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Identification of the *cis*-Thymine Glycol Moiety in Oxidized Deoxyribonucleic Acid†

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ABSTRACT: 5,6-Dihydroxy-5,6-dihydrothymine (thymine glycol) is formed in DNA by reaction with oxidizing agents and as a result of ionizing and near-ultraviolet radiation. We describe a rapid purification of *cis*-5,6-dihydroxy-5,6-dihydrothymine and *cis*-5,6-dihydroxy-5,6-dihydrothymidine (*cis*-thymidine glycol) and their use as markers in identifying the thymine glycol moiety in oxidized DNA. Both glycols were prepared by oxidation of [^{14}C]thymine and -thymidine with $KMnO_4$ followed by purification on Sephadex LH-20 (LH-20). [^{3}H]DNA was oxidized with $KMnO_4$ and the thymidine glycol in DNA identified by enzymatic digestion of the DNA followed by cochromatography of the digest with marker [^{14}C]thymidine glycol on LH-20. The cis conformation of the glycol was confirmed by the change in the elution pattern when borate rather than water was used as eluent. Alkaline hydrolysis of a mixture of [^{14}C]thymine glycol and oxidized [^{3}H]DNA followed by trichloroacetic acid precipitation and LH-20 chromatographic analysis of the neutralized supernatant yielded a complex pattern of radioactive degradation products with coincidence of one ^{14}C marker- and one [^{3}H]-DNA-derived peak. All applied radioactivity was recovered. This methodology should be useful in determining thymine glycol content of irradiated DNA and in elucidating the mechanism by which these altered residues are removed from cellular DNA by repair enzymes.

Ionizing and near-ultraviolet irradiation of DNA causes modification of thymine with the formation of saturated ring compounds (Hariharan & Cerutti, 1972, 1977). These modified thymines may be the major form of DNA base damage caused by ionizing radiation, and data exist which indicate that such modified thymines are removed from cellular DNA by an active enzymatic repair process (Hariharan & Cerutti, 1971; Mattern et al., 1973; Mattern & Welch, 1979; Painter & Young, 1972). Support for the latter hypothesis stems from reports that DNA which was exposed to ionizing radiation in vitro was attacked by DNA endonuclease activities which did not attack undamaged DNA (Bacchetti & Benne, 1975; Brent, 1973; Strniste & Wallace, 1975). Ring-saturated modified thymines can also be introduced into DNA by oxidation with osmium tetroxide (OsO_4) and potassium permanganate ($KMnO_4$) (Iida & Hayatsu, 1971; Hariharan & Cerutti, 1974; Hariharan et al., 1977), and poly(dA-dT) and DNA oxidized by OsO_4 were attacked by the same endonuclease activities which acted upon irradiated DNA (Gates & Linn, 1977; Hariharan & Cerutti, 1974; Nes & Nissen-Meyer, 1978). Therefore, it is possible that some, if not all, of the ring-saturated thymines are substrates for the enzymatic initiation of DNA excision-repair. Oxidation of DNA by OsO_4 and $KMnO_4$ is reported to yield only *cis*-5,6-dihydroxy-5,6-dihydrothymine (*cis*-thymine glycol, TG)[1] (Burton & Riley, 1966; Iida & Hayatsu, 1971; Hariharan et al., 1977). Thus, it is most probable that this particular modified thymine in DNA is acted upon by a repair *N*-glycosylase and/or DNA endonuclease.

The existing method for identifying TG in DNA is an alkali-acid treatment which recovers only about 20% of the ring-saturated modified thymines and does not distinguish between TG and other ring-saturated thymines (Hariharan & Cerutti, 1974). We have developed a method for the rapid purification of both *cis*-thymine and -thymidine glycols and have used them as markers in the analysis of chemical and enzymatic hydrolysates of oxidized DNA.

Experimental Procedures

Materials

[*methyl*-^{14}C]Thymine (49.6 mCi/mmol), [*methyl*-^{14}C]-thymidine (48.2 mCi/mmol), and [*methyl*-^{3}H]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. *E. 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.

