Conformation of 2-[(Deoxyguanosin-8-ylacetyl)amino]fluorene Differs in Protein-Free Deoxyribonucleic Acid and Chromatin†

Jeff Ross,‡ Gérard Metzger, and Harold Werbin*

ABSTRACT: There is substantial evidence that modification of protein-free DNA with 2-[(acetoxyacetyl)amino]fluorene results in the formation of 2-[(deoxyguanosin-8-ylacetyl)-amino]fluorene adducts in which the fluorene ring inserts between the bases and the guanosine is shifted to the outside of the helix creating localized regions of denaturation. It was therefore of interest to determine the conformation of similarly induced adducts in chromatin and nucleosome core particles by circular dichroism, thermal denaturation, and single-strand-specific endonuclease susceptibility. Fluorenyl residues in modified chromatin and core particles gave rise to a less intense circular dichroism signal at 300 nm than those in purified DNA: ~−190 vs. ~−280 deg cm^2/nmol of fluorene residues, respectively. Levels of modification from 1.37 to 2.87 fluorenyl residues/100 nucleotides that produced a decrease of the melting temperature from 0.8 to 2.0 °C in protein-free DNA had no effect upon the melting temperature of core particles. Adducts were not removed from modified core particles and chromatin by *Neurospora crassa* endonuclease as they were from modified DNA, even though this enzyme at the same concentration (12 units/A_{260} unit) recognized single-stranded regions introduced into the core particles by mild DNase I digestion. These data demonstrate minimal interaction of the fluorenyl residues with the DNA helix and provide no evidence for their induction of locally denatured regions. Hence, the existence of different conformations for modified bases in chromatin and protein-free DNA is proposed. The implications of this nondenaturing conformation with respect to the in vivo repair of these lesions are discussed, as well as the possibility that modified bases adopt a Z-DNA-like structure in modified chromatin as they do in modified poly(dG-dC)·poly(dG-dC).

The carcinogen AAF[1] binds to cellular macromolecules following metabolic activation to esters of *N*-OH-AAF (Miller, 1970). The synthetic ester *N*-AcO-AAF binds in vivo and in vitro to DNA yielding two guanine adducts: *N*-(deoxyguanosin-8-yl)-2-AAF (C-8 derivative) and 3-(deoxyguanosin-N^2-yl)-2-AAF (N^2 derivative) (Kriek, 1974). In vitro, the C-8 derivative predominates, accounting for approximately 80% of the bound fluorene. Formation of the C-8 derivative is followed by rotation of the guanosinyl-AAF adduct around the glycosidic bond, such that the planar fluorene ring stacks between the adjacent base pairs and the guanine moiety protrudes. This phenomenon, termed "base displacement" (Levine et al., 1974) or "insertion–denaturation" (Fuchs, 1975), results in a local region of denaturation around the C-8 derivative in DNA, recognizable by ss-specific endonucleases (Fuchs, 1975; Yamasaki et al., 1977a). The interactions of the fluorene ring with the bases in the interior of the helix induce optical activity detectable as a CD signal at 300 nm (Fuchs & Daune, 1972).

In contrast to the C-8 derivative, the N^2 derivative is not efficiently removed from *N*-AcO-AAF-modified DNA by ss-specific endonucleases (Yamasaki et al., 1977a). The fluorene ring of this adduct is situated in the minor groove of the DNA and produces no helix distortion (Bleland, 1978).

N-AcO-AAF binds to DNA in chromatin to yield the same adducts as with protein-free DNA, most of the binding occurring in the linker DNA, although the core DNA is still reactive (Metzger et al., 1976; Kaneko & Cerutti, 1980). Modification of chromatin does not grossly affect its micrococcal nuclease sensitivity or digestion pattern (Metzger et al., 1976), and nucleosomes can be reconstituted in vitro on to *N*-AcO-AAF-modified DNA (Yamasaki et al., 1977b). Thus modification with *N*-AcO-AAF does not greatly distort the chromatin structure. Although the C-8 derivative is formed in chromatin, it is not excised by the ss-specific nuclease S_1 as it is in *N*-AcO-AAF-modified protein-free DNA (Metzger et al., 1976). This observation, coupled with the results of this study, leads us to propose that the conformation of C-8 derivatives in modified chromatin differs from that in modified protein-free DNA and that the base displacement or insertion–denaturation model is not applicable to modified chromatin.

