Identification of the *cis*-Thymine Glycol Moiety in Chemically Oxidized and γ-Irradiated Deoxyribonucleic Acid by High-Pressure Liquid Chromatography Analysis†

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ABSTRACT: 5,6-Dihydroxy-5,6-dihydrothymine (thymine glycol) is formed in DNA by chemical oxidants and ionizing radiation. We describe the separation of thymine glycol, 5,6-dihydroxy-5,6-dihydrothymidine (thymidine glycol), thymine, and thymidine by high-pressure liquid chromatography (HPLC). Enzymatic hydrolysates of chemically oxidized or γ-irradiated single-stranded DNA were cochromatographed with ^{14}C-containing marker compounds. In chemically oxidized DNA, thymidine glycol was the major derivative formed. In addition, there were four rapidly eluting thymine-derived components. In irradiated DNA, thymidine glycol constituted about 5% of the modified thymines, and the rapidly eluting fractions were proportionately increased. DNA isolated from γ-irradiated and nonirradiated HeLa cells grown in the presence of [^3H]thymidine was subjected to enzymatic hydrolysis and HPLC analysis. In control DNA, 0.3% of the thymines were modified. Thirty-six kilorads of γ radiation caused a 30% increase in thymine damage. Thus, most of the base damage was due to internal β radiation from incorporated [^3H]thymidine. The chromatographic patterns of irradiated and nonirradiated samples were qualitatively the same, but the yields of some products increased 2-fold, while others remained unchanged. A comparison of the HPLC profiles of hydrolysates of in vitro oxidized and irradiated DNA with those of the cellular DNA revealed one fast eluting peak to be absent in cellular DNA, suggesting that it was formed only in single-stranded DNA. In cellular DNA, the major modified thymine was a more hydrophobic derivative not formed by in vitro radiation nor chemical oxidation. As in in vitro irradiated DNA, thymidine glycol constituted 5% of the modified thymines. The presence of *cis*-thymidine glycol in hydrolysates was confirmed by chromatography on Sephadex LH-20 using water and borate as eluants.

Thymine glycol[1] and other ring-saturated thymine derivatives are formed in DNA by the action of ionizing and near-ultraviolet irradiation (Hariharan & Cerutti, 1972, 1977; Teoule et al., 1974, 1977) and also by oxidation with $KMnO_4$ and OsO_4 (Beer et al., 1966; Iida & Hayatsu, 1971; Hariharan & Cerutti, 1974, 1977; Frenkel et al., 1981). These compounds seem to be removed from irradiated cellular DNA by repair processes (Hariharan & Cerutti, 1971; Painter & Young, 1972; Mattern et al., 1973; Mattern & Welch, 1979) and are removed from oxidized DNA by enzyme activities isolated from bacteria (Hariharan & Cerutti, 1974; Demple & Linn, 1980).

We previously described a rapid purification of thymine glycol and thymidine glycol and demonstrated the applicability of that method to the determination of thymidine glycol content of oxidized DNA (Frenkel et al., 1981). Chromatography of LH-20 separated thymidine and thymine glycols from thymidine and thymine. However, thymine glycol could not be separated from thymidine glycol on a short analytical column. Furthermore, the method could not be applied to DNA containing a few modified thymines because the large thymidine peak overlapped the much smaller glycol peak.

We now describe the separation of thymine and thymidine glycols and thymine and thymidine by HPLC and the analysis of enzymatic hydrolysates of oxidized and γ-irradiated DNA. HPLC followed by LH-20 chromatography also enabled us to detect thymidine glycol in the ^3H-labeled DNA of HeLa cells.

Experimental Procedures

Materials

[*methyl*-^{14}C]Thymine (49.6 mCi/mmol), [*methyl*-^{14}C]-thymidine (48.2 mCi/mmol), and [*methyl*-^3H]thymidine (6.7 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Sephadex LH-20 and Sephadex G-50 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. *Escherichia coli* W 3110 (*thy*$^-$) J. Cairns strain was obtained from the *E. coli* Genetic Stock Center at Yale University School of Medicine, New Haven, CT. Cell and bacteria growth media, calf serum, and L-glutamine were purchased from GIBCO, Grand Island, NY. OsO_4 was obtained from Sigma, St. Louis, MO, and $KMnO_4$ from Fisher Scientific Co., Fair Lawn, NJ.