Methods

Chromatography on Sephadex LH-20 (LH-20). All assays, unless otherwise specified, requiring chromatography on LH-20 were performed on 1.5 × 25 cm columns with either water or 0.05 M sodium borate titrated with boric acid to pH 8.6 as eluents. These columns will be referred to as water and borate LH-20 columns. Fractions of 1 mL (1 mL/min) were collected in liquid scintillation vials, unless absorbance of the samples was to be determined. Samples were counted in 20%

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[1] Abbreviations used: thymine glycol (TG), 5,6-dihydroxy-5,6-dihydrothymine; thymidine glycol, 5,6-dihydroxy-5,6-dihydrothymidine; BSA, bovine serum albumin; Cl_3CCOOH, trichloroacetic acid; NMR, nuclear magnetic resonance; IR, infrared; UV, ultraviolet, TLC, thin-layer chromatography; high-pressure LC, high-pressure liquid chromatography; LH-20, Sephadex LH-20.

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Bio-Solv (Beckman) in a Nuclear Chicago Mark II scintillation counter. The same water and borate LH-20 columns were reused for many assays with high reproducibility. When new columns were prepared, reproducibility was within one fraction.

Synthesis, Purification, and Characterization of cis-5,6-Dihydroxy-5,6-dihydrothymine and cis-5,6-Dihydroxy-5,6-dihydrothymidine. The synthesis and purification of thymine and thymidine glycols detailed here are a modification of the method developed by Iida & Hayatsu (1970, 1971). Thymine (0.5 g; 4 mmol) or thymidine (1.0 g; 4 mmol) was dissolved in 200 mL of 0.2 M ammonium chloride titrated with ammonium hydroxide to pH 8.6 (NH_4Cl–NH_3 buffer). [*methyl*-^{14}C]Thymine or [*methyl*-^{14}C]thymidine (1 μCi) was added, the mixture was cooled in an ice-water bath, and then it was treated with 80 mL of cold 0.07 M $KMnO_4$ for 5 min with vigorous mixing. The reaction was stopped by addition of 1 M sodium metabisulfite until there was no red tint, and the mixture was left at 4 °C for 30–60 min. Manganese dioxide was separated by filtration. If the solution was still yellow-brown, it was again treated with solid metabisulfite until clear. The filtrate was concentrated in a flash evaporator under high vacuum at 30 °C until the first precipitate appeared. In order to prevent extraction of manganese ions, potassium phosphate buffer (1 M K_2HPO_4, 5 mL, pH 8.6) was added to the concentrate followed by addition of an excess of acetone until salts precipitated and could be separated by filtration. The filtrate was concentrated under vacuum to about 1 mL and then chromatographed on Sephadex LH-20 (2.5 × 75 cm, in water). A total of 250 fractions were collected [2 mL/(fraction·min)]. Thymine glycol eluted between fractions 139 and 152 and thymidine glycol between 140 and 155 as determined by absorbance at 230 nm and radioactivity. Peak fractions were pooled and concentrated under high vacuum at 30 °C, and the products were recrystallized from water–ethanol and methanol, respectively, with yields of 60% for thymine glycol and 50% for thymidine glycol. The purity of both was determined by high-pressure liquid chromatography (high-pressure LC, Beckman, Model 330) on a reverse-phase Altex column (Ultrasphere-ODS, 5-μm particle, 4.6-mm i.d.) with water or 0.05 M potassium phosphate buffer, pH 4.5, as eluents, monitored by UV absorption and by radioactivity. Melting points of thymine glycol (214–216 °C) and thymidine glycol (191–193 °C) were in agreement with those previously reported (Iida & Hayatsu, 1970; Howgate et al., 1968). Thymine glycol was assayed by thin-layer chromatography with 250-μm Avicel F cellulose plates (Analtech, Inc.) and 1-butanol–water (86:14 v/v) as the eluent. Thymine and urea were used as standards. After detection of thymine with a UV lamp (Mineralight), the plates were sprayed with 0.5 M NaOH in ethanol and immediately viewed under UV light. Thymine glycol, with its ring opened by the alkaline treatment, was detected as a dark spot. Then, spraying with 5% titanous chloride caused urea to be visualized as a lavender-colored spot. The R_f of thymine glycol under these conditions was 0.27 while those of urea and thymine were 0.33 and 0.53, respectively. These can be compared to 0.21, 0.25, and 0.50, respectively, obtained by Iida & Hayatsu (1970) with ascending chromatography on Toyo filter paper no. 53 with the same eluent. An infrared (Perkin-Elmer 421 infrared spectrophotometer) spectrum of thymine glycol run as a KBr pellet had bands at 3430, 3350, 3235, 1740, 1705, 1670, 1170, 1110, 1090, and 1055 cm^{-1}, similar to those reported by Iida & Hayatsu (1970). The nuclear magnetic resonance spectrum (Varian, 60 MHz) of thymine glycol determined in hexadeuteriodimethyl sulfoxide with tetramethylsilane as an internal standard had signals at δ 1.28 (5-CH_3, s), 4.40 (6-H, dd, 4, 5), 5.3 (5-OH, s), 6.04 (6-OH, d, 4), 8.12 (1-NH, d, 5), and 10.05 (3-NH, s). These δ values were comparable to those obtained by Howgate et al. (1968) and Iida & Hayatsu (1970). When deuterated water was added to the NMR sample, signals corresponding to 5-OH, 6-OH, 1-NH, and 3-NH disappeared while the doublet of doublets corresponding to 6-H became a singlet, thus providing further evidence that the product obtained was thymine glycol.

