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Methylmercury(II)-Induced Histone Perturbations in Nuclei Isolated from Calf Thymus

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SUMMARY

Intact nuclei isolated from calf thymus were first incubated for 2 hr at 37° with CH_3HgOH, ranging in concentration from 0.087 μM (pM 7.06) to 8.7 mM (pM 2.06) (pM ≡ − log $[CH_3HgOH]_{added}$), followed by a 1-hr treatment at the same temperature with 0.1% formaldehyde as contact-site cross-linking agent. Histones, isolated subsequently from the nuclei, were subjected to electrophoresis in acid-urea gels and their migration pattern was evaluated with the help of an integrating gel scanner. While the histones isolated from CH_2O-treated nuclei, whether in absence of CH_3HgOH or in its presence at concentrations up to about 32 μM (pM 4.5), contained three cross-linked species (X-1, X-2, X-3) besides their monomeric forms H1, H3, H2B, H2A, and H4, those isolated from nuclei not treated with CH_2O, either in the presence or absence of CH_3HgOH, or isolated from CH_2O-treated nuclei that had been kept at CH_3HgOH concentrations above 1 mM (pM < 3), did not contain the three cross-linked species but displayed the monomer composition of the histones obtained from completely untreated nuclei. In the concentration range of about pM 4.5–3, there exists a "sigmoidal" decline in the aggregate amounts of the cross-linked species X-1, X-2, and X-3 which is accompanied by a concomitant increase in the amounts of monomeric histones. Using information available from the literature, the three cross-linked species X-1, X-2, and X-3 have been tentatively identified as representing the histone dimers H2A-H2B, α-H2B-H4, and β-H2B-H4, respectively. The disappearance of the cross-linked forms does not take place gradually but commences within a narrow organomercurial concentration range. We believe this to be an indication of conformational changes taking place in the histone "core" of chromatin at pM < 4.5 under the experimental conditions given, but we cannot dismiss the possibility that methylmercury simply blocks suitable protein side chains (viz., ε-NH_2 of lysine) that are needed for CH_2O cross-linking, i.e., those which represent histone-histone contact sites in the "core." Whether acting via conformational alterations or chemical blockage, either event will affect the biological activity of the constituent chromosomal DNA, and the demonstration that CH_3HgOH interferes with contact-site cross-linking could be the first clue to the mechanism of its genotoxicity at the molecular level.

INTRODUCTION

A number of workers have shown that the administration of inorganic (1–5) or organic mercury (1, 3) to rats leads to the accumulation of non-negligible quantities of Hg(II) in the nuclei of kidney and liver, the two organs usually selected for testing in animal intoxication studies. Recent work from several laboratories (3, 5–8) provides compelling evidence that chromatin is mostly responsible for the intranuclear mercury binding.

Chromatin, whose basic structure has become known in recent years (9–11), makes up most of the genome of eukaryotes. Its interaction with mercury, particularly with organic mercury (viz., methylmercury(II)), may very well constitute the primary event in a chain that results in the embryogenic damages observed in a variety of animal species subsequent to the administration of methylmercury (12–17).

Chromatin is now known to be constructed of subunits termed nucleosomes. A nucleosome consists of a well-defined length of duplex DNA, about 200 base pairs long, complexed with two copies each of the slightly lysine-rich histones H2A and H2B and the arginine-rich histones H3 and H4. The eight histones are bound tightly to each other, forming a globular core, with the duplex DNA wrapped probably in two "superhelical" turns around the protein core. A fifth class of histones, H1

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(together with H5 in, for instance, nucleated erythrocytes), is not part of the nucleoprotein core but is bound at a peripheral location on the nucleosome (9–11). What remains unresolved at present is the mechanism by which nucleosomes regulate the biological activity of their constituent DNA. Indications are that their conformational flexibility, and, thus, the precise arrangement of the eight histones in the core, is indispensable to their proper functioning.