Methods

Synthesis and Purification of cis-Thymine Glycol and cis-Thymidine Glycol. Synthesis and purification of ^{14}C-containing glycols was accomplished as previously described (Iida & Hayatsu, 1970, 1971; Frenkel et al., 1981). These two compounds were further purified by HPLC (Beckman, model 330) on a preparative reverse-phase 5-μm Ultrasphere-ODS column (10 mm i.d. × 25 cm) with water (deionized, glass distilled) as eluant. Fractions containing thymine or thymidine glycol were combined, and water was removed (under 30 °C) by flash evaporation (Buchler Instruments).

Preparation of E. coli [^3H]*DNA. E. coli* W 3110 (*thy*$^-$) J. Cairns strain was grown in minimal A medium according

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[1] Abbreviations used: thymine glycol, 5,6-dihydroxy-5,6-dihydrothymine; thymidine glycol, 5,6-dihydroxy-5,6-dihydrothymidine; HPLC, high-pressure liquid chromatography; LH-20, Sephadex LH-20; PBS, phosphate-buffered saline; UV, ultraviolet; AP, apurinic/apyrimidinic; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; rd, rad; krd, kilorad.

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to DeLucia & Cairns (1969) and [^{3}H]DNA isolated by the method of Marmur (1961). The purified DNA was dissolved in 0.1 M NaCl, 0.02 M Tris-HCl, and 0.001 M EDTA, pH 7.5, buffer (Frenkel et al., 1981). The specific activity of the DNA was 1800 cpm/μg, assuming that 1 A_{260} unit = 50 μg of DNA/mL.

Oxidation of [^{3}H]DNA. The double-stranded *E. coli* [^{3}H]DNA was denatured by heating at 100 °C for 5 min followed by rapid cooling in an ice–water bath. Oxidation of the resultant single-stranded DNA with $KMnO_4$ was carried out at pH 8.6 in ice–water as previously reported (Iida & Hayatsu, 1971; Frenkel et al., 1981) and with 0.4% OsO_4 at 55 °C as described by Hariharan & Cerutti (1974). The oxidized DNA was purified by Sephadex G-50 filtration with 0.15 M KCl, 0.01 M K_2HPO_4, and 0.001 M EDTA, pH 8.0, buffer as eluant.

γ Irradiation of DNA. Double-stranded *E. coli* [^{3}H]DNA was dialyzed at 4 °C against 0.001 M potassium phosphate buffer, pH 7.4, heat denatured, and irradiated at room temperature by a "Gammator" ^{137}Cs source at a dose rate of 600 rd/min for 2 h. The dose rate was determined with thermoluminescent dosimeters (Harshaw Chemical Co., Solon, OH) calibrated against a standard ^{60}Co source.

γ Irradiation of HeLa Cells and Isolation of [^{3}H]DNA from Irradiated and Control Cells. HeLa cells were grown at 37 °C in spinner bottles in minimum essential medium (MEM; Joklik modified) supplemented with 10% calf serum and L-glutamine (to 0.002 M). When there were 3×10^5 cells/mL, 750 μCi of [^{3}H]thymidine were added to 600 mL of cells. There were 7×10^5 cells/mL after 24 h. The medium was removed by centrifugation at 1500 rpm at 4 °C for 10 min (International Centrifuge IEC, Model PR-2). Cells were washed once with PBS, resuspended in 600 mL of the growth MEM supplemented with thymidine (6 mL of 0.001 M thymidine in water), and grown at 37 °C for 2 h. Cells were again centrifuged at 1500 rpm at 4 °C for 10 min, washed once with cold PBS, and, after resuspension in 60 mL of cold PBS, divided into two tubes. One tube was placed in an ice-water bath for 1 h, while the other, also in an ice-water bath, was irradiated by a ^{137}Cs source for 1 h (36 krd). The cells from both tubes were then centrifuged, and the DNA was isolated according to Marmur (1961). The purified DNA had an A_{260}/A_{280} ratio of 1.85 and specific activity of 1.5×10^5 dpm/μg.

Enzymatic Hydrolysis of Oxidized and Irradiated DNA. [^{3}H]DNA oxidized with $KMnO_4$ or OsO_4 or γ-irradiated was enzymatically digested to 2′-deoxyribonucleosides as described by Frenkel et al. (1981). The hydrolysates were precipitated with 5 volumes of acetone (HPLC grade, Fisher Scientific Co.) and centrifuged (Sorvall RC-2 with SS-34 rotor) at 7000 rpm, and supernatants were removed and evaporated to dryness. The residues were dissolved in water and, after filtration through a Millipore 0.22-μm filter, were ready for the HPLC analysis.

