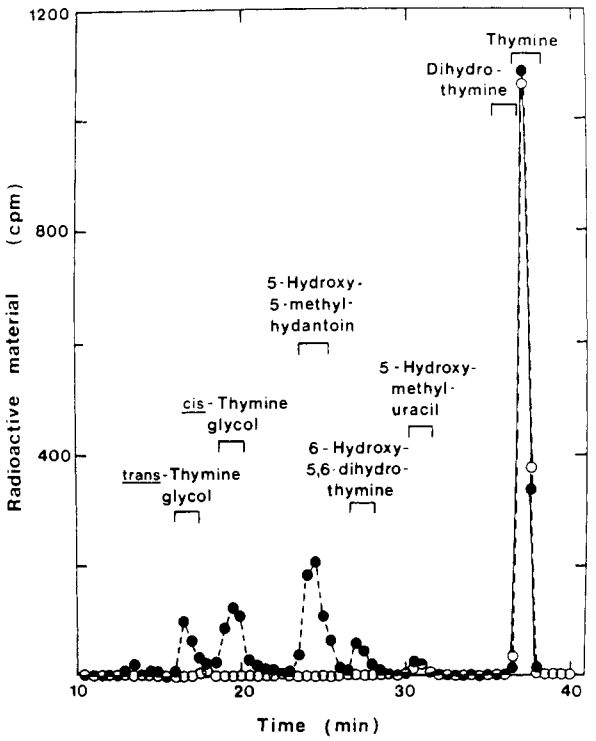

FIGURE 1: HPLC profile of the ethanol-soluble material released by endonuclease III from nick-translated [^{3}H]thymidine-labeled DNA irradiated with a dose of 200 Gy under N_2. Reverse-phase HPLC was performed with H_2O as eluant. Authentic markers were eluted as indicated. (●) Enzyme added; (○) no enzyme.

markers and by analysis in four different paper chromatography systems as well as by isomerization by heating followed by rechromatography. The third peak was identified as 5-hydroxy-5-methylhydantoin (0.16 pmol), again confirmed by paper chromatography in four different systems together with an authentic marker. The fourth, smaller peak (0.04 pmol) was tentatively identified as 6-hydroxy-5,6-dihydrothymine from its chromatographic properties. However, we have not ruled out that this peak could contain a mixture of the two different monohydroxythymine hydrates, i.e., 5-hydroxy-6-dihydrothymine and 6-hydroxy-5,6-dihydrothymine. The cis and trans forms of the latter derivative elute at similar positions (Cadet et al., 1982). No dihydrothymine was formed (Figure 1). Furthermore, no radioactive material eluted at the position of methyltartronylurea (11.5 min), showing that this compound was not a major radiation product (Figure 1).

Similar product spectra were obtained with DNA irradiated with a dose of either 200 or 600 Gy in the presence of O_2, except that an even higher proportion of 5-hydroxy-5-methylhydantoin was present. Moreover, a monohydroxydihydrothymine derivative was not observed in this case; it seems likely that dihydroxyhydrothymines (thymine glycols) would be the dominant ring-saturated compounds formed in the presence of O_2. A typical chromatogram is shown in Figure 2. A small amount of γ-radiation-induced methyltartronylurea (2–3% of the total radiation products) could be identified by two-dimensional paper chromatography after irradiation of DNA under oxygen.

Irradiation of Intracellular DNA. To evaluate the thymine lesions formed in irradiated human cells, lymphoma cells containing [^{3}H]thymidine-labeled DNA were irradiated at 966 Gy·min^{-1} (total dose 2000 Gy) at 0 °C and immediately lysed for DNA preparation. An HPLC profile of the material released by endonuclease III from the purified DNA (about 0.01% of the total radioactivity) is shown in Figure 3a. The