We have shown recently that the addition of CH_3HgOH to buffered solutions of calf thymus chromatin dramatically affects its thermal stability, and from the changes observed in the integral and differential melting profiles it was concluded that methylmercury at low concentrations (1–10 μM), and prior to its denaturing the double helix of the chromosomal DNA, interferes with the binding of DNA by histones H3 and H4 which is followed, at methylmercury concentrations between 10 and 32 μM, also by its interfering with the complexing of the DNA by the less basic histones H2A and H2B (18). As a sequel to the above work, we report now on the effect of CH_3HgOH on the spatial arrangement of the several histone classes in intact calf thymus nuclei, using formaldehyde as a contact-site cross-linking reagent (19). Formaldehyde has been used by a number of investigators to probe the arrangement of histones in chromatin (cf. refs. 19 and 20).

MATERIALS AND METHODS

Materials. Methylmercuric hydroxide, CH_3HgOH (more than 97% pure), was purchased from Alfa Products, Ventron Corporation (San Leandro, Calif.). Its handling has been described (21, 22). All other chemicals were of reagent grade. Doubly distilled water was used throughout the investigation.

Isolation of nuclei. Calf thymus was obtained from the local slaughterhouse. The organ, within 3 min of excision, was immersed in an ice-cold solution of 300 mM sucrose, 50 mM Tris-HCl buffer, 25 mM KCl, 5 mM $MgCl_2$, and 1 mM PMSF[2] (prepared as a 100 mM stock solution in isopropanol), pH 6.4 (4°), and transported embedded in ice to the laboratory. It was then freed aseptically of all foreign tissue, cut into a number of 25-g portions, and each portion, after being freed of excess liquid, was quickly frozen by placing it between two discs of solid carbon dioxide. The freezing required 2–3 min per portion. The portions were kept at −80° until use.

Nuclei were isolated by using the method of Blobel and Potter (23) in the modification of Garrard and Hancock (24) (their method 3). The latter's modification consists primarily of replacing the Tris-HCl buffer with triethanolamine so that cross-linking agents can be employed which otherwise would react with the free amino group of Tris. Modifications on our part consisted of replacing KCl and $MgCl_2$ with K_2SO_4 and $MgSO_4$ so as to eliminate complexing of CH_3Hg^+ by Cl^- (21) as this can abstract the metal from the methylmercury-nucleoprotein complex. Thus, blending of a 25-g portion was performed in 300 mM sucrose, 50 mM triethanolamine, 12.5 mM K_2SO_4, 5 mM $MgSO_4$, and 1 mM PMSF, pH 6.4 (4°), whereby the pH was adjusted with the help of H_2SO_4. Prior thawing and mincing of the sample was performed at 0° in the same medium. Octanol (1 ml) was added to the blending medium (200-ml final volume), which very effectively suppressed foaming in the Waring Blendor. Isolation of nuclei took place at 4°.

Methylmercury treatment of nuclei. Purified nuclei were washed a minimum of six times by gentle dispersion and subsequent centrifugation for 10 min at 735 × g_{av} with the blending medium described above, but with pH adjusted now to 7.8 at 25°. This yields a pH of about 7.6 at 37°. The nuclei were then dispersed in 200 ml of the same medium at a concentration of 0.5 mg of DNA per milliliter, corresponding to 10 A_{260} units or about 7.4 × 10^7 nuclei per milliliter (25), and finally distributed in 10-ml aliquots over 20 glass-stoppered incubation vials. DNA concentrations were determined by pipetting small portions of suspended nuclei into 1 M NaOH and measuring the absorbance at 260 nm. An extinction coefficient of 27 (1/g·cm) was used (26).

Methylmercury stock solutions were prepared in such a way that the addition of 0.01 volume of stock to a given 10-ml portion of suspended nuclei yielded the desired final toxicant concentration. Final methylmercury concentrations ranged from 0.087 μM (pM 7.06) to 8.7 mM (pM 2.06), whereby pM ≡ − log $[CH_3HgOH]_{added}$. They increased in the sequence (pM): 7.06; 6.66; 6.36; 6.19; 6.06; 5.66; ... ; 4.06. Additional information is contained in Table 1.