To demonstrate that the synthesized [^{14}C]thymine glycol was the cis isomer, it was chromatographed on LH-20 with water and borate buffer, pH 8.6, as eluents, adding nonradioactive thymine as the UV-absorbing marker (Figure 1a). The thymine glycol peak eluted at fraction 30 in water and at fraction 24 in borate. Thymidine glycol eluted in the same fractions as the free base. Elution of thymine and thymidine (fractions 41 and 37, respectively) was not affected by the eluent. The *trans*-thymine glycol was prepared by heating the *cis*-thymine glycol at 95 °C for 5 h (Barszcz et al., 1963), and the mixture was chromatographed on both water and borate LH-20 columns.

Preparation of E. coli [^{3}H]DNA. *E. coli* W 3110 (*thy*–) J. Cairns strain (DeLucia & Cairns, 1969) was grown by inoculating a 50-mL pot of minimal A medium and incubating it with aeration at 37 °C overnight. The minimal A medium contained per liter: 7 g of K_2HPO_4, 3 g of KH_2PO_4, 0.5 g of sodium citrate, 1 g of $(NH_4)_2SO_4$, and 0.1 g of $Mg_2SO_4 \cdot 7H_2O$. This was supplemented with thymine (10 μg/mL) and with casamino acids (0.5%) and glucose (0.25%), respectively. A 2.5-mL aliquot of an overnight growth was added to 50 mL of minimal A medium supplemented with the same amount of casamino acids and glucose as above. The concentration of thymine was reduced to 0.5 μg/mL, and after addition of 0.2 mL (0.2 mCi) of [*methyl*-^{3}H]thymidine, bacteria were grown with aeration at 37 °C for two doublings, monitored at 480 nm. Medium was removed by centrifugation, and bacteria were washed with 0.15 M NaCl and 0.1 M EDTA, pH 8.0, solution. [^{3}H]DNA was 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. The specific activity of DNA was 1800 cpm/μg, assuming that 1 A_{260} unit = 50 μg of DNA/mL.

Oxidation of [^{3}H]DNA. Double-stranded [^{3}H]DNA isolated from *E. coli* W 3110 (*thy*–) J. Cairns strain was heated at 100 °C for 5 min followed by rapid cooling. Oxidation of single-stranded DNA which resulted from this procedure was carried out in 0.3 M NH_4Cl–NH_3 buffer, pH 8.6, by treatment with 0.0015 M $KMnO_4$ in an ice–water bath for 5 min (Iida & Hayatsu, 1971). The reaction was stopped by addition of 1 M sodium metabisulfite (10–20 μL) until the solution became colorless. A control DNA sample was treated identically, but without $KMnO_4$. The salts were removed by passing the reaction mixture through a small Sephadex G-50 column with 0.15 M KCl, 0.01 M K_2HPO_4, and 0.001 M EDTA, pH 8.0, buffer. DNA was excluded from this column and collected in 0.25-mL fractions. The peak fractions were combined and used as substrate for subsequent experiments.

