Aberrant and Nonrandom Methylation of Chromosomal DNA-binding Proteins of Colonic Epithelial Cells by 1,2-Dimethylhydrazine

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ABSTRACT

The alkylating carcinogen 1,2-dimethylhydrazine (DMH) induces colonic tumors with high incidence. The sites of in vivo modification of target macromolecules were studied using [methyl-3H]DMH. Carcinogen binding to subcellular fractions of colonic epithelial cells was analyzed at time intervals ranging from 10 min to 36 hr, with particular emphasis on the alkylation of nuclear constituents. DMH modifies not only nucleic acids but also histones and other DNA-binding proteins in the target cells. Separation of the 3H-methylated amino acids showed aberrant methylation patterns after exposure to [3H]DMH, as compared with methylations observed with [methyl-3H]methionine as a natural methyl group donor. DMH-modified histone H1 contained methylated lysine, arginine, and histidine residues not normally found, and other histones had abnormal methylarginine contents. High-mobility-group proteins and other nuclear proteins contained methyllysine residues not normally detected. Proteins known to be associated with template-active and more accessible DNA sequences, such as the high-mobility group proteins and the multiacetylated forms of histones H3 and H4, were preferentially damaged after exposure to [3H]DMH. The result suggests that carcinogen-induced chromosomal damage is not random and may selectively affect proteins in the actively transcribing or replicating genes in the target cells. That damage affects the amino acids most likely to be involved in DNA binding.

INTRODUCTION

DMH is an alkylating carcinogen which induces adenocarcinomas of the colon (12, 17, 38), malignant hemangioendotheliomas in the liver (12, 26), and kidney tumors (12, 17). The distribution of tumors in rats depends upon the dosage, schedule, and route of administration of the carcinogen. Given in repeated large dosages, DMH is particularly effective in inducing colonic tumors (12, 17, 38). It requires enzymatic activation to produce the metabolites which eventually alkylate the macromolecular constituents of the target cells (13, 38). The molecules modified in colonic cells include DNA (4, 18, 19, 37, 47), RNA (18), and cellular proteins (1). Hawks and Magee (18) established that methyl groups derived from [methyl-3H]DMH are transferred to colon DNA to form 7-methylguanine. This identifies which amino acids are modified in proteins in the more accessible template-active regions of the chromatin.

While it is generally accepted that persistent DNA damage by alkylating carcinogens and other mutagens is the major cause of malignant transformation, it is also evident that epigenetic changes play an important role in tumor promotion, progression, and in the expression of the malignant phenotype. Carcinogen-induced damage to proteins which regulate gene structure and function could have serious consequences for cell growth and differentiation. A variety of carcinogens are known to interact with chromosomal proteins in vivo and in vitro to form persistent adducts which would be expected to have altered DNA affinities (3).

The present paper deals with the characterization of such adducts in colonic epithelial nuclei following the injection of [methyl-3H]DMH. It identifies which amino acids are modified in histones, and other nuclear proteins in the target cells. Evidence is also presented that chromosome damage by the alkylating carcinogen is not random but preferentially affects proteins in the more accessible template-active regions of the chromatin.

MATERIALS AND METHODS

Synthesis of [methyl-3H]DMH. [3H]DMH was prepared according to the method of Horisberger and Matsumoto (20), starting with 4 g of N,N'-dibenzoylhydrazine (Aldrich Chemical Co., Milwaukee, Wis.) and successively methylating with 100 mCi of [3H]methyl iodide (specific activity, 100 mCi/mmol; New England Nuclear Inc., Boston, Mass.) and dimethyl sulfate. After removal of the benzoyl residues in HCl, the product, [methyl-3H]DMH, was crystallized from ethanol. The yield was 1.3 g (60%) with a specific activity of 50 μCi/mg.