HPLC and Sephadex LH-20 (LH-20) Analysis. ^{14}C-Containing thymine and thymidine glycols and thymine and thymidine were separated on an analytical 5-μm Ultrasphere-ODS column (4.6 i.d. × 25 cm) with water as eluant on a Beckman HPLC, Model 330. The initial flow rate was 0.5 mL/min and, after 10 min, was increased to 2 mL/min. Fractions were collected at 0.5-min intervals (Figure 1). Enzymatic hydrolysates of chemically oxidized or γ-irradiated [^{3}H]DNA were injected into the preparative, 5-μm Ultrasphere-ODS column with water as an eluant and chromatographed together with ^{14}C-containing markers and additional nonradioactive thymine and thymidine as UV markers. The flow rate was 2 mL/min. Fractions were collected at 0.5-min intervals [1 mL (0.5 min)$^{-1}$ fraction^{-1}] and, starting with fraction 71, at 1-min intervals [2 mL min^{-1} fraction^{-1}]. The column was cleaned and conditioned after each analysis with a water–acetonitrile gradient by using a Beckman Model 330 gradient elution accessory equipped with microprocessor–controller, Model 421. Fractions were collected in scintillation vials in a LKB 2112 "RediRack" fraction collector and, after addition of Ready-Solv HP liquid scintillation cocktail (Beckman), counted in a Nuclear Chicago Mark II scintillation counter. When necessary, some fractions were collected in test tubes, concentrated on the flash evaporator, and then chromatographed on LH-20 with either water or borate buffer, pH 8.6, as eluant (Frenkel et al., 1981).

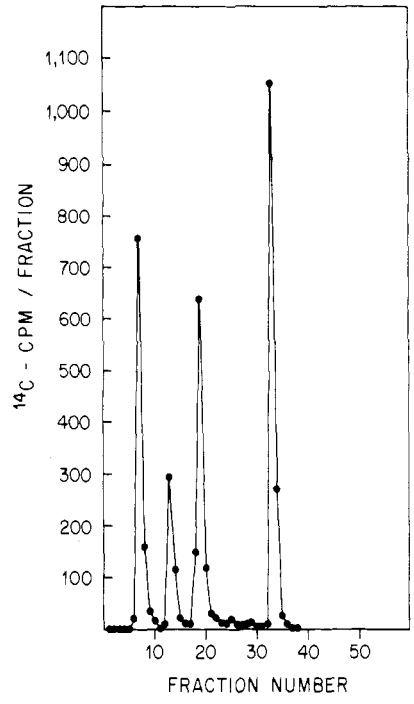

FIGURE 1: Chromatography of [^{14}C]thymine glycol, [^{14}C]thymidine glycol, [^{14}C]thymine, and [^{14}C]thymidine on an analytical ODS HPLC column with water as eluant. Details are given under Methods.

Results

Thymine glycol, thymidine glycol, thymine, and thymidine were separated on a reverse-phase analytical ODS HPLC column using water as eluant. As can be seen in Figure 1, these compounds were separated within 40 fractions in less than 20 min. Thymine glycol eluted in fraction 8 (4 min), thymidine glycol in fraction 13 (6.5 min), thymine in fraction 19 (9.5 min), and thymidine in fraction 33 (16.5 min).

So that enzymatic hydrolysates of oxidized DNA could be analyzed, a preparative ODS column was used. This allowed for the injection of larger volumes and also improved the separation of the marker compounds. Thymine glycol eluted in fraction 17, thymidine glycol between fractions 40 and 46, thymine between fractions 50 and 60, and thymidine between fractions 90 and 110, which correspond to retention times of 8.5, 20–23, 25–30, and 60–80 min, respectively (Figure 2). Figure 2 also shows a profile of an enzymatic hydrolysate of $KMnO_4$-oxidized ^{3}H-labeled DNA cochromatographed with the ^{14}C-containing standards. Thymidine was excluded from this and subsequent figures because there were no radioactive peaks eluting between fraction 80 and thymidine. The small difference in elution time between ^{3}H-labeled peaks and the ^{14}C-containing marker compounds is due to the "isotopic