Identification of cis-Thymidine Glycol in Oxidized DNA by Enzymatic Hydrolysis. *cis*-[^{14}C]Thymidine glycol (2300 cpm) was added to the oxidized [^{3}H]DNA (18 000 cpm), and the solution was made 0.015 M with respect to $MgCl_2$. This was followed by treatment at 37 °C with DNase I (200 units mL; 2 h), alkaline phosphatase (40 units/mL; 1 h), spleen and snake venom phosphodiesterases (0.02 and 0.3 unit/mL, re-

spectively; 1 h), and then alkaline phosphatase again (40 units/mL) overnight. Macromolecules were precipitated with 5% Cl_3CCOOH and centrifuged, and the supernatant was neutralized with 1 M K_2HPO_4 (0.5 volume of the supernatant). The radioactivity of one-eighth of the total volume was determined, and three-fourths of the volume was applied to a water or borate LH-20 column. Recovery of applied radioactivity from the columns was nearly 100%.

Rapid Identification of cis-Thymine Glycol in Oxidized DNA by Alkaline Hydrolysis. *cis*-[^{14}C]Thymine glycol (2000 cpm) was added to oxidized [^{3}H]DNA (18 000 cpm) and treated with 0.1 M NaOH at 37 °C for 20 min, 60 min, and 20 h. The reaction was stopped by the addition of 0.1 volume of 1 M HCl. The mixture was precipitated with 5% Cl_3CCOOH following addition of 0.1 mg of BSA. After centrifugation, the supernatant was neutralized with 0.5 volume of 1 M K_2HPO_4. The radioactivity of one-eighth of the total volume was determined, and three-fourths of the volume was applied to a water or borate LH-20 column. Recovery of applied radioactivity from the columns was nearly 100%.

Results

The oxidation of thymine and thymidine in dilute $KMnO_4$ resulted in the formation of *cis*-5,6-dihydroxy-5,6-dihydrothymine and *cis*-5,6-dihydroxy-5,6-dihydrothymidine. The oxidation was carried out under conditions described by Iida & Hayatsu (1970, 1971). The purification step was changed from an 8-day chromatographic procedure with a cellulose column and water-butanol as the eluent to a 4-h chromatographic procedure on a Sephadex LH-20 column with water as the eluent.

The purity and identification of the compounds were ascertained by melting point determinations, TLC, and IR and NMR spectroscopy. All of these data were in agreement with those reported by Iida & Hayatsu (1970) and Howgate et al. (1968). Further proof of structure came from the NMR spectrum following addition of D_2O. The spectrum showed disappearance of the signals assigned to exchangeable protons, and the doublet of doublets became a singlet [δ 4.40 (6-H)].

The elution pattern of the *cis*-glycol on LH-20 in borate was different than that in water while that of thymine or thymidine remained unchanged (Figure 1a). Heating of the *cis*-thymine glycol at 95 °C for 5 h resulted in conversion of 20% of the material into a second component which did not change mobility in borate (Figure 1b). This demonstrated the formation of a *trans*-glycol, as expected.

After the two *cis*-glycols were synthesized, it was determined whether $KMnO_4$ oxidation of DNA indeed yielded the same modification of thymine moieties. The [^{3}H]thymine-labeled single-stranded DNA was oxidized with $KMnO_4$ and then enzymatically digested to deoxyribonucleosides. [^{14}C]Thymidine glycol was added to DNA prior to the digestion. Elution profiles in both water and borate showed coincidence of the marker [^{14}C]thymidine glycol with a ^{3}H-containing peak (Figure 2). The large peak in both profiles was thymidine as determined by cochromatography with unlabeled thymidine and monitored by ultraviolet absorbance. The profile with water as eluent showed an additional ^{3}H-containing peak which may be another thymine oxidation product. In borate, the coincident ^{14}C–^{3}H-containing peak moved forward, indicating it was a *cis*-glycol. This change in mobility resulted in the *cis*-glycol eluting together with the other oxidized thymine derivative which did not change mobility. Thus, the borate column shows only one peak other than thymidine (Figure 2B).