Experimental Procedures

Reagents and Enzymes. All chemicals were reagent grade. [9-^{14}C]-*N*-AcO-AAF (38 mCi/mol) and unlabeled *N*-AcO-AAF were synthesized by the method of Lotlikar et al. (1966) and were purified by dry column chromatography on silica gel (E. Merck). The purified compound migrated as a single spot (R_f = 0.42) when analyzed by TLC on silica gel developed with chloroform–acetone (19:1). Additional [9-^{14}C]-*N*-AcO-AAF (49.6 mCi/mmol) utilized in some of the experiments was supplied by the NCI Chemical Carcinogen Reference Standard Repository, a function of the Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Neurospora crassa ss-specific endonuclease and DNase I were from Sigma Chemical Co. Micrococcal nuclease and proteinase K were products of Worthington Chemical Corp. and Boehringer Mannheim, respectively.

Preparation of Calf Thymus Chromatin and Rat Liver Nuclei. The preparation of calf thymus chromatin has been described elsewhere (Metzger & Werbin, 1979). Isolation of

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[1] Abbreviations: AAF, 2-(acetylamino)fluorene; ss, single strand; ds, double strand; CD, circular dichroism; *N*-AcO, *N*-acetoxy; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; NaDodSO$_4$, sodium dodecyl sulfate; TLC, thin-layer chromatography; NMR, nuclear magnetic resonance.

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nuclei from male Sprague-Dawley rat livers and all subsequent operations were carried out at 0–4 °C. Freshly excised livers were perfused and rinsed with buffer A1. Buffer A1 consists of buffer A of Hewish & Burgoyne (1973) (15 mM Tris-HCl, pH 7.4, 15 mM NaCl, 15 mM 2-mercaptoethanol, 60 mM KCl, 0.5 mM spermidine, and 0.15 mM spermine) supplemented with 0.34 M sucrose, 2 mM Na_2EDTA, 1 mM EGTA, and 1 mM PMSF. After the livers were minced with scissors in fresh buffer A1 and homogenized in a glass homogenizer with a Teflon pestle, the homogenate was filtered through eight layers of gauze and centrifuged for 10 min at 8000*g*. The pellet was resuspended by homogenization in buffer A1 containing 0.5% (v/v) Triton X-100. The suspension, 20 mL, was placed in 50-mL polypropylene centrifuge tubes, underlayered with 10 mL of buffer A2, which contains buffer A with the addition of 1.37 M sucrose, 1 mM EDTA, 0.5 mM EGTA, and 1 mM PMSF, and centrifuged at 10000*g* for 20 min. The nuclear pellet was washed twice by suspension and resedimentation in buffer A1. The nuclei, examined by phase-contrast microscopy, were spherical with well-defined nucleoli and were free of gross cytoplasmic contamination.

Preparation of Nucleosome Cores from Rat Liver Nuclei. The nuclei were resuspended in buffer O (0.3 M sucrose, 50 mM triethanolamine, 25 mM KCl, 4 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 mM PMSF, pH 7.0) (Todd & Garrard, 1977) at a concentration of about 50 A_{260} units/mL. Micrococcal nuclease was added to 0.8 unit/A_{260} unit and the suspension incubated at 37 °C for 3 min with gentle swirling. The digestion was terminated by addition of EDTA to 10 mM and by chilling to 0 °C. The nuclei were sedimented at 8000*g* for 10 min and lysed by resuspension in 0.2 mM EDTA (pH 7). The membranes and insoluble nuclear material were sedimented at 8000*g* for 5 min, providing soluble chromatin in the supernatant.

Nucleosome cores were prepared from the soluble chromatin according to Lutter (1978) except that Sephacryl S-300 (Pharmacia) was used as the gel-filtration medium. Nucleosome cores were concentrated by vacuum dialysis against 10 mM Tris-HCl and 1 mM EDTA (pH 7.5) to about 10 A_{260} units/mL and stored at 4 °C.

Purification of DNA. DNA was extracted from chromatin samples by incubating them with proteinase K (25 μg/A_{260} unit at 37 °C for 3 h). The samples were then extracted with phenol–chloroform–isoamyl alcohol (24:24:1 v/v), and phenol was removed from the DNA phase by diethyl ether extraction and extensive dialysis.

Modification of Chromatin, Nucleosome Cores, and DNA with N-AcO-AAF. Samples were saturated with dry argon gas, and the required amount of *N*-AcO-AAF, which was dissolved in a minimum volume of argon-saturated ethanol or dimethyl sulfoxide, was added. Different degrees of modification were obtained by increasing the carcinogen to DNA ratio in the reaction mixture. The reaction was allowed to proceed at 37 °C in the dark for 3 h. Unbound fluorenyl hydrolysis products were removed by three ether extractions, and the samples were dialyzed. Control samples free of *N*-AcO-AAF were run simultaneously.