Administration of [methyl-3H]DMH. Male CFN-Wistar rats weighing 100 g (Charles River Laboratories, Lake Vico, N. J.) were given i.p. injections of 300 μCi of [3H]DMH in a stock solution containing 6 mg/ml in 0.9% NaCl solution-1 mM EDTA, pH 6.5 (60 mg/kg body weight). For studies of normal methylation patterns, 100-g rats were given injections of 1 mCi of [methyl-3H]methionine (specific activity, 11 mCi/mmol; New England Nuclear). For studies of the kinetics of DMH modification of DNA, RNA, and colonic nuclear proteins, 4 rats were used for each time point. In tests for the RNase sensitivity of the [3H]DMH-labeled cytosolic fraction, 10 rats were used. For histone and HMG fractionations, groups of 30 rats were used in parallel labeling experiments using [3H]DMH and [methyl-3H]methionine. The animals were killed by cervical dislocation at the time points indicated in the various experiments.

Cell Fractionation. Colons were excised, and the epithelial layers were removed by scraping with a razor blade, as described previously (9). The tissue was washed in Earle’s balanced salt solution, and a small aliquot was solubilized in 1% SDS in 0.9% NaCl containing 0.32 M sucrose, using a Dounce-type homogenizer with a pestle clearance of 0.06 to 0.14 mm. After filtration through nylon bolting cloth, the suspension was centrifuged at 3300 x g for 10 min in a Sorvall SS34 rotor to separate the crude nuclear pellet from the "cytosolic" supernatant fraction.

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1 This research was supported by Grant CA 14906 from the National Cancer Institute, administered by the National Large Bowel Cancer Project; by NIH Grant GM 17383; and by American Cancer Society Grant NP-228K.

2 The abbreviations used are: DMH, 1,2-dimethylhydrazine; HMG, high-mobility-group proteins; SDS, sodium dodecyl sulfate.

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Analysis of [3H]DMH-labeled Cytosol. Four equal aliquots of the cytotoxic fraction were analyzed to determine the nuclease or protease sensitivities of the incorporated 3H-methyl groups. As groups, the first aliquot was dialyzed against 0.2 M sodium borate, pH 7.5, and Pronase (RNase-free) was added at an enzyme:substrate ratio of 1:100. After 4 hr at 37°C, the reaction was stopped by the addition of 10% SDS to a final concentration of 0.1%. The solution was then dialyzed against 0.1% SDS in 5 mM Tris-HCl, pH 7.3 (Buffer T). A second cytotoxic aliquot was dialyzed against 10 mM Tris-HCl, pH 7.8, containing 10 mM NaCl and 3 mM MgCl₂, and DNase I (Worthington, Freehold, N. J.) was added at a concentration of 10 Kunitz units/ml. After 1 hr at 37°C, the reaction was stopped and the mixture dialyzed as before. A third cytotoxic aliquot was dialyzed against 5 mM Tris-HCl, pH 7.5, and then treated with RNase A; (500 µg/ml Worthington) for 1 hr at 37°C. The reaction was stopped, and the mixture was dialyzed as above. A fourth aliquot (control) was dialyzed directly against Buffer T. Each fraction was analyzed by exclusion chromatography on 1.0- x 25-cm columns of Sephadex G-25 which had been equilibrated with Buffer T. Elution of the columns was monitored by measuring the absorbance at 230 nm and the 3H activity of 0.5-ml fractions.

Isolation of Colonic Epithelial Nuclei. The crude nuclear pellet was suspended in 50 mM Tris-HCl, pH 7.2-25 mM KCl-5 mM MgCl₂-1 mM CaCl₂ buffer containing 2 µm sucrose and centrifuged at 50,000 x g for 60 min (9). The purified nuclei were washed twice with 0.14 M NaCl prior to extraction of the histones and other protein fractions.

