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RAPID ISOLATION, HYDROLYSIS AND CHROMATOGRAPHY OF FORMALDEHYDE-MODIFIED DNA

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SUMMARY

Deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine were reacted with formaldehyde. High-performance liquid chromatographic (HPLC) analysis indicated that each deoxynucleoside had formed one major product. With the exception of the thymidine product, these adducts were analyzed by nuclear magnetic resonance spectroscopy and identified as hydroxymethyl derivatives at the exocyclic amines. Calf thymus DNA was incubated with [^{3}H]formaldehyde and, after purification, enzymatically hydrolyzed to nucleosides. HPLC analysis indicated the presence of a substantial proportion of non-covalently bound formaldehyde and the following hydroxymethyl adducts, listed in order of decreasing concentration: N^6-hydroxymethyldeoxyadenosine $\gg$ N^4-hydroxymethyldeoxycytidine > N^2-hydroxymethyldeoxyguanosine. Incubation of Chinese hamster ovary (CHO) cells with [^{3}H]formaldehyde resulted in metabolic incorporation of the formaldehyde into purines and pyrimidines plus an appreciable concentration of formaldehyde noncovalently associated with the DNA. However, HPLC analysis clearly indicated the presence of N^6-hydroxymethyldeoxyadenosine in the CHO cell genome.

INTRODUCTION

The reaction of formaldehyde with nucleic acids to form hydroxymethyl derivatives was first described by Fraenkel-Conrat in 1954 [1]. This interaction, which can occur with both exocyclic and endocyclic amino groups, has been extensively reviewed by Feldman [2] and Auerbach et al. [3]. More recently, McGhee and Von Hippel, in an elegant series of studies [4—7], demonstrated that formaldehyde reacted preferentially with A · T rich regions in DNA and that the interaction was preceded by an unstacking of the polynucleotide. In addition, they found that although thymidine reacted much faster with formaldehyde, the adduct formed from adenosine was quantitatively more important. This is due to differences in the stabilities of the adducts;

endocyclic hydroxymethyl derivatives, such as N3-hydroxymethylthymidine, have very short lifetimes ($t_{1/2}$ ca. 0.1 sec at 40°C), whereas exocyclic N^6-hydroxymethyladenosine is a comparatively more stable adduct with a $t_{1/2}$ of 100 min at 40°C.

Given sufficient time, the hydroxymethyl derivatives can react with another amine function to yield methylene diadducts [2, 3]. These diadducts are much more stable than the hydroxymethyl monomers and Chaw et al. have developed techniques for their analysis in DNA [8]. It should be noted, however, that in vitro formation of formaldehyde diadducts requires prolonged reaction times (e.g., 40 days) and rather high formaldehyde concentrations.

Another type of diadduct involving DNA is a methylene protein–DNA crosslink [2, 3]. These products are thought to form through an initial interaction of formaldehyde with an amine function on proteins followed by reaction with the polynucleotide. This type of adduct may be important in vivo because the interaction of formaldehyde with amino acids has been reported to increase the rate of reaction of formaldehyde with DNA [9, 10]. The structures of these protein—DNA crosslinks have not been elucidated. Furthermore, although the interactions of formaldehyde with isolated macromolecules have been studied extensively, the identity of the products obtained in intact cells has been inferred only through indirect evidence [11—17].

An additional DNA lesion that results from formaldehyde treatment is single-strand breaks. This type of damage, which appears to be dependent upon DNA repair processes, has been observed in yeast [18] and mammalian cells [14—17].

Of the products formed from the interaction of formaldehyde, one class, the hydroxymethyl adducts, has not been investigated in cells. The relative instability of these hydroxymethyl products has probably hindered their analysis and yet these adducts could be responsible for some of the biological effects produced by formadehyde. We have, therefore, developed methods for the analysis of these derivatives in mammalian cells.