Digestion of DNA Single Strands. Samples to be digested with *N. crassa* endonuclease were dialyzed against 10 mM Tris-HCl (pH 8.0)–1 mM $MgCl_2$, with or without 200 mM NaCl. Digestions were carried out at 37 °C, and samples taken at various times were mixed with 3.5 volumes of 4 M NaCl and 1.5 volumes of 1.9 M $HClO_4$ and left at 0 °C for 30 min. The samples were then centrifuged at 12800*g* in an Eppendorf microcentrifuge for 5 min. The percentage of acid-soluble nucleotides was calculated from the absorbance of the supernatant at 260 nm multiplied by 0.6 to correct it for hyperchromicity.

Digestion of Nucleosome Cores with DNase I and N. crassa Endonuclease. Nucleosome cores (9.7 A_{260} units/mL in 10 mM Tris-HCl and 1 mM EDTA, pH 7.5) were made 10 mM in $MgCl_2$ and warmed to 37 °C. After removal of a zero-time aliquot, 25 μg of DNase I/mL was added and incubation continued at 37 °C. At various times, samples were removed, made 20 mM in Na_2EDTA, and chilled on ice. An aliquot of each sample was used to determine the extent of DNA hydrolysis. For separation of the partially digested nucleosomes from the remaining DNAse I, the remainder of each sample was placed on a column of Sephacryl S-300 (200 mm × 8 mm) and eluted with a mixture of 10 mM Tris-HCl (pH 8.0) and 2 mM EDTA. The effluent was monitored at 260 nm.

Nucleosome core peak fractions were pooled, their absorbance at 260 nm was measured, and the samples were made 10 mM in $MgCl_2$ and 200 mM in NaCl. They were warmed to 37 °C and 12 units of *N. crassa* endonuclease/A_{260} unit was added. Incubation was continued for 4 h at 37 °C, and then the percentage of acid-soluble nucleotides was determined.

Determination of the Extent of Modification. The amount of labeled *N*-AcO-AAF bound to various samples was measured by liquid scintillation counting in 3a40 cocktail (Koch-Light Laboratories, Ltd.). The counts were corrected for quenching by the H number method (Horrocks, 1977). The amount of unlabeled *N*-AcO-AAF bound to samples was determined from the ratio of absorbances at 305 and 260 nm (Fuchs & Daune, 1972).

The DNA was quantitated by its absorbance at 260 nm assuming a molar absorptivity/nucleotide of 6700 L M^{-1} cm^{-1}. The extent of modification was expressed as the number of fluorenyl residues per 100 nucleotides.

TLC of Guanosinyl-AAF Adducts. DNA purified from *N*-AcO-AAF-modified nucleosome cores was depurinated by mixing it with an equal volume of 1.0 N HCl and by heating the mixture at 75 °C for 2 h in a sealed glass tube. Then the sample was diluted with 10 volumes of water and placed on a C-18 SEP-PAK cartridge (Waters Associates, Inc.). The column was washed with 10 mL of water, and the fluorenyl derivatives were eluted with 1 mL of methanol. A 200-μL aliquot of the eluate was chromatographed on a Whatman K1F silica gel TLC plate in a butanol–acetic acid–water (100:20:50 v/v) system.

Circular Dichroism. Circular dichroism spectra were taken on a Cary 61 CD spectrophotometer equipped with a Varian Spectrosystem 100 to digitize the values at each wavelength. Spectra of modified calf thymus chromatin and rat liver nucleosomes, and the DNA extracted from them, were measured in 10 mM Tris-HCl (pH 8.0). The dissociated chromatin samples were obtained by adding 0.1 volume of a 20% solution of $NaDodSO_4$ to the native sample.

Thermal Denaturation. Nucleosome and DNA samples were thermally denatured in 10 mM Tris-HCl (pH 7.5)–1 mM EDTA. Absorbance at 260 nm and temperature were monitored on a Beckman-Gilford 2000 spectrophotometer. The data are presented as the percent of the maximal hyperchromicity as a function of temperature. The melting temperature (T_m) of a sample was the temperature at which 50% of its maximmal hyperchromicity was attained.