Extraction and Separation of Nuclear Proteins. Histones and other nuclear proteins were extracted in 5 volumes of 0.25 M NaCl. The histones were separated from other proteins in the acid extract by ion-exchange chromatography on 0.5- x 10-cm columns of Bio-Rex-70 (200 to 400 mesh; Bio-Rad, Inc., Richmond, Calif.). Two procedures were used. (a) For simple separations of the more acidic components of the extract, the proteins were applied in 0.1 M sodium phosphate buffer, pH 7.4, containing 6 M urea, 0.1% 2-mercaptoethanol, and 0.4 M guanidine-HCl (Buffer B) and eluted in Buffer B. Histones and HMG proteins were then eluted from the column in buffer containing 4 M guanidine-HCl. (b) For separation of the histone fractions and HMG proteins, the Bio-Rex-70 column was eluted at a 0.8 to 4 M linear gradient of guanidine-HCl in 0.1 M sodium phosphate, pH 7.4-6 M urea:0.1% 2-mercaptoethanol (23, 39).

Alternatively, HMG proteins and histone H1 were extracted from the total acid-soluble protein fraction in 0.75 M HClO₄. Histone H1 was precipitated by the addition of 3.5 volumes of acetone. The supernatant solution was adjusted to 0.07 M in HCl, and the HMG proteins were precipitated by the further addition of 6.5 volumes of acetone (42).

The nuclear nonhistone proteins remaining after acid extraction were solubilized in 0.1 M sodium phosphate buffer, pH 7.2, containing 6 M urea, 0.1% 2-mercaptoethanol, and 0.4 M guanidine-HCl (23). The extract was chromatographed on Bio-Rex-70 to remove minor basic protein contaminants.

Protein Determinations. Protein concentrations were routinely measured by the method of Lowry et al. (28). The amounts of individual histone fractions were determined by their absorption at 230 nm, using highly purified histones as standards.

Histone Interaction with [3H]DMH. Total histones (1 mg/ml in Buffer B) were mixed with [3H]DMH (0.1 µCi/mg histone). After 1 hr at 0°C, the mixture was applied to a Bio-Rex-70 column, and the column was washed with Buffer B. The histones were eluted in buffer containing 4 M guanidine-HCl and analyzed for 3H activity by scintillation spectrometry. The same chromatographic procedure was applied to 3HDMH alone to establish that the free carcinogen did not bind to the column under these conditions.

Electrophoretic Separation of Nuclear Proteins. Histones and HMG proteins were separated by electrophoresis in SDS-polyacrylamide gels according to the procedure of Laemmli (22) or in acid:urea: polyacrylamide gels by the procedure of Ruiz-Carrillo et al. (41). Protein bands in SDS gels were stained by Coomassie blue, and those in acid:urea gels were stained with Amido Black 10B. Relative protein concentrations were estimated by quantitative densitometry of the stained gels at 575 and 615 nm, respectively (41). The 3H activity in individual bands was determined by cutting out the band, oxidizing it in a Packard Model 350 sample oxidizer, and measuring the 3H activity of the oxidation product, tritiated H₂O. Separation of histone H3 and H4 subfractions which differed in degree of acetylation was achieved by electrophoresis in acid:urea:polyacrylamide gels (41). The specific activities of each subfraction were determined by dividing the observed cpn by the absorbance of each band at 615 nm.

Amino Acid Analysis. Histones and other nuclear protein fractions were desalted by dialysis, lyophilized, and hydrolyzed in 6 N HCl under N₂ at 110°C for 24 hr. The amino acids were separated by ion-exchange chromatography on columns of PA 35 resin (Beckman), eluting in 0.35 M citrate buffer, pH 5.85, at 28°C (15). The elution positions of 3H-methylated amino acids were compared with those of authentic standards obtained from Calbiochem-Behring Corp. (La Jolla, Calif.). Eluates were monitored by absorbance at 570 nm after reaction with ninhydrin (46) and by counting successive 1-ml aliquots.

DNA Extraction and Analysis. The nuclear pellets remaining after extraction of the proteins were washed with 5% HClO₄ at 4°C. Each pellet was treated with 10% HClO₄ at 75°C for 20 min and centrifuged. Aliquots (2 ml) of the supernatant were analyzed for DNA content by the Burton modification of the Dische diphenylamine reaction (10) and for 3H activity by scintillation spectrometry.