MATERIALS AND METHODS

Chemicals

Formaldehyde was obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.) as a 37% aqueous solution which contained 10—15% methanol as a preservative. [^{3}H] Formaldehyde (specific activity, 85 mCi/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.) as 1% aqueous solution. Deoxyguanosine, deoxyadenosine, deoxycytidine, calf thymus DNA (Type I), RNase A (Type XII-A), RNase T_1 (grade IV), proteinase K (Type XI), lysozyme (grade 1), bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane (Bis-Tris), sodium dodecyl sulfate, and alkaline phosphatase (Type III-S) were acquired from Sigma (St. Louis, MO, U.S.A.). Thymidine and nuclease P_1 were obtained from Calbiochem-Behring (LaJolla, CA, U.S.A.). 2,4-Dinitrophenylhydrazine was purchased from MCB Manufacturing Chemists (Gibbstown, NJ, U.S.A.).

Instrumentation

High-performance liquid chromatography (HPLC) was conducted with a Waters Assoc. system consisting of two 6000A pumps, a U6K injector, a 440 UV detector and an automated gradient controller. UV spectra were recorded with either a Cary 210 or a Gilford 2400-2 spectrometer. Radioactivity was measured in Scintisol (Isolabs, Akron, OH, U.S.A.) using a Searle Mark III liquid scintillation counter. ^{1}H-Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker WM 500 spectrometer.

Formaldehyde analysis

Formaldehyde was analyzed by HPLC as its 2,4-dinitrophenylhydrazone derivative [19]. The formaldehyde (ca. 10 μg) was added to a solution of 12 mg 2,4-dinitrophenylhydrazine in 20 ml 0.2 *M* hydrochloric acid. The mixture was incubated 30 min at 37°C and then extracted three times with 10-ml aliquots of chloroform. The combined chloroform extracts were washed twice with 2 *M* hydrochloric acid, twice with water and then evaporated under reduced pressure. The residue was dissolved in ethanol and analyzed by reversed-phase HPLC using a 10 μm μBondapak C_{18} column (300 × 3.9 mm) and a 30-min linear gradient of 20—100% methanol at 2 ml/min. The absorbance was monitored at 340 nm and the amount of 2,4-dinitrophenylhydrazone was quantified by comparison to an authentic standard [20] which eluted at 18.5—19.0 min. When [^{3}H]formaldehyde was assayed, 30-sec fractions were collected for measurement of radioactivity.

Preparation of hydroxymethyldeoxynucleoside standards

Solutions of 10 m*M* deoxynucleoside and 670 m*M* formaldehyde in 100 m*M* potassium phosphate buffer, pH 7.0, were incubated for 24 h at 37°C. HPLC analysis of the solutions, using the conditions outlined below, indicated the presence of two major UV-absorbing peaks from each incubation. In each case, the early eluting peak corresponded to the starting material while the second peak contained the modified deoxynucleoside. The product from each incubation was collected for spectral characterization.

Reaction of calf thymus DNA with [^{3}H]formaldehyde

A 400-μl solution consisting of 650 μg DNA and 2 μg [^{3}H]formaldehyde in 40 m*M* sodium acetate buffer, pH 4.5, was incubated 20 h at 37°C. The mixture was then cooled on ice, and the DNA was precipitated by addition of 30 μl of 1 *M* sodium chloride followed by 800 μl ice-cold ethanol. The DNA was recovered by centrifugation and dissolved in 400 μl of 5 m*M* Bis-Tris—0.1 m*M* EDTA buffer, pH 7.1. After determination of the specific activity, the DNA was again precipitated, collected by centrifugation and dissolved in the Bis-Tris buffer.

Enzymatic hydrolysis of calf thymus DNA and HPLC separation of products

The DNA solutions (400 μl) were hydrolyzed by addition of 20 μl 20 m*M* zinc sulfate, 20 U nuclease P_1 and 0.5 U alkaline phosphatase [21]. After incubating 1 h at 37°C, 75 μl 1 *M* Tris—HCl, pH 8.0, was added and the hydrolysis was continued one additional hour. The solution was centrifuged

with a Beckman microfuge at 22,000 *g* for 1 min and aliquots of the supernatant were analyzed directly by HPLC. Individual adducts were separated with a 10 μm μBondapak C_{18} reversed-phase analytical column (300 × 3.9 mm) with a linear gradient of 10 m*M* ammonium acetate, pH 5.3, to 20% methanol in 10 m*M* ammonium acetate, pH 5.3, over 30 min at a flow-rate of 2 ml/min [21]. The absorbance was monitored at 254 nm and 30-sec fractions were collected for analysis of radioactivity.