Results

Metzger et al. (1976) have shown that ss-specific nuclease S_1 does not digest *N*-AcO-AAF-modified chromatin as it does

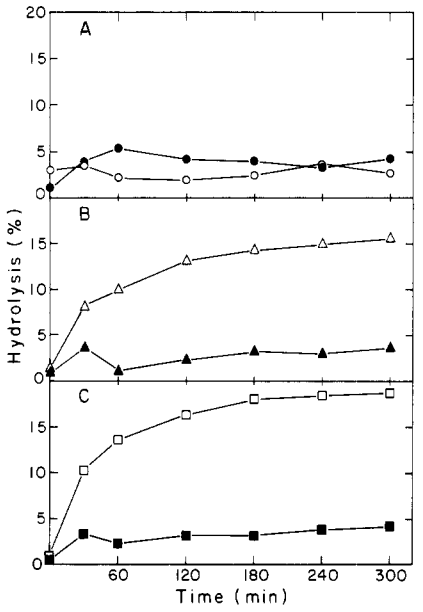

FIGURE 1: Digestion of modified nucleosome cores (closed symbols) and purified DNA isolated from the modified cores (open symbols) by *N. crassa* endonuclease. (A) No modification; (B) 1.36 DNA-bound fluorenyl residues/100 bases; (C) 2.87 DNA-bound fluorenyl residues/100 bases.

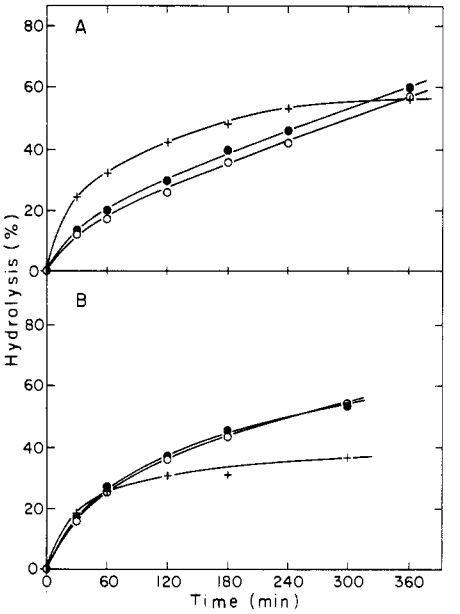

FIGURE 2: Digestion of unmodified and modified (A) DNA and (B) chromatin by *N. crassa* endonuclease. (○) Acid-soluble nucleotides from unmodified samples; (●) acid-soluble nucleotides from samples having 1.23 fluorenyl residues/100 bases; (+) acid-soluble fluorenyl residues.

modified protein-free DNA. Yamasaki et al. (1977a) found that while this enzyme preferentially removes C-8 adducts from *N*-AcO-AAF-modified DNA, it also removes some N^2 derivatives. In contrast, they reported that ss-specific endonuclease from *N. crassa* liberates only the C-8 derivatives. When this enzyme is used to digest modified chromatin, the concentration of salt must be low to avoid dissociation of histone H1 and nonhistone proteins. However, under low-salt conditions the enzyme will digest ds DNA in addition to ss DNA but still preferentially digests ss DNA 5–8 times as fast as ds DNA (Fraser, 1980). Thus, by using a low level of enzyme (0.05 unit/A_{260} unit) at low ionic strength, it should be possible to detect solely denatured regions in the modified chromatin. Under these conditions, neither the control (untreated) nor chromatin that had 1.23 ± 0.03 DNA-bound fluorenyl residues/100 bases was hydrolyzed. In contrast, protein-free DNA extracted from the modified chromatin was digested (data not shown).

Purified nucleosome cores, lacking histone H1 and nonhistone proteins, tolerate ionic strengths high enough to inhibit the activity of the *N. crassa* endonuclease on ds DNA. Thus, *N*-AcO-AAF-modified nucleosome cores were dialyzed against 10 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, and 200 mM NaCl, prior to incubation with *N. crassa* endonuclease (Figure 1). Under these conditions, heat-denatured DNA was rapidly digested while native DNA, native core particles, and core particles with 1.36 and 2.87 DNA-bound fluorenyl residues/100 bases were not. DNA extracted from the modified core particles was hydrolyzed, showing that the lack of hydrolysis of the modified core particles cannot be attributed to an absence of the C-8 derivatives. This was confirmed by TLC analysis of fluorene adducts in the DNA isolated from [^{14}C]-*N*-AcO-AAF-modified chromatin, which revealed two radioactive components with R_f values of 0.67 and 0.80, in excellent agreement with those reported for the C-8 and N^2 derivatives (Westra et al., 1976).