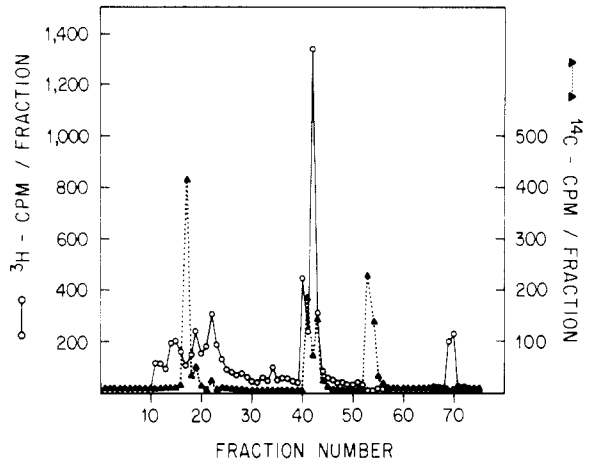

FIGURE 2: Chromatography of the enzymatic hydrolysate of $KMnO_4$-oxidized [^{3}H]DNA (○) and [^{14}C]thymine glycol, thymidine glycol, and thymine (▲) on a preparative ODS HPLC column with water as eluant.

effect" (Klein, 1966; Weinstein et al., 1976). This effect becomes more pronounced with increasing elution time. The extent of oxidization of $KMnO_4$ of this DNA sample was approximately 20%. Of 24 000 cpm of [^{3}H]DNA hydrolysate applied to the column, 2400 cpm were recovered as thymidine glycol (fractions 40–44) and 2400 in four rapidly eluting peaks (fractions 12–25). The additional small peak seen in the HPLC profile (fraction 70) was present in samples of control DNA and may represent a product of ^{3}H radiation; it generally constituted 0.5–1.5% of total applied radioactivity. The thymidine glycol peak split into two peaks which probably represent cis and trans isomers. This split was most apparent when stock ^{14}C-containing aqueous solutions were several weeks old. When a fresh solution was made, a single peak was seen. The presence of the two isomeric forms of thymidine glycol in water was also suggested by their separation on the preparative ODS column using 1% silver nitrate as eluant. Silver nitrate had been shown to effect separation of cis and trans isomers of olefins on reverse-phase HPLC columns (Schomburg & Zegarski, 1975). We applied this method to the analysis of a several-week-old solution of [^{14}C]thymidine glycol. A minor peak appeared in fraction 32 (16 min) while the major peak appeared in fraction 38 (19 min). Since we had previously determined that *cis*-thymidine glycol eluted in fraction 38, it is likely that the compound eluting in fraction 32 was *trans*-thymidine glycol. When eluted with water, the [^{3}H]thymidine glycol peak (Figure 2) also split into two, one of which was much larger than the other. The large peak (fraction 42) represented the cis isomer which is known to be formed by $KMnO_4$ oxidation of the thymine moiety (Iida & Hayatsu, 1971; Frenkel et al., 1981). Isomerization of the cis (Barszcz et al., 1963; Subbaraman et al., 1973; Teoule et al., 1974) to a small amount of what probably is the trans glycol (fraction 40) occurred during the overnight incubation at 37 °C while the oxidized DNA was subjected to enzymatic digestion. There was no coincidence of ^{3}H-containing material with [^{14}C]thymine glycol, indicating that during the overnight enzymatic digestion there was no release of intact thymine glycol from the DNA backbone. Similarly, there was no tritium present in the [^{14}C]thymine-containing fractions (53–55), showing that oxidation of DNA did not cause spontaneous release of unmodified thymine.

Figure 3 illustrates analysis of an enzymatic hydrolysate of OsO_4-oxidized [^{3}H]DNA. In this experiment, the thymidine glycol fractions showed a shoulder rather than two distinct peaks. This DNA contained 5% modified thymines which was

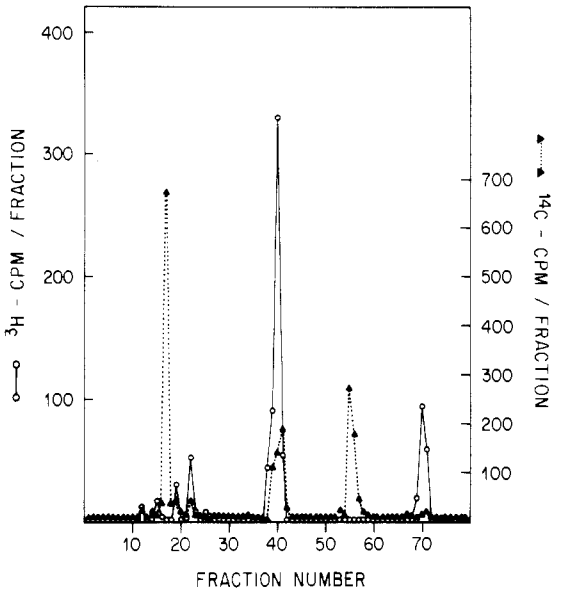

FIGURE 3: Chromatography of a mixture of the enzymatic hydrolysate of OsO_4-oxidized [^{3}H]DNA (○) and [^{14}C]thymine glycol, thymidine glycol, and thymine (▲) on a preparative ODS HPLC column with water as eluant.