The next set of experiments was designed to determine whether *cis*-thymine glycol could be detected in DNA by rapid

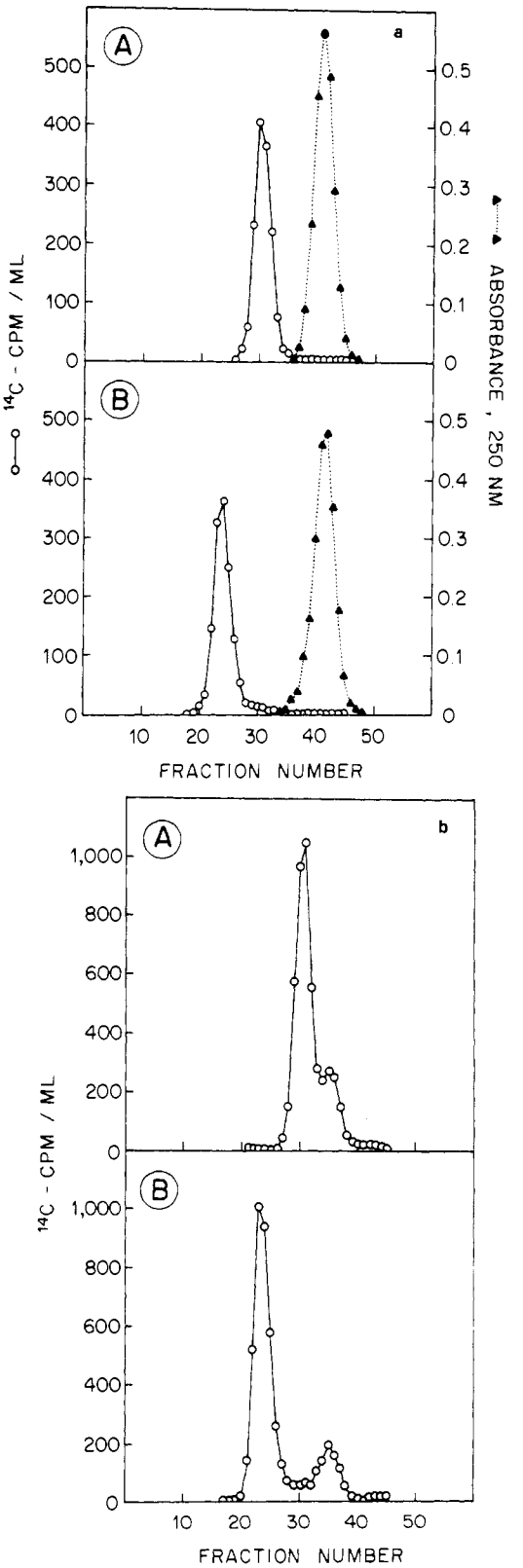

FIGURE 1: (a) Chromatography of *cis*-[^{14}C]thymine glycol (○) and thymine (▲) on Sephadex LH-20 (A) with water and (B) 0.05 M sodium borate, pH 8.6, as eluents. (b) Chromatography of *cis*- and *trans*-[^{14}C]thymine glycols on Sephadex LH-20 with (A) water and (B) 0.05 M sodium borate, pH 8.6, as eluents.

chemical hydrolysis. Samples of the same batch of oxidized DNA were subjected to alkaline hydrolysis for different time intervals. After Cl_3CCOOH precipitation, neutralized aliquots of the supernatant were assayed by liquid scintillation counting. Table I shows that all alkali-labile Cl_3CCOOH-soluble ^{3}H radioactivity was released within 20 min. Figure 3a–c depicts