Incubation of Chinese hamster ovary (CHO) cells with [^{3}H]formaldehyde, isolation of DNA and analysis of adducts

Suspension cultures of CHO-K_1-BH_4 cells (obtained from A.W. Hsie, Oak Ridge, TN, U.S.A.) were centrifuged and the cell pellet was resuspended at a concentration of approximately 1 × 10^7 cells/ml in Ham's medium F12, containing 25 m*M* N-2-hydroxyethylpiperazine-N′-ethanesulfonic acid buffer, pH 7.3 [22]. Samples were treated with 1 m*M* [^{3}H]formaldehyde or 2.5 m*M* and 5 m*M* formaldehyde for 2 h at 37°C. Aliquots were used to determine cell survival by relative cloning ability and the remaining cells from the [^{3}H]formaldehyde exposure were isolated by centrifugation for adduct analysis.

DNA was isolated using a modification of the technique of Gupta [23]. Cell pellets were suspended in 10 ml 1% sodium dodecyl sulfate—1 m*M* EDTA and homogenized for 30 sec with a Polytron (Brinkmann Instruments, Westbury, NY, U.S.A.) at speed setting 4—5. After centrifugation for 5 min at 3400 *g* to break the emulsion, the solution was treated with 5 mg proteinase K for 30 min at 37°C. After addition of 500 μl 1 *M* Tris—HCl, pH 8.0, the solution was gently extracted for 5 min with 10 ml phenol and then centrifuged at 3400 *g* for 5 min. (The phenol and all other organic solvents used in the DNA isolation and purification were previously saturated with 50 m*M* Tris—HCl, pH 8.) The aqueous phase was then sequentially extracted with 10 ml phenol—chlorform—isoamyl alcohol (25:24:1), followed by 10 ml chloroform—isoamyl alcohol (24:1). After adding 1.1 ml 5 *M* sodium chloride to the aqueous fraction, the DNA was precipitated with 11 ml ice-cold ethanol, washed twice with 5 ml 70% ethanol, and redissolved in 2 ml 1.5 m*M* sodium chloride- 0.15 m*M* trisodium citrate—1 m*M* EDTA buffer. The solution was treated with 40 μl 1 *M* Tris—HCl, pH 7.4, 600 μg heat-treated RNase A, and 300 U RNase T_1, and incubated for 15 min at 37°C. Following the addition of 250 μl 1 *M* Tris—HCl, pH 8, the mixture was extracted with 6 ml phenol—chloroform—isoamyl alcohol (25:24:1) and then with 6 ml chloroform—isoamyl alcohol (24:1). The DNA was precipitated by sequential addition of 600 μl 5 *M* sodium chloride and 6 ml ice-cold ethanol, washed twice with 70% ethanol, and dissolved in 4 ml 5 m*M* Bis-Tris—0.1 m*M* EDTA, pH 7.1, for analysis. The DNA was quantified by UV spectroscopy (48 μg/ml = 1.0 $A_{260\,nm}$) and its specific activity was determined by liquid scintillation spectrometry. Aliquots were enzymatically digested and analyzed for adducts by the technique used for calf thymus DNA.

RESULTS

Formaldehyde analysis

The purity of the formaldehyde was estimated by formation of its 2,4-dinitrophenylhydrazone derivative and analysis of this product by reversed-phase HPLC using a modification of the procedure of Mansfield et al. [19]. Based upon comparisons to an authentic standard, the recovery of formaldehyde, using this technique, was 103 ± 3%. When the [^{3}H] formaldehyde was analyzed in a similar manner, 93% of the radioactivity partitioned into the organic phase, and 90% of the radioactivity which migrated on the HPLC coeluted with the standard. The identity of the impurities is not known.