The observed lack of hydrolysis of modified chromatin and core particles could be accounted for either by the absence of local regions of denaturation around the C-8 derivatives or by the possible inaccessibility of the chromatin DNA to the endonuclease. For determination of the accessibility of the chromatin DNA to the nuclease, control and modified chromatin samples were incubated at a low ionic strength and a high enzyme concentration (12 units/A_{260} unit), conditions under which ds DNA and ss DNA are cleaved (Linn & Lehman, 1965). Digestion of both samples (Figure 2) argues against the inaccessibility of chromatin DNA. Comparison of the slopes of the initial digestion kinetics reveals no difference between the native and *N*-AcO-AAF-modified chromatin. If some ss regions had been created in the modified chromatin, the initial rate of hydrolysis would have been greater than that of the control, since, in addition to the hydrolysis of the ds DNA, the endonuclease would also have preferentially digested the ss regions. Additionally, the finding that the initial rate of release of fluorenyl residues from modified chromatin is identical with the rate of release of nucleotides implies that no preferential digestion of the modified regions occurred. In contrast, the initial rate of release of fluorenyl residues from the modified protein-free DNA was much greater than the rate of release of nucleotides.

A more direct approach to the question of accessibility of chromatin DNA to *N. crassa* endonuclease became apparent after the work of Riley (1980) appeared. He demonstrated that a brief digestion of chromatin with DNase I introduced ss gaps into the DNA. Nucleosome cores similarly treated could be tested for susceptibility to *N. crassa* endonuclease. This experiment was performed by digesting nucleosome cores for various times with DNase I and then redigesting with *N. crassa* endonuclease under conditions which were ss specific (Figure 3). It is clear that the ss regions introduced by DNase I can be recognized by the *N. crassa* endonuclease. All these findings are reconciled if the formation of *N*-AcO-AAF adducts in chromatin and nucleosome cores occurs without the base displacement that creates local denaturation.

This idea is supported by circular dichroism measurements of the signal at 300 nm of the fluorenyl residues in DNA. The measured CD spectra of modified DNA and chromatin is composed of the sum of component CD signals arising from the primary, secondary, and tertiary DNA structures, the fluorenyl–guanine adduct structure, and any local secondary

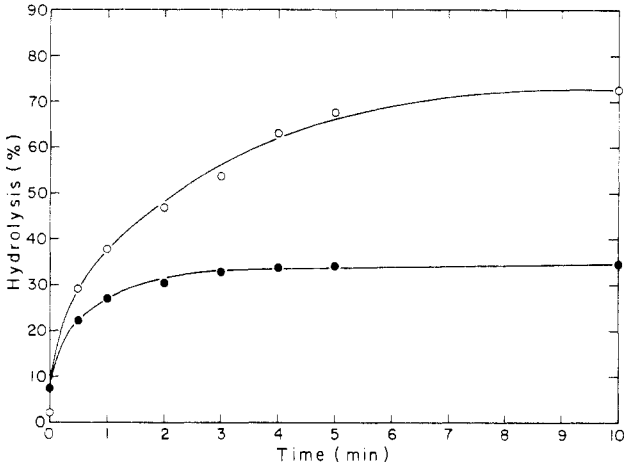

FIGURE 3: Consecutive digestion of nucleosome cores with DNase I and *N. crassa* endonuclease. (●) Acid-soluble nucleotides released by DNase I digestion; (○) acid-soluble nucleotides released by *N. crassa* digestion (4 h at 37 °C, 12 units of enzyme/A_{260} unit) of nucleosome cores isolated from the DNase I digest at the indicated times.

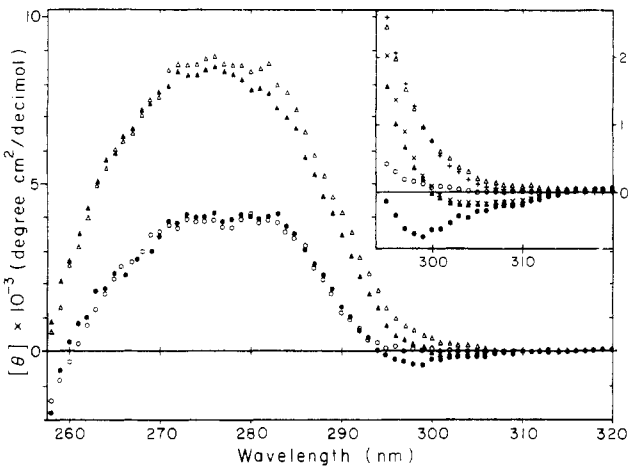

FIGURE 4: Circular dichroism spectra of chromatin and DNA. Unmodified (○) and modified (●) chromatin; unmodified (△) and modified (▲) DNA extracted from chromatin; unmodified (+) and modified (×) $NaDodSO_4$-dissociated chromatin. The modified samples had 1.08 DNA-bound fluorenyl residues/100 bases. Inset: enlargement of spectra between 295 and 320 nm.