Two experimental series were run: one consisted of 20 samples (including control); the other consisted of 8 samples (including control). Each series was run in triplicate. Methylmercury treatment of a given series lasted for 2 hr at 37° (thermostated water bath) whereby the samples were subjected to slight agitation so as to minimize settling of the nuclei. At the end of the 2-hr treatment period, 0.33 volume of formaldehyde, dissolved in the same suspension medium and with pH adjusted to 7.8, was added to each sample in rapid succession and incubation was continued for 1 additional hr. The final formaldehyde concentration amounted to 0.1% in each sample. Methylmercury treatment and cross-linking took place under a well-ventilated chemical hood, and disposable gloves were used in all operations. Controls consisted of (a) omitting methylmercury from the suspension of nuclei but performing CH_2O cross-linking in the indicated fashion; (b) omitting both CH_3HgOH and CH_2O (identical volumes of suspension medium were added instead); and (c) treating samples of nuclei with methylmercury at pM 3.06 and 2.06, respectively, but adding only suspension medium instead of CH_2O after 2 hr of methylmercury treatment.

After completion of methylmercury treatment and cross-linking, each vial was plunged into slushed ice and its content transferred quantitatively (with a rubber policeman) to a centrifuge tube, also kept in ice. Each vial was rinsed with 10 ml of ice-cold suspension medium, the rinses were added to the original solutions, and the nuclei were centrifuged for 10 min at 11,800 × g_{av}. After removal of the supernatant (with a Pasteur pipette), 10 ml of doubly distilled water, made 1 mM in PMSF, were added to each centrifuge tube and the pelleted nuclei were suspended. They were again subjected to centrifugation for 10 min at 11,800 × g_{av}, and the supernatant was

[2] The abbreviation used is: PMSF, phenylmethylsulfonyl fluoride.

TABLE 1

Effect of methylmercury(II) on the relative amounts of gel-electrophoresed monomeric and CH_2O cross-linked histones isolated from calf thymus nuclei

The relative amounts given as percentages were evaluated by integration under the curves. Since the percentages have been rounded off, they do not always add up to exactly 100%.

Sample no.	pM[a]	CH_2O	X-1	X-2	X-3	H1	H3	(H2B + H2A)	H4
		%	%	%	%	%	%	%	%
1	∞	0	—	—	—	14	17	48	19
2	∞	0.1	8	7	6	7	26	40	7
15	4.06	0.1	8	8	5	3	29	39	8
16	3.66	0.1	6	6	6	4	31	40	7
17	3.36	0.1	6	5	3	6	31	42	9
18	3.19	0.1	6	3	4	3	35	39	9
19	3.06	0.1	6	3	2	—[b]	36	38	11
20	3.06	0	—	—	—	12	14	56	18
21	2.99	0.1	2	2	2	3	33	48	10
22	2.69	0.1	—[b]	—[b]	—[b]	3	33	52	9
23	2.41	0.1	—	—	—	10	19	54	18
24	2.23	0.1	—	—	—	27	—[c]	60	13
25	2.06	0.1	—	—	—	19	—[c]	53	28
26	2.06	0	—	—	—	9	17	47	26

[a] pM ≡ − log $[CH_3HgOH]_{added}$.

[b] Present only in trace amounts (<1%).

[c] Co-migrating with (H2B + H2A) peak.

removed by aspiration. A minimum of three washing cycles with 1 mM PMSF were performed in this fashion. The nuclei were finally allowed to swell by leaving them overnight in 1 mM PMSF at 4°. It was our experience that the nuclei of Control b and Control c (*vide supra*) lysed "as expected," i.e., swelled readily to a gel that failed to sediment when subjected to centrifugation for 10 min at 11,800 × g_{av}. By contrast, all formaldehyde-treated nuclei, including Control a, released chromatin only very slowly and required prolonged extraction with 1 mM PMSF. Incidentally, the pH of 1 mM PMSF was that of doubly distilled water alone.

Histones were acid-extracted from the chromatin gel as described in the literature (27) and precipitated, after DNA removal, from solution by the addition of a 5-fold excess of acetone.