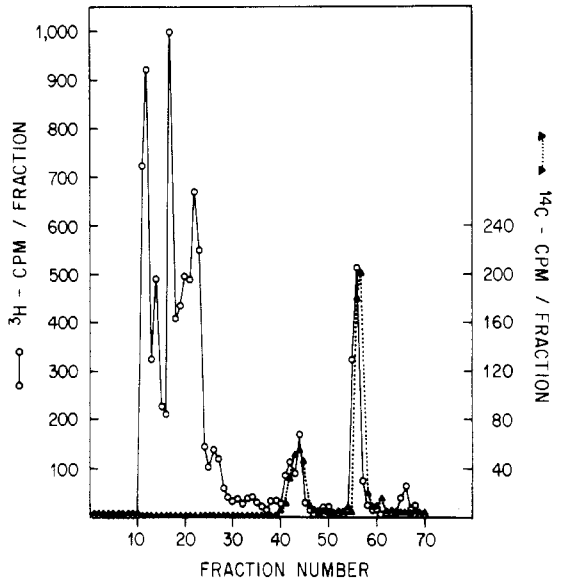

FIGURE 4: Chromatography of a mixture of the enzymatic hydrolysate of in vitro γ-irradiated [^{3}H]DNA (○) and [^{14}C]thymine glycol, thymidine glycol, and thymine (▲) on a preparative ODS HPLC column with water as eluant.

the maximum level of oxidation achieved with OsO_4 under the conditions described by Hariharan & Cerutti (1974). Thymidine glycol constituted 85% of the modified thymines, and the four early eluting peaks totaled 15%. Peak 70 amounted to about 1.5% of the total applied radioactivity and was comparable to that of $KMnO_4$-oxidized and control DNA.

Figure 4 shows the HPLC profile of an enzymatic hydrolysate of single-stranded DNA exposed to 72 krd of γ irradiation. The DNA was subjected to enzymatic digestion immediately after irradiation without purification. The majority of ^{3}H-containing products eluted as five peaks between fractions 10 and 30 and contained 15% of the applied radioactivity. There was a peak at fraction 17 which is the elution position of thymine glycol. However, the complexity of the pattern precluded its unambiguous identification. Thymidine glycol was identified in fraction 41–45 and thymine, in fraction 55–58. They constituted 1% and 2%, respectively, of the

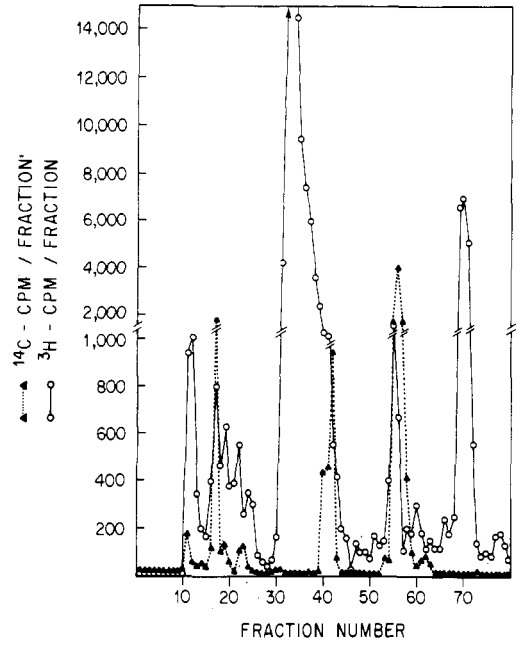

FIGURE 5: Chromatography of a mixture of the enzymatic hydrolysate of [^{3}H]DNA (O) isolated from control HeLa cells and [^{14}C]thymine glycol, thymidine glycol, and thymine (▲) on a preparative ODS HPLC column with water as eluant.