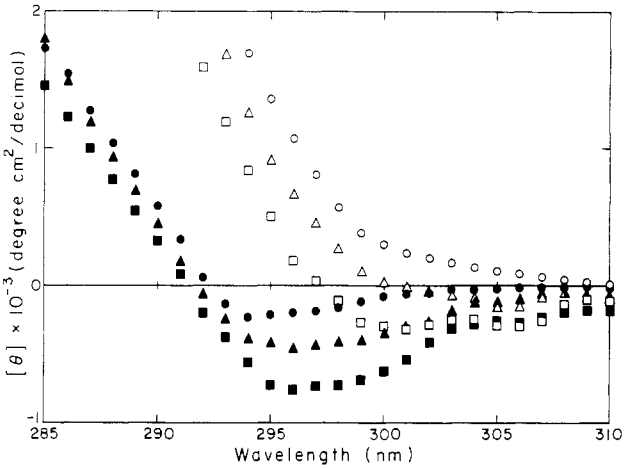

FIGURE 5: Circular dichroism spectra of nucleosome cores and DNA. Unmodified nucleosome cores (●) and extracted DNA (○); nucleosome cores (▲) and extracted DNA (△) having 1.36 fluorenyl residues/100 bases; nucleosome cores (■) and extracted DNA (□) having 2.87 fluorenyl residues/100 bases.

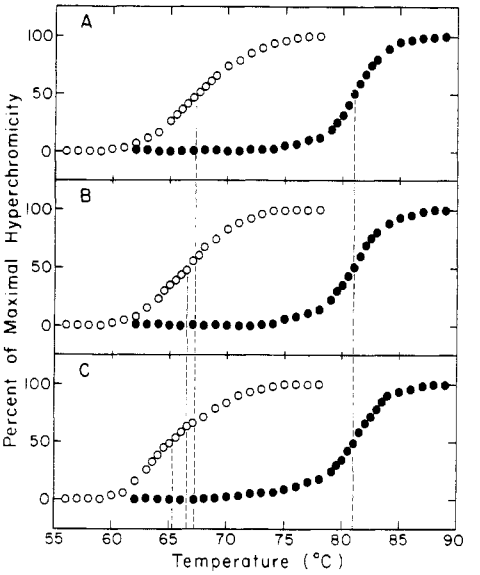

FIGURE 6: Thermal denaturation profiles at 260 nm of nucleosome cores (●) and DNA extracted from them (○) modified with 0 (A), 1.36 (B), and 2.87 (C) fluorenyl residues/100 bases. The T_m of each curve is indicated by a dashed line.

conformational changes induced at the site of modification. In addition, since AAF–protein adducts are produced in modified chromatin, these could contribute to the observed spectrum. However, since the CD spectra of $NaDodSO_4$-dissociated *N*-AcO-AAF-modified chromatin and protein-free DNA extracted from it are the same between 250 and 320 nm (Figure 4), the presence of fluorenyl residues bound to protein does not contribute to the signal at 300 nm.

By subtracting the CD signal of the unmodified control DNA and chromatin from those of the corresponding modified samples, the contributions of the primary, secondary, and tertiary structures of the DNA are eliminated. Further, since the protein-free DNA was obtained from the modified chromatin, the number and type of AAF–guanine adducts in both are identical. Thus any differences in the CD intensities of modified DNA and chromatin, after subtraction of the corresponding controls, reflect only differences in the local secondary structure at the modification site. After this correction, the absolute value of the AAF signal is lower in chromatin (-185 ± 6 deg cm^2/nmol of DNA-bound fluorenyl residues) than in protein-free DNA or in $NaDodSO_4$-dissociated chromatin (-288 ± 7 deg cm^2/nmol of DNA-bound fluorenyl residues). Hence, the DNA-bound fluorenyl derivatives produce a CD signal 45–65% more intense in modified protein-free DNA than in modified chromatin, indicating that the fluorene ring of the C-8 derivative interacts less strongly with the DNA helix in chromatin than in free DNA.