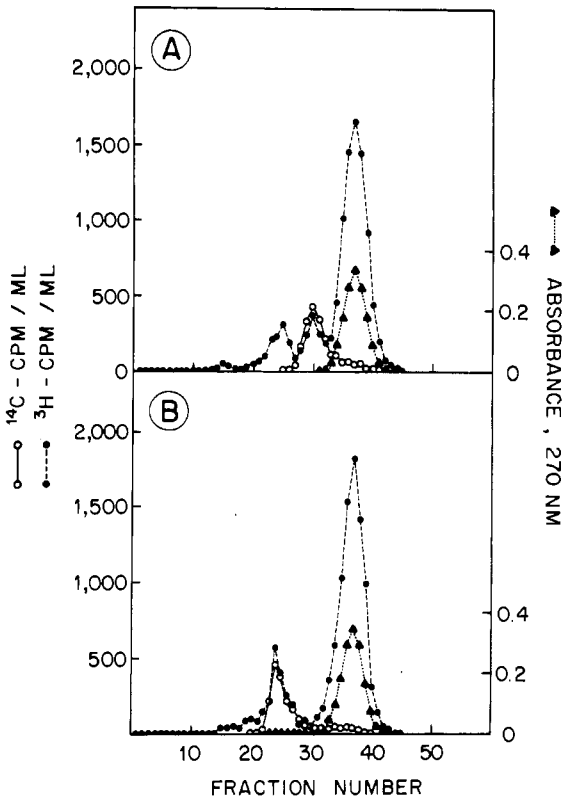

FIGURE 2: Chromatography of the enzymatic hydrolysate of oxidized [^{3}H]DNA (●) containing 2'-deoxyribunucleosides, thymidine (▲), and *cis*-[^{14}C]thymidine glycol (○) on Sephadex LH-20 with (A) water and (B) 0.05 M sodium borate, pH 8.6, as eluents.

LH-20 chromatographic elution patterns of the hydrolysates of oxidized DNA which had been treated with alkali for 20 min, 60 min, and 20 h, respectively. Radioactivity derived from the [^{3}H]DNA appeared in two major and two minor peaks with the two minor peaks virtually disappearing after 20 h of alkaline hydrolysis. [^{14}C]Thymine glycol derived radioactivity showed a complex pattern of at least three peaks in both water and borate, one of which coincided with the most hydrophilic DNA-derived peak. This complex pattern persisted even after 20 h of alkaline hydrolysis, and none of the peaks changed mobility in borate when compared to water.

Table I: Alkali Lability of Oxidized DNA[a]

hydrolysis time	experiment 1 ^{3}H (cpm)	experiment 1 ^{14}C (cpm)	experiment 2 ^{3}H (cpm)	experiment 2 ^{14}C (cpm)
20 min	454	244	498	240
60 min	465	249	524	251
20 h	472	187	546	255

[a] $KMnO_4$-oxidized [^{3}H]DNA (18 000 cpm) samples were treated with 0.1 M NaOH at 37 °C in the presence of *cis*-[^{14}C]thymine glycol (2000 cpm). After indicated times, reaction mixtures were neutralized with 1 M HCl and precipitated with 5% Cl_3CCOOH. After centrifugation, supernatants were neutralized with 1 M K_2HPO_4. The radioactivity of one-eighth of the total volume was determined. The remaining three-fourths of the total volume was chromatographed on Sephadex LH-20 (Figure 3a-c).

Comparison of the two methods of hydrolysis revealed that of a total of 12 400 ^{3}H cpm in the enzymatic digest 10 000 were released as thymidine, approximately 1200 as thymidine glycol, and 1200 as the other most rapidly moving peak on the LH-20 water column (Figure 2A) (19% oxidation products) while alkaline hydrolysis of the same DNA sample yielded approximately 3600–4000 alkali-labile Cl_3CCOOH-soluble counts (Table I) from a total of 18 000 cpm (20–22% oxidation products). Thus, the percent of alkali-labile Cl_3CCOOH-soluble ^{3}H radioactivity was about the same as the total percent of ^{3}H radioactivity contained in thymidine glycol and another thymine oxidation product in the enzymatic digest. This suggests that alkaline hydrolysis released all modified thymines from oxidized DNA. Furthermore, approximately half of the ^{3}H radioactivity in the alkaline hydrolysate was contained in the first major peak which coincided with a [^{14}C]thymine glycol derived peak while the remaining half did not coincide with any ^{14}C-containing peak (Figure 3). We suggest that the first major ^{3}H-containing peak plus the two minor peaks are *cis*-TG derived, and the other major, most hydrophobic peak has derived from the nonthymidine glycol product seen in the