Gel electrophoresis. Electrophoresis was performed by using the LKB Model 2117 Multiphore horizontal-slab gel electrophoresis apparatus (LKB-Produkter AB, Bromma, Sweden) driven by the Pleuger CVC-A power supply (Pleuger, Antwerp-Brussels, Belgium). Rectangular gels of the size 250 × 115 × 2 mm were used. Each sample was applied to a rectangular 10 × 5 mm area (filter paper); sample sizes ranged from 5 to 50 μl depending on the histone concentration of each sample.

Gels were prepared according to the method of Panyim and Chalkley (28): the 0.9 M acetic acid-2.5 M urea-15% polyacrylamide gel system was used. Gels were pre-electrophoresed at 200 V until a constant power output of 10 W had been reached. This required about 2 hr. Electrophoresis was performed at constant power (about 11 W), starting with a 200-V setting. Electrophoresis was terminated when the blue component of methyl green reached the opposite side of the gel. A 5% solution of methyl green in 0.9 M acetic acid was used as tracking dye. About 8 hr were needed to complete a run.

Gels were stained overnight at room temperature in 0.1% Coomassie blue-11% trichloroacetic acid-4% sulfosalicylic acid-33% methanol. Destaining was done also at room temperature in 25% ethanol-8.4% acetic acid. Staining and destaining solutions were stirred magnetically.

Histone samples were prepared by dissolving the acetone-precipitated and vacuum-dried histones in 6 M urea-10% mercaptoethanol (29) and applying them in this form to the gel. Histone electrophoresis patterns were evaluated by using the Gelman DCD-16 Model 39431 digital computing densitometer (Gelman Instrument Company, Ann Arbor, Mich.). Stained gels were scanned at 600 nm.

RESULTS

In Figs. 1 and 2 are displayed the densitometer tracings of histones isolated from calf thymus nuclei that had been in contact with methylmercury in the presence or absence of the contact-site cross-linking reagent formaldehyde. Sample 1 (Fig. 1) shows the well-known five classes of histones—H1, H3, H2B, H2A, and H4—as they can be obtained via acid extraction from totally untreated nuclei (Control b; see Materials and Methods). Although histones H2B and H2A, after destaining, always were visible to the bare eye as two individual bands, the densitometer failed to separate them into individual peaks: at best, a shoulder became discernible, as seen, for instance, in Sample 11, or a minute indentation appeared at the apex of the peak, viz., Sample 15.

Formaldehyde treatment of the nuclei in the absence of methylmercury produces the histone migration pattern shown in Sample 2 (Fig. 1, Control a). Besides the monomeric forms of histones H1, H3, (H2B + H2A), and H4, one sees three cross-linked forms. They have been denoted X-1, X-2, and X-3; their possible composition is discussed below. Formaldehyde treatment appears to alter slightly the electrophoretic properties of the histone monomers: the H3 peak becomes clearly separated from the (H2B + H2A) peak, and both the H1 and (H2B + H2A) peaks migrate slightly faster than the correspond-