RNA Extraction and Analysis. RNA was extracted in 10 mM sodium phosphate buffer, pH 7.5, containing 0.1% SDS and separated by the phenol procedure of Greenberg and Perry (16). RNA concentrations were measured by the orcinol reaction (30), and 3H activities were measured by scintillation spectrometry.

RESULTS

[methyl-3H]DMH Modification of Colonic Macromolecules. The uptake of radioactive DMH into colonic epithelial cells was measured at successive times following i.p. injection of 300 µCi of [methyl-3H]DMH into rats. Chart 1 compares the kinetics of labeling of the total tissue with the labeling of nuclei isolated from the tissue. The uptake of radioactive DMH into the tissue is rapid and peaks within 10 min. The radioactivity of the nuclear fraction (after washing to remove unbound DMH) rises...
gradually for 6 hr and remains essentially constant for the next 30 hr (Chart 1).

These radioactivity measurements do not discriminate between \(^\text{H}\) activities due to the presence of noncovalently bound DMH (or its metabolites) and the \(^{3}\text{H}\) activities due to the alkylation of cellular constituents by \(^{3}\text{H}\)-methyl groups. To study the covalent modifications of cytoplasmic macromolecules, the cytosolic fraction obtained after 8 hr of exposure to the carcinogen was dialyzed (to remove free \(^{3}\text{H}\)DMH and low-molecular-weight metabolites) and then analyzed by exclusion chromatography on Sephadex G-25 to determine the size distribution of the bound radioactivity. Chart 2A shows that most of the \(^{3}\text{H}\) activity passes through Sephadex G-25 in the exclusion peak, indicating its presence on large molecules. Treatment of the dialyzed cytosolic fraction with RNase converts nearly all of the radioactivity to low-molecular-weight products that are retained on the column (Chart 2B). Corresponding treatments of the labeled cytosol with DNase or with Pronase did not have this effect (data not shown). It follows that alkylated RNA is the major radioactive component in the cytoplasm of \(^{3}\text{H}\)DMH-treated colonie epithelial cells. The specific activity of the total RNA isolated by the phenol procedure (19,800 cpm/mg) is far greater than that of the total cellular protein (1,300 cpm/mg), or mixed nuclear proteins (3,700 cpm/mg), or DNA (2,800 cpm/mg).

**[\(^{3}\text{H}\)]**DMH Uptake into DNA and Nuclear Proteins. The kinetics of labeling of different macromolecules in the nuclei of \(^{3}\text{H}\)DMH-treated colonie epithelial cells are compared in Chart 3, in which the activities of the various fractions are expressed on a nuclear basis, i.e., as cpm/µg DNA. The specific activity of the DNA reaches a maximum at 6 hr after injection of the carcinogen and declines only slightly during the next 30 hr (Chart 3). The extensive labeling of colonie DNA seen here is in accord with earlier observations (4, 17–19, 27, 37, 40, 47) and is known to involve the formation of 7-methylguanine (18, 19, 36, 40, 47) and \(^{O}\)-methylguanine residues (11, 27, 36, 40, 47).

Histone-labeling kinetics differs from that of the DNA. The maximum \(^{3}\text{H}\) activity of the chromatographically purified basic proteins is observed at very early times. This early labeling may be due in part to DMH binding by the histones rather than to their covalent modification by the transfer of \(^{3}\text{H}\)-methyl groups from the activated carcinogen. In in vitro studies, we have observed an immediate interaction between histones and \(^{3}\text{H}\)DMH, which is not broken on subsequent ion-exchange purification of the histones on Bio Rex-70 (data not shown). However, at later times after DMH injection, most of the histone-associated radioactivity is due to protein methylation. For example, at 8 hr, 67% of the \(^{3}\text{H}\) activity in the histone fraction is recovered as methylated amino acids (see Chart 4). All histone classes are modified in the DMH-treated cells but not to the same extent. Chromatographic separations (23, 39) of the colonie histones at 8 hr show that the nucleosomal core histones H3 and H4 have a somewhat higher specific activity (362 cpm/mg) than do histones H2A plus H2B (198 cpm/mg) or istone H1 (204 cpm/mg). A more detailed analysis of the covalent modification of colonie histones by \(^{3}\text{H}\)DMH is presented below.