CD spectra of *N*-AcO-AAF-modified nucleosome cores and extracted DNA (Figure 5) are qualitatively similar to those seen with chromatin. The intensity of the 300-nm band, again relative to the DNA-bound fluorenyl residues, is -172 ± 35 deg cm^2/nmol of AAF in modified nucleosome cores and -287 ± 18 deg cm^2/nmol of AAF in protein-free DNA. These values, as well as those for the chromatin, are constant for levels of modification varying between 1 and 3 DNA-bound fluorenyl residues/100 bases.

Comparison of thermal denaturation profiles of modified nucleosome cores with those of protein-free DNA extracted from them provides further evidence for the reduced level of base displacement in modified chromatin. In protein-free *N*-AcO-AAF-modified DNA, helix destabilization produced by base displacement results in a decrease of the T_m when compared to that of unmodified DNA. Although the reso-

lution of our data does not permit a fine-structure analysis of the nucleosome core denaturation, it is evident that modification produces no significant decrease in the T_m (Figure 6), while DNA isolated from the modified core particles exhibits a decrease in T_m dependent upon the extent of modification.

Discussion

There is considerable physical and chemical evidence that the formation of the C-8 derivative in protein-free DNA leads to base displacement and local denaturation of the double helix. There is a linear decrease of the T_m with an increase in the extent of modification (Troll et al., 1969; Fuchs & Daune, 1971; Kapuler & Michelson, 1971) as well as an increase in the initial rate of formaldehyde unwinding (Fuchs & Daune, 1974). Also, analyses of CD spectra (Grunberger et al., 1970) and NMR spectra (Nelson et al., 1971) have indicated that the modified guanine rotates around the glycosidic bond from an anti to a syn conformation, and electric dichroism measurements (Fuchs et al., 1976) have shown that the fluorene ring is approximately perpendicular to the helix axis in modified DNA. Further, the local regions of denaturation produced can be cleaved by ss-specific nucleases (Fuchs, 1975; Yamasaki et al., 1977a).

Our findings that insertion of fluorenyl residues between the bases is prevented do not support the base displacement model for *N*-AcO-AAF-modified chromatin and nucleosome cores. This follows from the absence of ss-specific nuclease susceptibility, the less intense AAF signal in the CD spectra, and the lack of thermal helix destabilization in modified chromatin and nucleosome cores compared to their unmodified counterparts. While these results could be trivially explained if C-8 derivatives were not produced upon modification of chromatin with *N*-AcO-AAF, the facts that in each experiment the DNA extracted from the modified chromatin or nucleosome cores served as the basis for comparison and that C-8 derivatives were identified by TLC negate this objection.

These results mimic those observed when 7-iodo-*N*-AcO-AAF binds to protein-free DNA. Although this analogue binds to carbon atom 8 of guanine, fluorenyl insertion apparently is prevented by the bulky iodine atom on the fluorene ring. The 7-iodo-AAF derivative is not recognized by ss-specific endonuclease (Fuchs, 1975) and exhibits a lower induced optical activity than AAF at 300 nm (Lefèvre et al., 1978).

While Metzger et al. (1976) found that modified protein-free DNA was hydrolyzed by S_1 nuclease and that modified chromatin was not, in agreement with our observations, DNA extracted from their modified chromatin was not hydrolyzed. The latter finding is not in accord with ours but may have resulted from the different digestion conditions required for *N. crassa* endonuclease (pH 8.0, 1 mM Mg^{2+}) and S_1 nuclease (pH 4.6, 1 mM Zn^{2+}), as well as differences in the levels of DNA modification.

Because the C-8 and not the N^2 derivative is removed by repair systems in vivo (Kriek, 1972), it is generally accepted that the former in chromatin creates a locally denatured region recognizable by repair enzymes (Amacher et al., 1977; Ahmed & Setlow, 1977; Yamasaki et al., 1977a).

Our data do not support this contention but can be reconciled with the repair observed in vivo by the proposal that repair enzyme systems recognize protruding fluorenyl residues or local conformational changes. The recent isolation of a protein that lacks nuclease or polymerase activity that binds to AAF-modified DNA but not to ss DNA or UV-irradiated DNA (Moranelli & Lieberman, 1980) lends credence to this proposal and suggests that recognition and excision of AAF adducts may be separate events, possibly mediated by separate proteins.

AAF adducts are repaired much more efficiently from linker regions than from core regions of chromatin (Tlsty & Lieberman, 1978; Kaneko & Cerutti, 1980). These results might be explained by assuming that base displacement is prevented in the core but not in the linker, providing for differential recognition of adducts in these regions (Kaneko & Cerutti, 1980). An alternate explanation is more in accord with our data. Both modified chromatin and core particles show almost identical molar fluorenyl ellipticities, and neither is susceptible to ss-specific endonuclease, reflecting similar conformations for the AAF adducts in the linker and core. It is therefore more likely that the recognition of AAF adducts is as efficient in core as in linker DNA but that the excision enzyme has restricted access to the former, analogous to that observed with micrococcal nuclease.