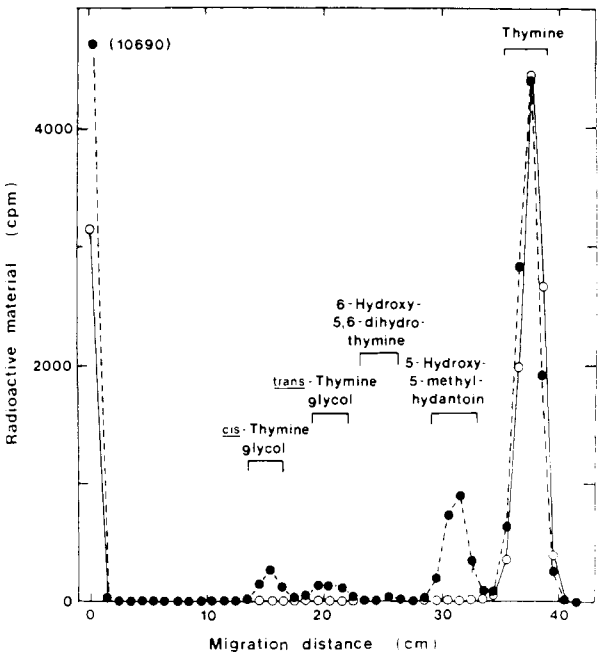

FIGURE 2: Paper chromatographic analysis of the ethanol-soluble material released by endonuclease III from [^{3}H]thymidine-labeled DNA irradiated in the presence of O_2 with a dose of 600 Gy. The paper was developed with system I (see Experimental Procedures). Brackets indicate reference compounds. (●) Enzyme added; (○) no enzyme.

enzyme liberated *cis*- and *trans*-thymine glycol, as well as a monohydroxythymine hydrate. However, the results differed from those obtained with irradiated purified DNA in that 5-hydroxy-5-methylhydantoin was not observed. This could be due to the more hypoxic environment, with selective quenching of the formation of 5-hydroxy-5-methylhydantoin, or possibly to very rapid repair of this particular lesion in vivo. The DNA from irradiated cells contained many strand breaks, leading to a high "background" in the HPLC profile of oligonucleotides which were identified as such by paper chromatography. For this reason, the material enzymatically liberated from DNA isolated from irradiated cells was also analyzed directly by paper chromatography (Figure 3b), with similar results.

DISCUSSION

In the present work, damaged thymine residues have been enzymatically liberated from γ-irradiated DNA and identified and quantitated by chromatographic analysis (Figure 4). The DNA glycosylase employed for this purpose acts on ring-saturated and ring-fragmented thymines in double-stranded DNA, but it does not release altered pyrimidines in which the 5,6 double bond is retained, such as uracil and 5-(hydroxymethyl)uracil (Katcher & Wallace, 1983; Breimer & Lindahl, 1984; Hollstein et al., 1984). The latter compound, which presumably retains unaltered coding properties and therefore may be of little biological relevance, is one of the major γ-irradiation products of thymine in DNA (Teebor et al., 1984), but it would not have been released here.

Teoule and co-workers (Teoule et al., 1977, 1978) have characterized in detail the various thymine radiation products in hydrolysates of DNA γ-irradiated in aerated solution. The main thymine lesions observed here by enzymatic analysis of irradiated DNA are among those described by Teoule et al.; these derivatives are the *cis*- and *trans*-thymine glycols and 5-hydroxy-5-methylhydantoin. In addition, radiation under hypoxic conditions generated a monohydroxydihydrothymine, probably 6-hydroxy-5,6-dihydrothymine, in agreement with