The \(^{3}\text{H}\) activity of the nonhistone proteins remaining after acid extraction of the nuclei exceeds that of the histones and follows a time course similar to that observed for DNA methylation (Chart 3). At least 80% of the radioactivity in the nonhistone protein fraction at the 8-hr time point is attributable to covalent modification of the constituent amino acids by the radioactive carcinogen. The nature of those modifications is described below.

**Aberrant Patterns of Nuclear Protein Methylation of \(^{3}\text{H}\)DMH.** The transfer of \(^{3}\text{H}\)-methyl groups from the activated carcinogen to the nuclear proteins of the colonie epithelium is a complex phenomenon involving multiple sites of modification and differing susceptibilities of different protein classes. We have analyzed the nature of the protein modifications in \(^{3}\text{H}\)DMH-treated cells and compared them with the normal meth-
ylation patterns observed using [methyl-3H]methionine as a natural methyl group donor. The results summarized in Chart 4 indicate that important differences exist in the proteins of control and carcinogen-treated cells, as revealed by their contents of methylated amino acids. Chart 4 shows the elution profile of the basic amino acids, lysine, histidine, arginine, and their methylated derivatives, as separated by ion-exchange chromatography of the acid hydrolysates of the colonic nuclear proteins. Chart 4A compares the normal and DMH-induced methylation patterns of the histone plus HMG protein fraction separated by chromatography on Bio Rex-70 columns. Normally, histones H3 and H4 in this fraction contain mono- and dimethyllysine residues but contain few, if any, methylated arginine residues. Whereas the HMG proteins contain appreciable amounts of dimethylarginine but no methylated lysine residues (8). The distribution of radioactively labeled amino acids after injection of [methyl-3H]methionine confirms the presence of both methylated lysine and arginine residues (Chart 4A). However, when the histones and HMG proteins are separated before amino acid analysis, it becomes clear that DMH-induced methylation patterns are not normal. For example, histone H1, which is not normally methylated, is modified by the carcinogen with the formation of mono- and dimethyllysine residues, 3-methylhistidine, and N°,N°-dimethylarginine (Table 1). The HMG proteins, which normally contain N°,N°-dimethylarginine but not methylated lysine residues (8), contain both mono- and dimethyllysine after the injection of [3H]DMH (Table 1).

Similar aberrations in amino acid methylation patterns are seen in other nuclear proteins of DMH-treated colonic epithelial cells. Chart 4B compares the methylation patterns using [methyl-3H]methionine and [3H]DMH as precursors. There is no detectable labeling of the lysine residues of the nuclear non-histone protein fraction when [3H]methionine is used as a natural methyl group donor, but lysine residues are methylated after exposure to the alkylating carcinogen (Chart 4B). Both radioactive methionine and DMH serve as precursors in the methylation of arginine residues in the colonic nuclear non-histone protein fraction. It is not known whether the carcinogen modifies arginine residues that are not normally methylated, but considering the abnormal methylations of histone H1 in DMH-treated animals, this seems probable.

In addition to these well-characterized methylations of histones and other nuclear protein fractions, DMH induces some unknown modifications of nuclear protein structure. These are indicated by X and Y in the elution profiles of Chart 4. Neither modification corresponds in elution position to monomethylamine, a known breakdown product of dimethylarginine (35).