Preparation of formaldehyde—deoxyribonucleoside standards

HPLC analysis of the deoxyribonucleoside—formaldehyde reaction mixtures indicated the presence of one major product from incubation with each of the deoxynucleosides. Each adduct, with the exception of the thymidine derivative, was isolated for characterization by UV and NMR spectroscopy. The spectral parameters are given in Table I. The UV spectra are consistent with the previously reported values for the formaldehyde adducts [2]. In each case, the NMR spectra confirmed the hydroxymethyl substitution at the exocyclic amines. Although a product was observed from the reaction of thymidine with formaldehyde, it was formed in insufficient quantity and was too labile for characterization.

Reaction of [^{3}H] formaldehyde with calf thymus DNA

Calf thymus DNA was incubated with [^{3}H] formaldehyde for 20 h. The DNA was precipitated and then reprecipitated until constant specific activity was obtained. The enzymatically digested DNA was analyzed by HPLC which indicated two major bands of radioactivity (Fig. 1). The first migrated with the void volume and accounted for 85% of the radioactivity applied to the column; the second band coeluted with N^6-hydroxymethyldeoxyadenosine and contained 7% of the activity. In some experiments, small amounts of radioactivity coeluted with N^4-hydroxymethyldeoxycytidine (0.6%) and with N^2-hydroxymethyldeoxyguanosine (0.2%).

The HPLC profile indicated that the enzymatic hydrolysis of the DNA was complete because UV absorbance was associated only with the deoxynucleoside monomers and the hydroxymethyldeoxynucleoside markers. The absence of UV absorbance in the vicinity of the void volume suggested that the radioactivity eluting in this region might be due to nonreacted [^{3}H] formaldehyde. To examine this possibility, the modified DNA was incubated with 2,4-dinitrophenylhydrazine and the products formed were analyzed by HPLC. Of the radioactivity applied to the column, more than 85% migrated with the 2,4-dinitrophenylhydrazone standard, while the remaining activity eluted as a single peak slightly later.

McGhee and Von Hippel have reported rapid removal of residual noncovalently bound formaldehyde from polynucleotides by use of a Bio-Rad P2 column [6]. Repeated attempts to use this technique were not successful; in each case all the radioactivity applied to the column eluted with the DNA.

TABLE I

UV AND ^{1}H-NMR SPECTRAL PARAMETERS OF HYDROXYMETHYL DEOXYRIBONUCLEOSIDE ADDUCTS

Compound	UV*	NMR**	
		Chemical shift (δ)	Assignment
N^6-Hydroxymethyldeoxyadenosine	263	2.29	H2'a
		2.72	H2'b
		3 57	H5'ab
		3.87	H4'
		4.41	H3'
		4.93***	-$\underline{CH_2}$-
		6.35	H1'
		8.26***	H8
		8 41***	H2, -N$\underline{H}$CH$_2$-
N^2-Hydroxymethyldeoxyguanosine	253 (shoulder, 275)	2 20	H2'a
		2.55	H2'b
		3.50, 3.54	H5'a,b
		3.80	H4'
		4.30	-CH$_2$O$\underline{H}$
		4 35	H3'
		4.76	-$\underline{CH_2}$-
		4.85	3'$\underline{O}$H
		5 25	5'OH
		6 18	H1'
		6.99	-N$\underline{H}$CH$_2$-
		7 92	H8
N^4-Hydroxymethyldeoxycytidine	271	1.90	H2'a
		2.09	H2'b
		3 57	H5'ab
		3 76	H4'
		4.19	H3'
		4 72	-$\underline{CH_2}$-
		5 75	H5
		6.15	H1'
		7.81	H6
		8.15	-N$\underline{H}$CH$_2$-

*UV spectra were recorded in water and are reported as the maximum absorbance in nm.

**^{1}H-NMR spectra were recorded at 500 mHz in dimethylsulfoxide-d_6 and are referenced to internal tetramethylsilane. The adducts formed from reaction of formaldehyde with the deoxynucleosides were isolated by HPLC. Due to the adducts' lability, some decomposition occurred during the preparation of the samples for NMR analysis. Therefore, the products were analyzed as mixtures of the adducts and their nonmodified nucleosides. Resonance assignments were made through comparisons to nonmodified deoxynucleosides.

***These signals were unusually broad when spectra were obtained at 300°K. When measured at 325°K, the resonances were dramatically sharpened A similar broadening has been reported for H2 of an N^6-substituted deoxyadenosine [24].