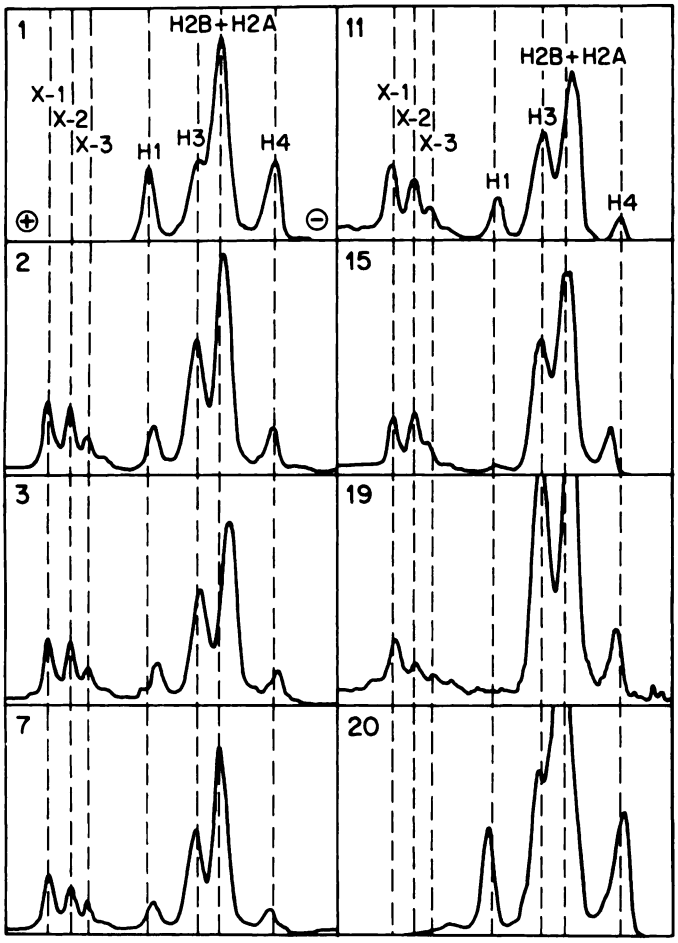

FIG. 1. *Densitometer tracings of the acetic acid-urea gel electrophoresis pattern of histones isolated from calf thymus nuclei that had first been incubated for 2 hr at 37° with varied concentrations of CH_3HgOH followed by a 1-hr treatment with 0.1% CH_2O at the same temperature*

Electrophoresis is always from left to right or from (+) to (−). Sample 1, pM ∞, 0% CH_2O; Sample 2, pM ∞, 0.1% CH_2O; Sample 3, pM 7.06, 0.1% CH_2O; Sample 7, pM 6.06, 0.1% CH_2O; Sample 11, pM 5.06, 0.1% CH_2O; Sample 15, pM 4.06, 0.1% CH_2O; Sample 19, pM 3.06, 0.1% CH_2O; Sample 20, pM 3.06, 0% CH_2O (pM ≡ − log $[CH_3HgOH]_{added}$). The "height" (ordinate) of each frame corresponds to 1.2 A_{600} units with the exception of those of Samples 15, 19, and 20, where the total ordinate corresponds to 0.6 A_{600} units. For further details see text.

ing peaks of Control b. On the other hand, the "improved" separation of H3 from (H2B + H2A) can also be caused by the disappearance of H2B from the (H2B + H2A) peak as a result of its being consumed in cross-linking. As is pointed out further below, formaldehyde cross-linking has a pronounced effect on the relative proportions of the histone monomers (cf. Table 1).

Samples 3, 7, 11, 15, and 19 depict, respectively, the electrophoresis patterns of histones isolated from nuclei kept at pM 7.06, 6.06, 5.06, 4.06, and 3.06 (pM ≡ − log $[CH_3HgOH]_{added}$) prior to their incubation with 0.1% formaldehyde. As can be seen in Fig. 1, increasing concentrations of methylmercury have little effect on the electrophoretic mobilities of the five histones, including the cross-linked products, and the migration pattern displayed by Control a is preserved in the treated samples. This pattern continues to exist with respect to the histone monomers at still higher organomercurial levels, for instance at pM 2.99 and 2.69 (data not shown), or at pM 2.41 (Sample 23, Fig. 2), but major alterations take place in the case of the cross-linked products. Samples 21–25 show that there is a sudden decrease and ultimate disappearance of the cross-linked products from the gels (Table 1; Fig. 2). Only at pM 2.06, the highest organomercurial concentration employed, were also noted changes in the electrophoresis pattern of some of the histone monomers. Thus, H4 failed to electrophorese as a narrow band and H3 moved up so closely to the (H2B + H2A) peak that its presence is indicated only by the small indentation on the left-hand side near the apex of the major band (Sample 25, Fig. 2). Since nuclei treated in an identical manner with methylmercury at pM 2.06, albeit in the absence of formaldehyde (Control c; Sample 26, Fig. 2), produced histones that migrated like those of Control b (cf. Sample 1, Figs. 1 and 2), we conclude that methylmercury treatment of the nuclei per se does not introduce the alterations. Incidentally, this is not unex-