**Nonrandom Modifications of Proteins in Chromatin by [3H] DMH.** We compared the specific 3H activities of the total histones, HMG-1, HMG-2, and residual nonhistone proteins in colonic epithelial cell nuclei 8 hr after injection of [3H]DMH. The normal methylation patterns of the same proteins were determined in paired rats receiving [methyl-3H]methionine. As shown in Chart 5, the specific activities of the 4 nuclear protein fractions are not very different when [3H]methionine is used as

<table>
<thead>
<tr>
<th>Site of modification</th>
<th>Histone H1</th>
<th>HMG fraction</th>
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</thead>
<tbody>
<tr>
<td>ε-N-Monomethyllysine</td>
<td>285</td>
<td>824</td>
</tr>
<tr>
<td>ε-N-Dimethyllysine</td>
<td>190</td>
<td>754</td>
</tr>
<tr>
<td>ε-N-Trimethyllysine</td>
<td>84</td>
<td>Trace</td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>773</td>
<td>520</td>
</tr>
<tr>
<td>X</td>
<td>96</td>
<td>640</td>
</tr>
<tr>
<td>Y</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>N°,N°-Dimethylarginine</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>N°-Monomethylarginine</td>
<td>230</td>
<td></td>
</tr>
</tbody>
</table>

*Modified amino acids listed in order of elution from ion-exchange column.*

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**Table 1**

<table>
<thead>
<tr>
<th>Aberrant methylation of HMG proteins and histone H1 by [methyl-3H]DMH</th>
<th>Specific activity (cpm/3 mg of protein hydrolyzed)</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>230</td>
</tr>
</tbody>
</table>

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**Chart 4.** Ion-exchange separation of the basic amino acids (lysine, histidine, and arginine) and their methylated derivatives in acid hydrolysates of the histone plus HMG protein fraction (A) and nonhistone nuclear proteins (B). The distribution of radioactivity in the methylated amino acids is shown after injection of the natural methyl group donor, [methyl-3H]methionine (8) or the radioactive carcinogen [methyl-3H]DMH (C). ---, absorbance at 570 nm.

---

**Chart 4A.** Ion-exchange separation of the basic amino acids (lysine, histidine, and arginine) and their methylated derivatives in acid hydrolysates of the histone plus HMG protein fraction (A) and nonhistone nuclear proteins (B). The distribution of radioactivity in the methylated amino acids is shown after injection of the natural methyl group donor, [methyl-3H]methionine (8) or the radioactive carcinogen [methyl-3H]DMH (C). ---, absorbance at 570 nm.
the precursor. However, the methylation of these nuclear proteins by [3H]DMH is not uniform; HMG-1 and HMG-2 are preferentially damaged by the alkylating carcinogen and are nearly 3 times more radioactive than are the histones (Chart 5). These HMG proteins are known to be preferentially localized in the more accessible regions of chromatin, as judged by their early release during limited endonuclease digestions of a variety of chromatin (6, 25, 29, 48). Their relatively high 3H-methyl content, as compared with that of the more randomly distributed histones, indicates that carcinogen damage to chromosomal proteins is more likely to involve the accessible, template-active regions than the transcriptionally inert regions of chromatin.

To test this view, we compared the levels of DMH-induced methylation of histone subfractions that differ in their degree of acetylation. The rationale for this approach is that the multiaetylated forms of histones H3 and H4 are known to be localized in the transcriptionally active regions; conversely, a loss of histone acetyl groups is associated with a suppression of RNA synthesis (2, 41, 43, 45, 49). Histone fractions which differed in their degree of acetylation were separated electrophoretically from colonie epithelial nuclei 8 hr after injection of [3H]-DMH. Three experiments were performed, comparing the specific 3H activities of the fast-moving (unacetylated) forms of the histones with the specific activities of their respective di- and triacylated forms. In histone H3, the carcinogen-derived radioactivity of the diacylated protein molecules ranged from 240 to 300 cpmp/A615 nm unit, while the corresponding activities of the nonacylated H3 molecules ranged from 60 to 140 cpmp/A615 nm unit. For histone H4, the 3H activities of the diacylated forms (ranging from 103 to 322 cpmp/A615 nm unit) were 3- to 5-fold higher than those of the unacylated H4 molecules in the same cells (35 to 60 cpmp/A615 nm unit). In one experiment, the triacylated forms of histone H4 (577 cpmp/A615 nm unit) were over 15 times more radioactive than were the parent forms (35 cpmp/A615 nm unit). Because of the very low concentrations of the triacylated forms of the histones, the measurement of their specific activities is not sufficiently accurate to warrant the generalization that the accessibility of the histones to carcinogen damage increases in direct proportion to their level of acetylation. However, the uniform trend toward higher 3H activities in the acetylated histones, as compared to the parent forms, in these 3 preliminary experiments, supports the view that DMH damage to chromosomal proteins is more likely to occur in the template-active regions of the chromatin of the target cell.