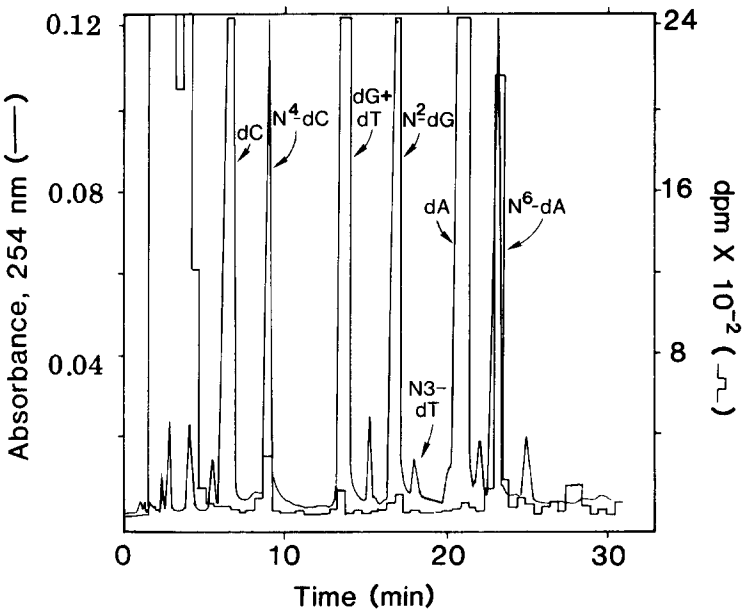

Fig. 1. Reversed-phase HPLC of adducts obtained from reacting [^{3}H]formaldehyde with calf thymus DNA. Following enzymatic hydrolysis, the solution was centrifuged and aliquots of the supernatant were analyzed directly. Synthetic hydroxymethyldeoxynucleosides were added to serve as UV markers. N^6-dA = N^6-hydroxymethyldeoxyadenosine; N^2-dG = N^2-hydroxymethyldeoxyguanosine; N^4-dC = N^4-hydroxymethyldeoxycytidine; N3-dT = N^3-hydroxymethylthymidine. The structure of the thymidine adduct has not been confirmed by spectral analysis. The adducts were separated by running a linear gradient of 10 m*M* ammonium acetate, pH 5.3, to 20% methanol in 10 m*M* ammonium acetate, pH 5.3, over 30 min at a flow-rate of 2 ml/min. (—), Absorbance at 254 nm of the hydrolyzed DNA plus the hydroxymethyldeoxynucleoside markers; (⎍⎍), radioactivity associated with the DNA.

In control experiments where [^{3}H]formaldehyde was applied in the absence of DNA, all the radioactivity was retained. When the enzymatic hydrolysate was applied to a P2 column, all of the radioactivity remained on the column as did all of the nucleosides. Prolonged drying of either the enzymatic hydrolysate or the DNA in vacuo also did not remove the noncovalently bound radioactivity.

Treatment of CHO cells with [^{3}H]formaldehyde

CHO cells were treated in suspension with either 1 m*M* [^{3}H]formaldehyde or 2.5 and 5 m*M* formaldehyde for 2 h at 37°C. After aliquots were removed to measure cell survival, the remainder of the [^{3}H]formaldehyde-treated cells was recovered by centrifugation to be used for adduct analysis.

The cell survival of the treated CHO cells, compared to control cultures, was 103, 9, and < 0.04% for 1, 2.5, and 5 m*M* formaldehyde, respectively.