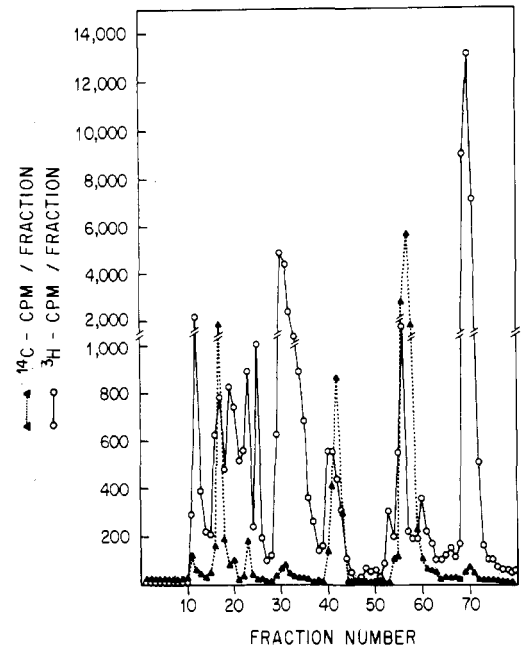

FIGURE 6: Chromatography of a mixture of the enzymatic hydrolysate of [^{3}H]DNA (O) isolated from γ-irradiated HeLa cells and [^{14}C]-thymine glycol, thymidine glycol, and thymine (▲) on a preparative ODS HPLC column with water as eluant.

applied ^{3}H radioactivity. The presence of thymine and the probably presence of thymine glycol indicated spontaneous release of bases from the DNA backbone.

Figures 5 and 6 show the profiles of enzymatic hydrolysates of ^{3}H-labeled DNA extracted from unirradiated (Figure 5) and γ-irradiated (Figure 6) HeLa cells. The extent of digestion of DNA of irradiated cells was greater than 99% while that of control DNA was 85%. This difference was manifest in the profile where a large peak of radioactivity was present in fractions 30–40 in the control sample and a much smaller one was present in the irradiated sample. We concluded that this large peak consisted of small oligonucleotides since the control sample was less digested than the irradiated sample. We reasoned that breaks in the DNA of irradiated cells rendered the DNA more susceptible to enzymatic digestion. The relative resistance to enzymatic digestion of unirradiated DNA may have been due to entanglement of DNA strands (Lehmann & Ormerod, 1970; Buhl et al., 1973). This hypothesis was supported by results of subsequent experiments in which vigorous shearing of DNA prior to enzymatic hydrolysis decreased fractions 30–40 by 90% while all other fractions were unchanged. We show these profiles to indicate the position of oligonucleotides and to emphasize the importance of as complete digestion as possible for satisfactory analysis of cellular DNA by HPLC. Even with 99% digestion, an oligonucleotide peak was present (Figure 6), and if large, this peak may overlap other peaks (Figure 5). Enzymatic hydrolysates of both control and irradiated cellular DNA displayed complex paterns which included five rapidly eluting peaks, one of which coincided with [^{14}C]thymine glycol (fractions 10–30), thymine (fractions 50–60), and a peak at fraction 70 similar to those present in the in vitro DNA experiments. Total modification of the irradiated DNA was about 0.4%. This included the putative oligonucleotide peak which represented 0.1% of the total, so the actual modification level was probably lower. The early eluting peaks constituted 0.07%, thymine 0.016%, and peak 70 0.2% of the total radioactivity. Approximately 0.02% of the ^{3}H radioactivity coincided with the [^{14}C]thymidine glycol, and one of the fast moving peaks eluted together with thymine glycol. In control DNA, the early eluting fractions constituted 0.05%, thymine 0.02%, and peak 70 0.15% of applied radioactivity. As in irradiated DNA, one of the fast moving peaks coincided with thymine glycol. The thymidine glycol peak was obscured by the large oligonucleotide fraction. Thus, comparison of the HPLC pattern of control and irradiated DNA indicated that most of the modification of thymine was probably the result of internal radiation caused by incorporated [^{3}H]thymidine.

Because of the possibility that the [^{3}H]thymidine glycol peak contained more than one thymine derivative, hydrolysates of control and irradiated DNA were analyzed by LH-20. Samples of the same control and irradiated DNA hydrolysates were again chromatographed on HPLC, and fractions 38–42 were collected. These fractions were then concentrated by flash evaporation, divided into two equal parts, and applied to LH-20 columns using water and borate as eluants. The results are shown in Figure 7. In control DNA (Figure 7a), approximately 50% of the ^{3}H radioactivity eluted earlier in borate (panel B) than in water (panel A). In irradiated DNA (Figure 7b), approximately 90% of the ^{3}H radioactivity eluted earlier in borate (panel B). This proved that *cis*-thymidine glycol was present in both control and irradiated DNA (Frenkel et al., 1981). The other products which did not change mobility in borate might be *trans*-thymidine glycol and/or other thymidine derivatives. The sum of radioactivity in fractions 20–24 (Figure 7a, panel B) was taken as the measure of thymidine glycol content of control DNA and fractions 20–25 (Figure 7b, panel B) of irradiated DNA. We estimated that thymidine glycol content of control DNA was 5.5 per 10^5 thymines and that of irradiated DNA 8 per 10^5. Similar LH-20 analysis of fractions eluting together with [^{14}C]thymine glycol revealed that there was less free thymine glycol than thymidine glycol present, with no significant difference in control and irradiated samples.