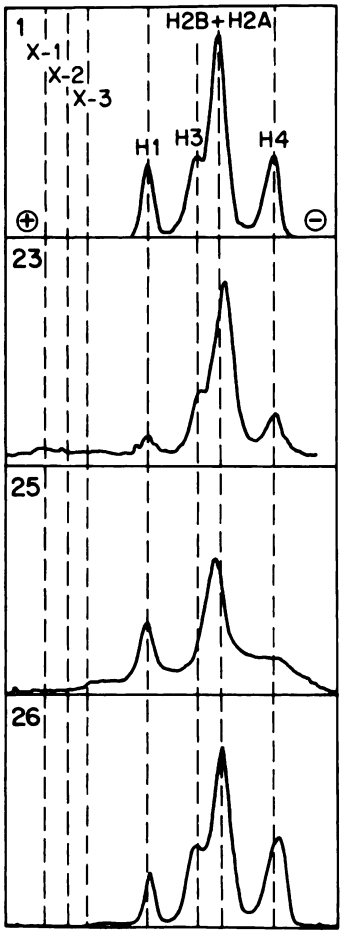

FIG. 2. *Densitometer tracings of the acetic acid-urea gel electrophoresis pattern of histones isolated from calf thymus nuclei that had first been incubated for 2 hr at 37° with varied concentrations of CH_3HgOH followed by a 1-hr treatment with 0.1% CH_2O at the same temperature*

The experimental details are those described in the legend to Fig. 1. Sample 1, pM ∞, 0% CH_2O; Sample 23, pM 2.41, 0.1% CH_2O; Sample 25, pM 2.06, 0.1% CH_2O; Sample 26, pM 2.06, 0% CH_2O. The "height" of each frame corresponds to 1.2 A_{600} units in Samples 1 and 26, 0.6 A_{600} in Sample 23, and 0.3 A_{600} in Sample 25. For further details see text.

pected since both the mode of histone isolation (extracting them with strong acid) and preparation for gel electrophoresis (dissolving them in 6 M urea-10% mercaptoethanol) should have removed all histone-bound organomercurial.

We have attempted to present also some quantitative information on the methylmercury-induced changes of the histone migration pattern elicited by formaldehyde, and we have done this by making use of the curve-integrating capabilities of the gel scanner. Thus, in Table 1 are given the relative amounts, expressed in percentage of the total area under the curves, of gel-electrophoresed histones isolated from treated and untreated nuclei. It is readily seen that formaldehyde treatment alone causes major alterations in histone distribution (cf. Samples 1 and 2): three new species are generated, X-1, X-2, and X-3, that migrate considerably more slowly than H1 and that make up about 20% of the total histones present. Their formation is at the expense of the histone monomers, particularly H4 and H2B (see also Fig. 1).

Methylmercury, in the concentration range pM 7.0–4.5, has little effect on the histone distribution caused by 0.1% formaldehyde alone. The distribution (Samples 3–14) follows quite closely the one given by Sample 2; we have therefore omitted the data from Table 1. The invariance can also be seen from Fig. 3, where we have plotted the aggregate amounts of species X-1, X-2, and X-3 as a function of methylmercury concentration in presence (○) and absence (●) of 0.1% formaldehyde. Also plotted are the amounts of (H2B + H2A) as a function of pM—in the presence (△) and absence (▲) of formaldehyde. The other monomers have been omitted from Fig. 3 for reasons of clarity. However, at methylmercury concentrations above 100 μM (pM < 4) there takes place the sudden decline of the amounts of cross-linked histones referred to above while the quantities of (H2B + H2A) increase (see also Table 1). In fact, at pM < 3, all cross-linked species have vanished and the distribution of the monomers assumes the proportions displayed by non-cross-linked histones.