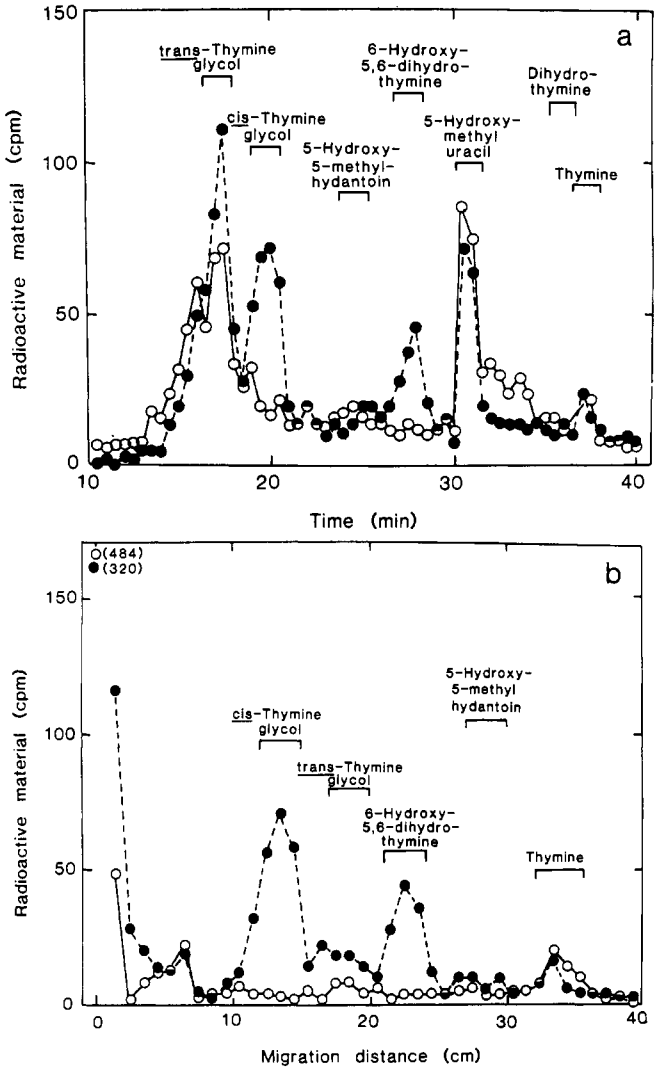

FIGURE 3: Analytical chromatography of the ethanol-soluble material released by endonuclease III from DNA which had been isolated from Raji cells irradiated under N_2 with a dose of 2000 Gy at 0 °C. (a) Reverse-phase HPLC. Authentic markers are indicated. (b) Paper chromatogram developed with system I: (●) enzyme added; (○) no enzyme.

the results of Nofre & Cier (1966) and Teoule et al. (1978). 5-Hydroxy-5-methylhydantoin, however, was not detected by us in DNA from cells γ-irradiated under hypoxic conditions.

Endonuclease III employed here was used previously to characterize the thymine lesions in DNA treated with an oxidizing agent, $KMnO_4$ (Breimer & Lindahl, 1984). In that case, it was found that the major base lesion after mild oxidation at neutral pH was the ring-fragmented derivative methyltartronylurea, observed earlier in $KMnO_4$-treated thymine and thymidine by Iida & Hayatsu (1970, 1971). Since that particular base derivative is acid and alkali labile (and is not retained significantly in most HPLC systems), it appeared that it might be a significant thymine lesion in γ-irradiated DNA which could have been overlooked previously. However, in contrast to our results with oxidized DNA, methyltartronylurea was only formed in trace quantities in γ-irradiated DNA. The two agents, a chemical oxidant and ionizing radiation, both generate thymine glycol and 5-hydroxy-5-methylhydantoin in DNA but differ in their product spectrum. Thus, methyltartronylurea is generated from thymine in DNA as a major lesion after $KMnO_4$ treatment but not after γ-irradiation. Another difference between these agents is that γ-irradiation causes opening of imidazole rings