The HPLC analysis of the enzymatic hydrolysate of $KMnO_4$-oxidized DNA confirms our previous report that thymidine glycol constituted 50% of the thymine derivatives. The other 50% which had eluted as a single peak on LH-20 (Frenkel et al., 1981) was resolved by HPLC into at least four

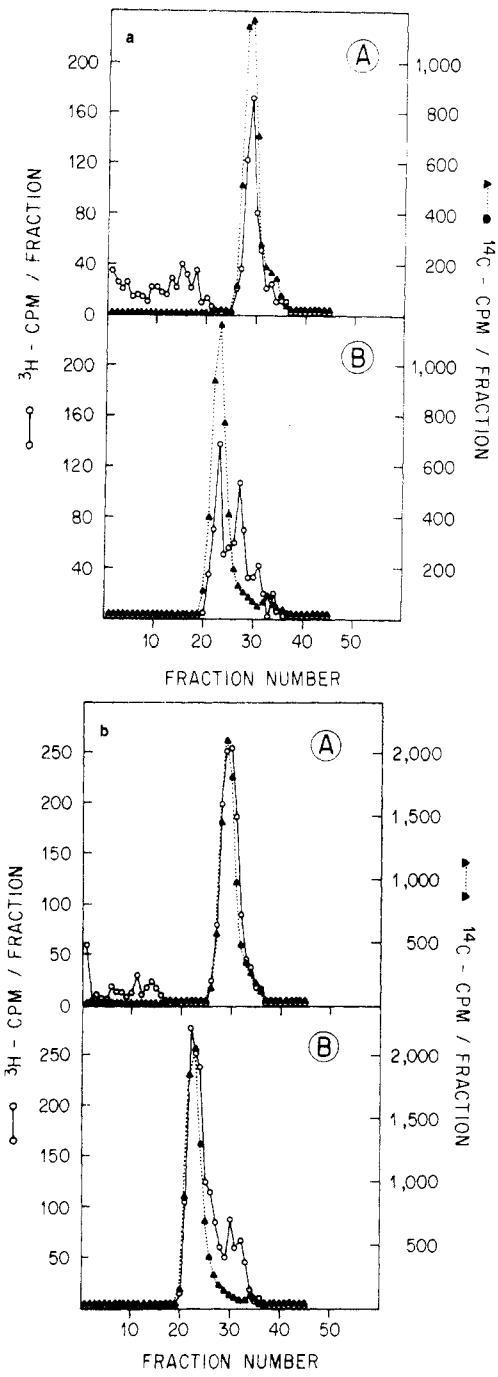

FIGURE 7: Chromatography on Sephadex LH-20 of pooled HPLC fractions 38–42 (a) from Figure 5 and (b) from Figure 6 (○) with [^{14}C]thymidine glycol (▲), with (A) water and (B) 0.05 M sodium borate, pH 8.6, as eluants.

components. Thymine glycol was not among them. The HPLC analysis of OsO_4-oxidized DNA demonstrated qualitative similarity to $KMnO_4$-oxidized DNA. However, there was proportionately more thymidine glycol formed (85%). This difference in relative amount of thymidine glycol formed may be a function of the degree of modification (20% and 5%, respectively) of the DNA. γ irradiation of DNA also caused the formation of thymidine glycol but in a smaller amount relative to the degree of thymine modification. Most of the products eluted before thymidine glycol. There was a peak at fraction 17 which was indicative of the presence of thymine glycol and at fraction 56 which coincided with thymine. Spontaneous release of bases from γ-irradiated DNA has been reported (Teoule et al., 1974) and attributed to labilization of N-glycosylic bonds caused by damage to the sugar phosphate backbone (Ward & Kuo, 1976). The fast moving components resolved into five peaks, including thymine glycol. The same components, except thymine glycol, were also present in the hydrolysates of chemically oxidized DNA. These may represent thymine oxidized to different degrees and/or open-ring products which retained the methyl group (Benn et al., 1960; Burton & Riley, 1966; Howgate et al., 1968; Iida & Hayatsu, 1970; Teoule et al., 1974).