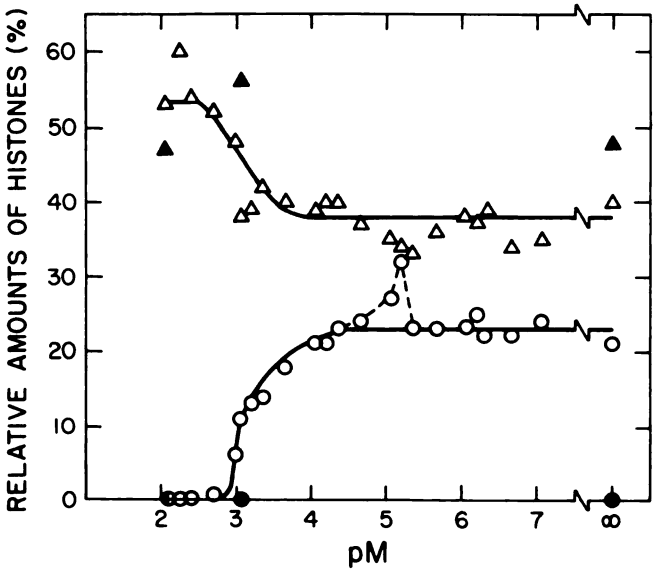

FIG. 3. *Relative amounts of histones (percentage) versus methylmercury concentration pM*

pM ≡ − log $[CH_3HgOH]_{added}$. Histones X-1, X-2, and X-3, ●, ○; histones (H2B + H2A), ▲, △. *Closed symbols* represent results obtained in absence of CH_2O and *open symbols* represent results obtained in presence of 0.1% CH_2O. ◐, overlap of *open* and *closed symbols*. For further detail see text.

DISCUSSION

The electrophoresis pattern of histones in acid-urea gels has been well documented (viz. refs. 27–30), and thus there is no need to prove this pattern here again. As far as the composition of the cross-linked species is concerned, Martinson and collaborators (30, 31) have shown that CH_2O cross-linking produces two H2B-H4 dimers (termed α and β) as the most prominent components and that a CH_2O-generated H2A-H2B dimer is possible also. Since they found that the H2A-H2B dimer migrates more slowly than the two H2B-H4 dimers in acid-urea gels, we tentatively identify X-1 with the H2A-H2B dimer and X-2 and X-3 with the α and β H2B-H4 dimers, respectively. Van Lente *et al.* (32) also found H2B-H4 and H2A-H2B dimers after formaldehyde treatment of intact nuclei as the most prominent gel components, but they worked with sodium dodecyl sulfate gels. Thus, our interpretation that X-1, X-2, and X-3 grow at the expense of H2B and H4, in particular, is fully corroborated by the findings of others, and since we found that the amounts of (H2B + H2A) varied in mirror-like fashion with those of the three cross-linked species (cf. Fig. 3), we believe this also to be a firm implication of H2A participating in cross-linking. The amounts of H4, incidentally, vary with pM in the manner displayed by (H2B + H2A): isolated from Control b nuclei, they comprise about 20% of the total area under the curve; isolated from 0.1% CH_2O-treated nuclei, and in the methylmercury concentration range pM ∞–4.2, their contribution drops to 6.3% ± 2.2% (SD) whereas at pM < 4, their percentage contribution increases again and reaches the original level.

The data presented in Table 1 and Fig. 3 show unambiguously that CH_3HgOH, at suitable concentrations, suppresses the formation of cross-linked histones while, at the same time, monomers are obtained in proportions that resemble closely those of histones extracted from totally untreated nuclei. The inhibition of cross-link formation is not a gradual process, as, for instance, the renewed increase in the amounts of (H2B + H2A) is not, but takes place within a narrow concentration range of added methylmercury. The following explanations can be given at this time. (a) The organomercurial causes large conformational changes in the nucleosomes of chromatin, so large indeed that CH_2O cannot any longer join together suitable side-chain residues via covalent one-carbon bridges (19) since they have been forced apart from each other beyond the distance of their van der Waals closest approach. (b) Methylmercury prevents histone cross-linking by binding directly to residues required for cross-linking without actually affecting nucleosome conformation. The exact sites of CH_2O-induced cross-links have not been identified as yet, neither in the H2A-H2B dimer nor in the two H2B-H4 dimers, but it seems to be certain that they involve the COOH-terminal regions of the histones and there, presumably, at least one lysine residue (19, 31). Thus, blockage of the ε-NH_2

groups of strategically located lysine residues by $CH_3Hg(II)$ will prevent H2B from interacting either with H4 (via its COOH-terminal region) or with H2A (via its middle region), even in absence of conformational changes (11). (c) Of course, there exists always the possibility that both modes of interaction contribute simultaneously to cross-link inhibition. Although previous work (18), dealing with the heat-induced denaturation of chromatin in presence of $CH_3Hg(II)$, tends to support the notion of methylmercury-induced conformational alterations in chromatin, we cannot, on the basis of the results obtained here, dismiss the possibility that merely chemical blockage occurs.