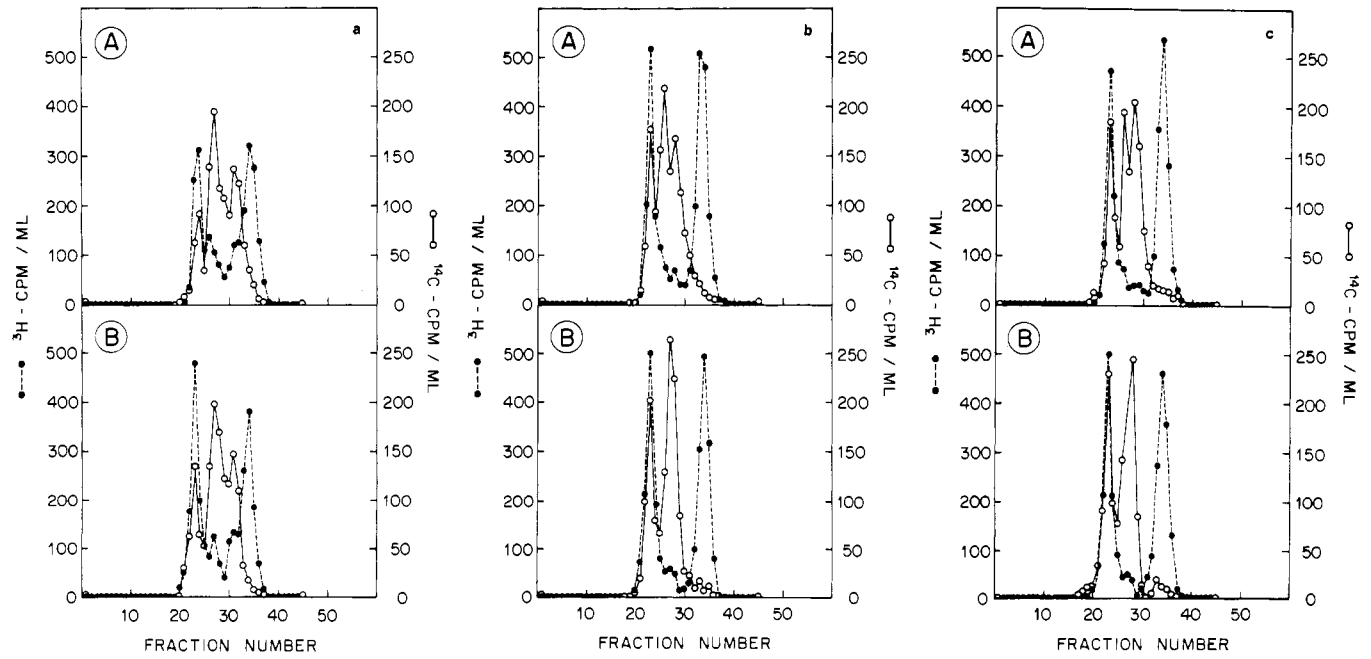

FIGURE 3: Chromatography of the alkaline hydrolysate of a mixture of oxidized [^{3}H]DNA (●) and *cis*-[^{14}C]thymine glycol (○) on Sephadex LH-20 with (A) water and (B) 0.05 M sodium borate, pH 8.6, as eluents. Alkaline hydrolysis at 37 °C for (a) 20 min, (b) 60 min, and (c) 20 h.