DISCUSSION

The use of [methyl-3H]DMH as a probe for covalent modifications of target macromolecules was first applied to studies of DNA modification (18, 19, 37). Our results confirm that colonie DNA is modified in DMH-treated animals but also indicate a more extensive modification of RNA in the cytosol. A variety of proteins, both nuclear and cytosolic, are targets for alkylation. We have focused on proteins in the nucleus which have known DNA-binding properties; these include the histones and HMG proteins. By comparing the normal patterns of protein methylation observed with [methyl-3H]methionine as a natural methyl group donor with those obtained with [methyl-3H]DMH as a prospective source of methyl carbonium ions, we reached the conclusion that the activated carcinogen methylates sites that are not normally modified. The methylation of lysine, histidine, and arginine residues in histone H1, for example, and the formation of methyllysine in the HMG proteins, represent changes in primary structure that could probably affect the DNA-binding properties of these important chromosomal proteins. Abnormal methylations of the nucleosomal core histones are also clearly evident in DMH-treated cells.

What is missing is definite information on how DMH-induced damage to histones or HMG proteins, or other nuclear proteins, might affect either the structure or the activity of the genetic material in the colonie epithelial cell. In the case of DNA damage, there are good indications of impaired template function. For example, the covalent modification of DNA by N-2-acetylaminofluorene has been observed to result in RNA chain termination (32), and DNA synthesis is also inhibited on templates containing adducts of N-acetoxyacetylaminofluorene (33). If DNA adduct formation presents an effective block to the progression of RNA or DNA polymerases, what are the likely consequences of hindered DNA-protein interactions or aberrant contacts induced by carcinogen-caused modifications of essential chromosomal proteins? Considering the evidence for a role of certain HMG proteins in transcription (2, 14, 24, 50) and in DNA replication (44), one might expect problems in polynucleotide chain initiation or elongation which could upset the normal balance of gene expression in the target nucleus and lead to a progressive change in phenotype. At present, there is no proof that DMH-induced damage to the chromosomal proteins is sufficient to initiate any aberrant patterns of transcription, but this is an important question which warrants further study.

A point of particular relevance to this question is the nonrandom distribution of carcinogen-induced damage to the chromatin of the target cell. One would expect that carcinogen-induced damage to the DNA would be highly dependent upon the shielding provided by DNA-binding proteins in different regions of the chromatin. This is what is observed in comparisons of the extent of modification in nucleosome "cores" and "linker" regions after short exposures to a variety of carcinogens, including aflatoxin B1 (5), benzo(a)pyrene (21), 7-bromomethylbenz(a)anthracene (34), and N-hydroxy-2-aminofluorene (31). The recent finding that a nucleosome-free control region of the SV40 viral chromosome is more susceptible to attack by N-acetoxyacetylaminofluorene than is the rest of the SV40 genome (7) is a particularly striking example of the importance of chromatin structure in the distribution of carcinogen-induced damage.
We find that DNA-binding proteins in the more accessible regions of chromatin are also more susceptible to carcinogen-induced modifications. This conclusion is based on: (a) the preferential damage to proteins of the high-mobility group as compared with the lower levels of alklylation of the more randomly distributed histones; and (b) the greater susceptibility of the multiacylated forms of histones H3 and H4 to methylation by [methyl-3H]DMDH, as compared with their nonacylated forms. Both results are indicative of nonrandom targeting of chromosomal proteins, with the greatest damage to proteins in transcriptionally active and replicating regions of the chromatin in the colonic epithelial cell.

REFERENCES


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