Analysis of the hydrolysate of control ^{3}H-labeled HeLa cell DNA revealed about 0.3% of the total thymidine radioactivity to be in thymine derivatives and a small amount of thymine. These derivatives and the labilization of thymine had to be caused by internal radiation from tritium in the methyl group of the thymine moiety. The effective nuclear dose from tritium can be estimated to be 450 rd, assuming one disintegration translates into 0.27 rd to the nucleus (*NCRP Rep.*, 1979) and the DNA content of a HeLa cell to be 15.7×10^{-12} g (Sober, 1968). The HPLC profiles of the hydrolysates of γ-irradiated and control DNA were qualitatively similar, with external radiation causing an overall increase of about 30% in modified thymines. However, the yields of some products increased 2-fold, while others remained unchanged. Thus, it appears that 36 krd of external radiation selectively increased the formation of only some of the products as determined by HPLC and LH-20 analyses.

The HPLC pattern of cellular DNA shows two interesting differences when compared to that of in vitro irradiated DNA. A peak at fraction 14 is conspicuously absent in cellular DNA, and the peak at fraction 70, present in small amounts in all ^{3}H-labeled *E. coli* DNA including control, is not increased by in vitro γ irradiation or chemical oxidation. Thus one product (peak 14) formed in single-stranded DNA by both chemical oxidants and γ radiation is not formed in cellular DNA. In contrast, peak 70, which is the major cellular thymine derivative, is not formed in single-stranded DNA by these agents. Thymidine glycol was estimated to constitute between 5% and 10% of the modified thymines formed by ionizing radiation both in vitro and in cellular DNA.

Discussion

The data presented here show the heterogeneity of thymine modification in DNA caused by γ radiation and reveal the presence of a thymine derivative in irradiated cellular DNA which was not formed by either chemical oxidation or γ irradiation of DNA in vitro (peak 70). Our results explain the "high radioactivity backgrounds" of ^{3}H-labeled control *M. radiodurans* DNA reported by Hariharan & Cerutti (1972). This background was probably due to a high level of thymine modification caused by incorporated [*methyl*-^{3}H]thymidine which we have shown is qualitatively similar to that caused by external γ radiation (Figures 5 and 6).

The extent of thymine modification in control cells which had received a dose of 450 rd from internal β radiation was very high compared to the small increase (30%) in thymine modification resulting from 36 krd of external γ radiation. This indicates that decomposition of the [*methyl*-^{3}H]thymine moiety in DNA cannot entirely be due to ionization produced by electron impact. Aronoff (1956) emphasized that the percent of actual decomposition of radioactive compounds frequently greatly exceeded that expected from calculated values. Person et al. (1976) differentiated local effects resulting from position of the ^{3}H label from radiation effects independent of the position of the label. Tritium in the 6 position of thymine was more mutagenic for *E. coli* than was ^{3}H in the 5-methyl group for a given amount of radioisotopic decay. Cleaver (1977) found these results to be true for eu-

karyotic cells as well and pointed out that these local transmutational effects were qualitatively similar to those produced by ionizing and β particle radiation. Our results support this conclusion since the changes induced by external radiation were qualitatively similar to those arising from incorporated [^{3}H]thymidine.

Some or all of these thymine derivatives may be removed from DNA by repair processes. It has been reported that *E. coli* endonuclease III contains *N*-glycosylase activity which releases thymine glycol (Demple & Linn, 1980). Breimer & Lindahl (1980) have discovered a bacterial *N*-glycosylase activity directed against the urea fragment of degraded thymine glycol which was retained on the sugar-phosphate backbone. No mammalian *N*-glycosylase activities directed against thymine glycol or its degraded residues have been yet identified, but it is possible that the mammalian endonucleolytic activities directed against chemically oxidized, γ-irradiated and heavily ultraviolet-irradiated DNA (Brent, 1973; Nes & Nissen-Meyer, 1978; Teebor et al., 1978) represent composite *N*-glycosylase and AP endonuclease activity. The method presented here is applicable to the determination of the mechanism of repair of thymine derivatives in cellular DNA. Soluble nuclear and cytoplasmic fractions and cell medium can be analyzed for content of modified thymines, the presence of which would reflect enzymatic and/or spontaneous removal of such derivatives from DNA. Cellular DNA can be extracted, digested, and analyzed to determine the rate at which these derivatives disappear, and modified residues which persist in DNA can also be identified.

Acknowledgments

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