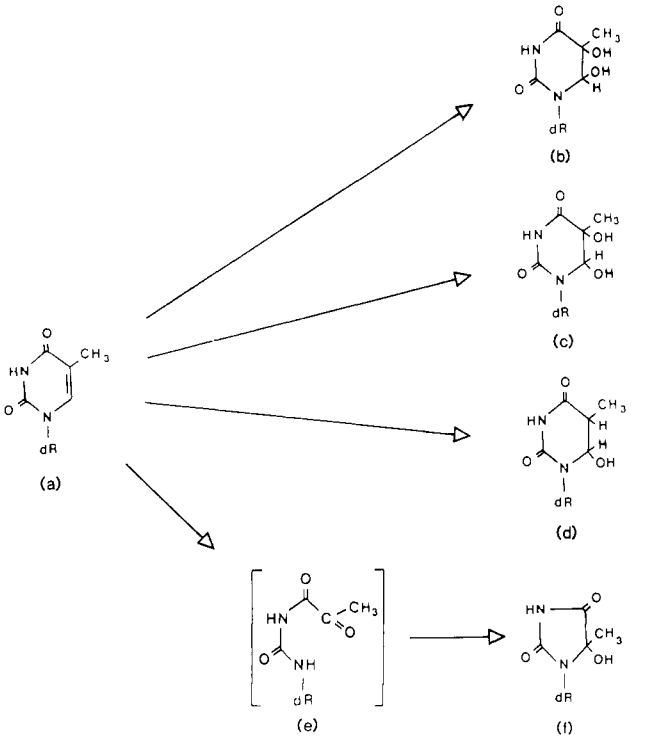

FIGURE 4: Major radiation products of thymine identified in double-stranded DNA by endonuclease III digestion. The compounds are shown attached to a deoxyribose moiety (dR). (a) Thymine; (b) *cis*-thymine glycol; (c) *trans*-thymine glycol; (d) 6-hydroxy-5,6-dihydrothymine; (e) pyruvylurea; (f) 5-hydroxy-5-methylhydantoin. The generation of compounds b–d from compound a occurred through labile hydroperoxy intermediates (Teoule et al., 1977). Compound e was probably derived by direct loss of C-6 following hydroxyl radical addition at this site; it cyclizes spontaneously into compound f.

of adenines in DNA (Bonicel et al., 1980; Breimer, 1984) while adenine residues are resistant to $KMnO_4$ oxidation (Jones et al., 1964; Breimer & Lindahl, 1980).

Cathcart et al. (1984) have recently described a noninvasive assay for the evaluation of oxidation damage to DNA in mammals based on measurements of thymine glycol in the urine. This seems to be an excellent strategy for several reasons, including the fact that mammalian cells possess a thymine glycol–DNA glycosylase similar to the *E. coli* endonuclease III (Breimer, 1983; Hollstein et al., 1984). In addition, thymine glycol would appear to be the only thymine lesion that is consistently detected in relatively large amounts in DNA γ-irradiated under either O_2 or N_2, in solution or intracellularly, and this compound is also a common lesion in chemically oxidized DNA.

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Registry No. Thymine, 65-71-4; *cis*-thymine glycol, 1124-84-1; *trans*-thymine glycol, 1431-06-7; 6-hydroxy-5,6-dihydrothymine, 13514-92-6; 5-hydroxy-5-methylhydantoin, 10045-58-6; methyltartronylurea, 90773-29-8; *E. coli* endonuclease III, 60184-90-9; oxygen, 7782-44-7.

REFERENCES

Barszcz, D., Tramer, Z., & Shugar, D. (1963) *Acta Biochim. Pol. 10*, 9–24.

Bonicel, A., Marriaggi, N., Hughes, E., & Teoule, R. (1980) *Radiat. Res. 83*, 19–26.

Breimer, L. H. (1983) *Biochemistry 22*, 4192–4197.