The yield of DNA from 3.85×10^8 cells was 3.3 mg with a specific activity of 1.90×10^5 dpm per mg DNA. Aliquots (340 μg) were enzymatically hydrolyzed and following centrifugation, the supernatant was analyzed by HPLC. Enzymatic hydrolysis released 91% of the radioactivity associated with the DNA and four radioactive bands were observed in the HPLC profile (Fig. 2). The first peak, containing 70% of the radioactivity, eluted with the void volume and based upon previous results was probably nonreacted formaldehyde. The second band (25%) coeluted with deoxyguanosine and/or

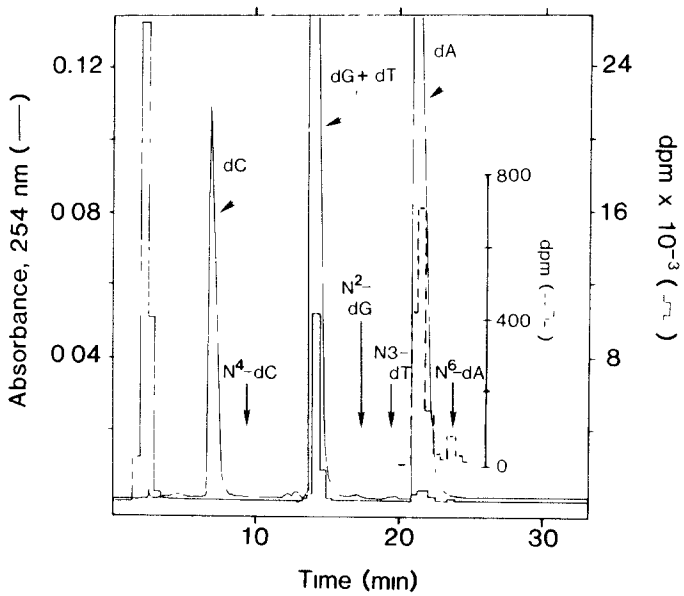

Fig. 2. Reversed-phase HPLC of supernatant from enzymatic hydrolysis of DNA isolated from CHO cells treated with [^{3}H]formaldehyde. Chromatographic conditions are identical to those used in Fig. 1. Under these conditions the hydroxymethyl adducts elute as follows: N^4-hydroxymethyldeoxycytidine, 9.0 min; N^2-hydroxymethyldeoxyguanosine, 17.2 min; N3-hydroxymethylthymidine, 19.2 min; N^6-hydroxymethyldeoxyadenosine, 23.8 min (—), Absorbance at 254 nm from the hydrolyzed CHO cell DNA; (⎍), radioactivity associated with the CHO cell DNA; (⎍), enlargement of the solid histogram ((included to emphasize the contributon from N^6-hydroxymethyldeoxyadenosine). The arrows indicate the elution volume of the hydroxymethyldeoxynucleoside standards.

thymidine. Subsequent analysis, using a program which would separate these two nucleosides, indicated that all of the activity was due to thymidine. The third peak (2%) coeluted with deoxyadenosine while the final band of radioactivity (0.2%) comigrated with N^6-hydroxymethyldeoxyadenosine. HPLC separation of twice the amount of DNA hydrolysate gave a concentration-dependent increase in all peaks. Assuming that the deoxyadenosine adduct had the same specific activity as the [^{3}H]formaldehyde, the level of binding in the CHO cells was 65 N^6-hydroxymethyldeoxyadenosines per 10^8 nucleotides. In a control experiment in which [^{3}H]formaldehyde-modified calf thymus DNA was subjected to the same DNA isolation procedure used for the CHO cells, the specific activity of the DNA decreased by 70% and the concentration of N^6-hydroxymethyldeoxyadenosine decreased by 60%. Thus, the concentration of N^6-hydroxymethyldeoxyadenosine in the CHO cell genome was probably at least twofold higher than that detected by the HPLC analysis.

DISCUSSION

The reaction of formaldehyde with deoxynucleosides has been reported to yield hydroxymethyl derivatives (see refs. 2 and 3 for pertinent references). Since these products are unstable, techniques had to be developed for the rapid isolation, hydrolysis and chromatography of formaldehyde-modified DNA.

In addition, since formaldehyde adducts have a polarity similar to nonmodified nucleosides and because metabolic incorporation of the formaldehyde into purines and pyrimidines could occur, the chromatographic system had to be able to resolve both modified and nonmodified deoxynucleosides.