Base displacement involves rotation of the modified guanine residue around its glycosidic bond, from an anti to a syn conformation. A recently described double-stranded conformation of DNA, designated Z-DNA, consists of alternating purine–pyrimidine nucleotides in which the purines adopt the syn conformation (Wang et al., 1979). When poly(dG-dC)·poly(dG-dC) is modified with *N*-AcO-AAF, the modified residues tend to undergo a transition to Z-DNA rather than base displacement (Sage & Leng, 1980; Santella et al., 1981). The modified guanines in this polymer are not excised by ss-specific endonucleases (Santella et al., 1981; Sage & Leng, 1981) and exhibit a reduced fluorenyl CD signal (Sage & Leng, 1981). While there is no evidence of Z-DNA existing in vivo,[2] it is conceivable that nucleotide sequences containing alternating purines and pyrimidines might tend to adopt a Z-DNA-like structure upon modification with *N*-AcO-AAF. This altered conformation could be highly localized, involving only a few bases, and be stabilized by DNA–histone interactions.

The binding of chemical carcinogens to cellular DNA represents a critical step in the initiation of carcinogenesis. A detailed knowledge of the conformation of the bound products will contribute to our understanding of this pathological process. Our data reveal that it cannot be assumed that the architecture of the DNA–AAF adducts in the cell is the same as that in protein-free DNA.

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[2] Added in proof: Immunological evidence for the existence of Z-DNA in *Drosophila* polytene chromosomes was published subsequent to the submission of this paper (Nordheim et al., 1981).

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Purification and Characterization of Nucleolar Ribonucleic Acid Methylase from Ehrlich Ascites Tumor Cells of Mice†

Masanobu Obara, Hideyasu Hirano,‡ and Ken Higashi*

ABSTRACT: RNA methylases in the extracts of mouse tumor cells were separated on a (diethylaminoethyl)cellulose column in two distinct activities. It was further revealed that the methylase from the isolated nucleoli corresponded to the peak eluted with lower concentration of salt and the extranucleolar nuclear methylase to that with higher salt concentration. Isolation of nucleoli from the purified nuclei was found to be profitable to a 10 times increase of specific activity of nucleolar methylase. The nucleolar methylase has been highly purified and characterized the properties of the enzyme. The purified nucleolar methylase was sedimented as a single component with a molecular weight of 130 000 on a sucrose density gradient. This enzyme is optimally active at pH 7.5 and sensitive to *N*-ethylmaleimide. The presence of a thiol-protecting reagent such as β-mercaptoethanol is necessary for its full activity. The enzyme required no divalent cations and its activity was suppressed in the presence of either 5 mM $MnCl_2$ or 10 mM $MgCl_2$. An apparent K_M value for *S*-adenosyl-L-methionine and a K_I value for *S*-adenosyl-L-homocysteine were 4×10^{-7} M and 1.0×10^{-6} M, respectively. Methyl-acceptance activities of homologous RNAs such as total cellular RNA, nucleolar RNA, and 45S ribosomal precursor RNA of Ehrlich tumor cells by nucleolar methylase from the same tumor cells were negligible, but heterologous RNA, prepared from *Escherichia coli*, served as a good substrate for the nucleolar methylase. On the other hand, hypomethylated RNA was prepared after the treatment of tumor cells with cycloleucine, an inhibitor of *S*-adenosylmethionine formation. The methyl-acceptance activities of both hypomethylated 18S and 28S rRNA were significantly greater than that of hypomethylated tRNA.

In eukaryotic organisms, rRNA precursor is synthesized and methylated in the nucleolus (Greenberg & Penman, 1966). This posttranscriptional modification is confined to rRNA sequences that are conserved during the processing of the precursor RNA (Weinberg et al., 1967). Moreover, all methylation sites within the conserved regions were detected in the specific primary sequence of rRNA (Khan & Maden, 1976). Therefore, elaborate recognition mechanisms may be involved during the modification of rRNA by methylases. Although the in vivo products of rRNA methylation in eukaryotic cells have been extensively studied (Maden & Salim, 1974; Khan et al., 1978), the purification of RNA methylase participating in the modification of ribosomal RNA has not

† From the Department of Biochemistry, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807, Japan. *Received June 3, 1981.*
‡ Present address: Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

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