Breimer, L. H. (1984) *Nucleic Acids Res. 12*, 6359-6367.
Breimer, L. H., & Lindahl, T. (1980) *Nucleic Acids Res. 8*, 6199-6211.
Breimer, L. H., & Lindahl, T. (1984) *J. Biol. Chem. 259*, 5543-5548.
Burton, K., & Riley, W. T. (1966) *Biochem. J. 98*, 70-77.
Cadet, J., Berger, M., & Voituriez, L. (1982) *J. Chromatogr. 238*, 488-494.
Cathcart, R., Schwiers, E., Saul, R. L., & Ames, B. N. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5633-5637.
Cerutti, P. A. (1976) in *Photochemistry and Photobiology of Nucleic Acids* (Wang, S. Y., Ed.) Vol. II, pp 375-401, Academic Press, New York.
Cunningham, R. P., & Weiss, B. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 474-478.
Demple, B., & Linn, S. (1980) *Nature (London) 287*, 203-208.
Glickman, B. W., Rietveld, K., & Aaron, C. S. (1980) *Mutat. Res. 69*, 1-12.
Hollstein, M. C., Brooks, P., Linn, S., & Ames, B. N. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4003-4007.
Iida, S., & Hayatsu, H. (1970) *Biochim. Biophys. Acta. 213*, 1-13.
Iida, S., & Hayatsu, H. (1971) *Biochim. Biophys. Acta 218*, 1-8.
Jones, A. S., Ross, G. W., Takemura, S., Thompson, T. W., & Walker, R. T. (1964) *J. Chem. Soc.*, 373-378.
Katcher, H. L., & Wallace, S. S. (1983) *Biochemistry 22*, 4071-4082.
Kato, K. I., Goncalves, J. M., Houts, G. E., & Bollum, F. J. (1967) *J. Biol. Chem. 242*, 2780-2789.
Levin, D. E., Hollstein, M., Christman, M. F., Schwiers, E. A., & Ames, B. N. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7445-7449.
Murahashi, S., Yuki, H., Kosai, K., & Doura, F. (1966) *Bull. Chem. Soc. Jpn. 39*, 1559-1562.
Nofre, C., & Cier, A. (1966) *Bull. Soc. Chim. Fr.*, 1326-1333.
Rigby, P. W. J., Dieckmann, M., Rhodes, C., & Berg, P. (1977) *J. Mol. Biol. 113*, 237-251.
Scholes, G. (1976) in *Photochemistry and Photobiology of Nucleic Acids* (Wang, S. Y., Ed.) Vol. I, pp 521-574, Academic Press, New York.
Swinehart, J. L., Lin, W. S., & Cerutti, P. A. (1974) *Radiat. Res. 58*, 166-175.
Teebor, G. W., Frenkel, K., & Goldstein, M. S. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 318-321.
Teoule, R., Bert, C., & Bonicel, A. (1977) *Radiat. Res. 72*, 190-200.
Teoule, R., Bonicel, A., Bert, C., & Fouque, B. (1978) *J. Am. Chem. Soc. 100*, 6749-6750.

Change of Conformation and Internal Dynamics of Supercoiled DNA upon Binding of *Escherichia coli* Single-Strand Binding Protein†

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ABSTRACT: The influence of *Escherichia coli* single-strand binding (SSB) protein on the conformation and internal dynamics of pBR322 and pUC8 supercoiled DNAs has been investigated by using dynamic light scattering at 632.8 and 351.1 nm and time-resolved fluorescence polarization anisotropy of intercalated ethidium. SSB protein binds to both DNAs up to a stoichiometry that is sufficient to almost completely relax the superhelical turns. Upon saturation binding, the translational diffusion coefficients (D_0) of both DNAs decrease by approximately 20%. Apparent diffusion coefficients (D_{app}) obtained from dynamic light scattering display the well-known increase with $\boldsymbol{K}^2$ ($\boldsymbol{K}$ = scattering vector), leveling off toward a plateau value (D_{plat}) at high $\boldsymbol{K}^2$. For both DNAs, the difference $D_{plat} - D_0$ increases upon relaxation of supercoils by SSB protein, which indicates a corresponding enhancement of the subunit mobilities in internal motions. Fluorescence polarization anisotropy measurements on free and complexed pBR322 DNA indicate a (predominantly) uniform torsional rigidity for the saturated DNA/SSB protein complex that is significantly reduced compared to the free DNA. These observations are all consistent with the notion that binding of SSB protein is accompanied by a gradual loss of supercoils and saturates when the superhelical twist is largely removed.

It is an established fact that supercoiling can influence the local structure of DNA in a variety of ways. Since the native superhelical twist of DNA is opposite to the sense of twist of the B-DNA helix, supercoiling generally destabilizes the B helix, making processes that decrease the superhelical density of covalently closed circular DNA chains such as cruciform formation (Lilley, 1980; Panayotatos & Wells, 1981; Mizuuchi et al., 1982; Lilley & Markham, 1983) or B- to Z-helix transition (Pohl & Jovin, 1972; Wang et al., 1979; O'Connor

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