When formaldehyde was reacted with deoxyadenosine, deoxycytidine and deoxyguanosine, one major adduct was detected in each instance. Although it was assumed that these were hydroxymethyl derivatives formed by reacting with the exocyclic nitrogens, with the exception of adenosine [4], this had not been unambiguously proven. Therefore, the major product from each reaction was isolated for spectral characterization. The UV spectra of the compounds were consistent with previously reported spectra [2] and NMR spectra contained a characteristic methylene doublet (ca. δ 4.7) which collapsed to a singlet upon irradiation of the adjacent amine proton. (It should be noted that NMR spectra have recently been reported on N-ethoxymethyl derivatives of ribonucleosides [25].) These data taken together indicate that the adducts are, indeed, exocyclic hydroxymethyl derivatives.

Incubation of [^{3}H] formaldehyde with calf thymus DNA yielded an apparent binding of seven formyl residues per 10^4 nucleotides. However, it became apparent that the great majority of this radioactivity was due to nonreacted formaldehyde. Attempts to remove this noncovalently bound material were unsuccessful. McGhee and Von Hippel [6] reported that a Bio-Rad P2 column could be used to lower the formaldehyde concentration to less than 1—2 μM, which was the limit of detection in their fluorescent analysis. In the absence of DNA, we found that the P2 column would remove more than 98% of the applied formaldehyde. With DNA present, however, the concentration of [^{3}H]-formaldehyde (ca. 3 μM) associated with the DNA did not change. Nevertheless, the HPLC procedure reported herein provided adequate resolution between the nonreacted formaldehyde and the adducts which were formed.

Initial attempts to separate the adducts were based upon acid hydrolysis and cation-exchange HPLC [26]. Although this did provide adequate resolution, we were concerned that the acidic conditions might destroy the relatively unstable hydroxymethyl derivatives. The procedure adopted was a modification of the enzymatic hydrolysis and reversed-phase HPLC developed by Kuo et al. [21]. By using enzymatic hydrolysis, neutral and mild conditions were maintained. When combined with the DNA isolation technique of Gupta [23], the entire analysis could be conducted within 6 h and complete resolution was obtained between the adducts, the nonmodified nucleosides, and formaldehyde.

Analysis of calf thymus DNA modified with [^{3}H] formaldehyde indicated that the majority of the activity was due to noncovalently bound formaldehyde. However, radioactivity was observed to comigrate with the synthetic hydroxymethyl derivatives of deoxyadenosine, deoxyguanosine, and deoxycytidine. Furthermore, the relative proportion of the adducts was N^6-hydroxymethyldeoxyadenosine $\gg$ N^4-hydroxymethyldeoxycytidine $>$ N^2-hydroxymethyldeoxyguanosine which is the anticipated order based upon the ease of formation of the adducts and their relative stability [4—7].

Incubation of CHO cells with 1 mM [^{3}H] formaldehyde gave an apparent binding to DNA of 3.2 formyl residues per 10^4 nucleotides. Upon hydrolysis,

more than 90% of the radioactivity was released and could be chromatographed. However, the majority of the radioactivity coeluted with formaldehyde, while a substantial proportion of the remainder was incorporated metabolically into nonmodified nucleosides, primarily thymidine. Nevertheless, N^6-hydroxymethyldeoxyadenosine was clearly present at a level of 65 adducts per 10^8 nucleotides. Other adducts may have been present in the CHO cell genome; however, if their relative proportion was similar to that found in the calf thymus DNA, the concentration of these adducts would have been below our limit of detection.

In conclusion, we have confirmed that the major adducts obtained from reacting formaldehyde with deoxynucleosides are hydroxymethyl derivatives at the exocyclic amines. Since these products are quite labile, we developed techniques for the rapid isolation, hydrolysis and chromatography of formaldehyde-modified DNA. We were able to demonstrate that the reaction of formaldehyde with isolated DNA yielded three adducts plus a substantial proportion of noncovalently bound formaldehyde. When CHO cells were incubated with formaldehyde, there was an appreciable amount of noncovalently bound formaldehyde associated with the DNA and, in addition, metabolic incorporation into the nucleoside bases had also occurred. However, we were also able to demonstrate clearly the formation of a hydroxymethyldeoxyadenosine adduct at a level similar to that reported for protein—DNA crosslinks [11] and single-stranded breaks [14]. The biological consequences which result from the formation of this adduct remain to be elucidated.

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