Table II: Oxidation of DNA by $KMnO_4$[a]

oxidation time (min)	% oxidation 0.0015 M $KMnO_4$	% oxidation 0.003 M $KMnO_4$
2.5		18
5	15	25
10	24	32
20	30	40

[a] [^{3}H] DNA was oxidized with 0.0015 and 0.003 M $KMnO_4$ at 4 °C for times indicated. The reaction was stopped by addition of metabisulfite and the DNA purified by passage through Sephadex G-50. Samples of oxidized DNA were treated with 0.1 M NaOH at 37 °C for 20 min, neutralized with 1 M HCl, and precipitated with 5% Cl_3CCOOH, and the radioactivity of the supernatant was determined. The ratio of ^{3}H cpm derived from the supernatant to the total cpm used for alkaline hydrolysis times 100 is the percent oxidation.

enzymatic digest on the LH-20 water column (Figure 2A).

Alkaline hydrolysis of DNA which had been exposed to increasing concentrations of oxidizing agent for increasing times revealed there was a concomitant increase in the degree of oxidation, as shown in Table II. In a separate experiment, samples of oxidized DNA were analyzed by the alkali–acid treatment of Hariharan & Cerutti (1974). Three samples oxidized for 10, 20, and 40 min by 0.0015 M $KMnO_4$ were analyzed. The percent of recovered radioactivity by the Hariharan and Cerutti method was 5.4, 5.8, and 5.9%, respectively. This method recovers only 20% of applied radioactivity. Hence, the estimate of actual modification was 27, 29, and 30%. Our method of alkaline hydrolysis followed by Cl_3CCOOH precipitation yielded 23, 27, and 31% modification. Thus, all three methods of hydrolysis (enzymatic, alkaline, and alkali–acid) yielded the same estimate of total thymine oxidation products. However, the use of ^{14}C markers and LH-20 chromatography allowed for quantitation of the actual *cis*-TG content.

Discussion

This is the first unambiguous demonstration that *cis*-thymine glycol is present in oxidized DNA. The rapid, simple purification of both thymine and thymidine glycols and the use of LH-20 as an analytical column make this methodology readily available to other workers. As little radioactivity as 500 ^{3}H cpm is sufficient to identify peaks from the column.

Analysis of alkaline hydrolysates of oxidized [^{3}H]DNA and synthetic [^{14}C]TG revealed that hydrolysis, be it for 20 min or 20 h, yielded a complex pattern of at least three products derived from the marker [^{14}C]TG while hydrolysis of [^{3}H]-TG-containing DNA yielded primarily one of these (Figure 3). This suggests that there are multiple sites for opening of the modified free heterocyclic base but that one of these sites is favored when the base is hydrolyzed from the sugar–phosphate backbone. Chromatographic analysis of the hydrolysate of the oxidized DNA showed a second thymine-derived product which was more hydrophobic than that derived from TG.

It has been shown that TG is most unstable at pH 13 with a half-life of only 4 min (Iida & Hayatsu, 1970). Roberts & Friedkin (1958) detected formation of acetol from TG at such a high pH by using the condensation reaction with *o*-aminobenzaldehyde. In another study (Benn et al., 1960), prolonged $KMnO_4$ oxidation of thymine at pH 9 led to the formation of not only TG and acetol but also pyruvaldehyde, pyruvic acid, pyruvate salts, sodium bicarbonate, formate, and acetate. This suggests that at pH 13 acetol is degraded unless trapped by a reagent such as *o*-aminobenzaldehyde. It is probable that alkaline hydrolysis caused the formation of a mixture of degradation products derived from TG and/or acetol, and these products were recovered from the LH-20 column.

Both enzymatic and alkaline hydrolyses yielded the same estimate of total thymine oxidation products, and both methods showed the presence of two major products in almost equal amounts. This is suggestive enough to conclude that the more hydrophilic major and two minor ^{3}H peaks of the alkaline hydrolysate which coincided with [^{14}C]TG-derived peaks represent breakdown products of TG in oxidized DNA. The nature of these products is a subject for future investigation.

This methodology is applicable to the determination of TG content of irradiated DNA, and synthetic marker TG should be useful in determining the mechanism of repair of this potentially significant DNA modification.

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