Irrespective of the exact nature of the interaction of methylmercury with the core histones, the demonstration that the organomercurial interferes at elevated levels with contact-site cross-linking and, thus, with the histone contacts in the core, could be the first clue to the mechanism of its action at the chromosomal level. The precise arrangement of the eight histones in the core is believed to be indispensable to the proper functioning of nucleosomes—for instance, to serve as "spools" in the supercoiling (i.e., packaging) of double-stranded DNA in chromosomes—and any interference with this packaging should be of consequence to the biological activity of the DNA. Future research will be concerned with using purified nucleosomes and DNA-free core histone octamers in order to gain further insights into the mode of interaction. One final note: we have observed that the amounts of H1 are affected by CH_2O also (cf. Fig. 1 and Table 1). We have no explanation for this at this time, as H1 is normally not implicated in cross-link formation with formaldehyde.

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REFERENCES

1. Ellis, R. W., and S. C. Fang. Elimination, tissue accumulation, and cellular incorporation of mercury in rats receiving an oral dose of ^{203}Hg-labeled phenylmercuric acetate and mercuric acetate. *Toxicol. Appl. Pharmacol.* **11**:104–113 (1967).
2. Norseth, T. The intracellular distribution of mercury in rat liver after a single injection of mercuric chloride. *Biochem. Pharmacol.* **17**:581–593 (1968).
3. Chanda, S. K., and M. G. Cherian. Isolation and partial characterization of a mercury-binding nonhistone component from rat kidney nuclei. *Biochem. Biophys. Res. Commun.* **50**:1013–1019 (1973).
4. Komsta-Szumska, E., J. Chmielnicka, and J. K. Piotrowski. Binding of inorganic mercury by subcellular fractions and proteins of rat kidneys. *Arch. Toxicol.* **37**:57–66 (1976).
5. Rozalski, M., and R. Wierzbicki. Binding of mercury by chromatin of rats exposed to mercuric chloride. *Environ. Res.* **20**:465–569 (1979).
6. Bryan, S. E., A. L. Guy, and K. J. Hardy. Metal constituents of chromatin: interaction of mercury *in vivo*. *Biochemistry* **13**:313–319 (1974).
7. Bryan, S. E., C. Lambert, K. J. Hardy, and S. Simons. Intranuclear localization of mercury *in vivo*. *Science* (*Wash. D. C.*) **186**:832–833 (1974).
8. Bryan, S. E., S. J. Simons, D. L. Vizard, and K. J. Hardy. Interactions of mercury and copper with constitutive heterochromatin and euchromatin *in vivo* and *in vitro*. *Biochemistry* **15**:1667–1676 (1976).
9. Kornberg, R. D. Structure of chromatin. *Annu. Rev. Biochem.* **46**:931–954 (1977).
10. Felsenfeld, G. Chromatin. *Nature* (*Lond.*) **271**:115–122 (1978).
11. McGhee, J. D., and G. Felsenfeld. Nucleosome structure. *Annu. Rev. Biochem.* **49**:1115–1156 (1980).
12. Spyker, J. M., and M. Smithberg. Effects of methylmercury on prenatal development in mice. *Teratology* **5**:181–190 (1972).
13. Olson, F. C., and E. J. Massaro. Effects of methylmercury on murine fetal amino acid uptake, protein synthesis and palate closure. *Teratology* **16**:187–194 (1977).
14. Dial, N. A. Methylmercury: some effects on embryogenesis in the Japanese medaka, *Oryzias latipes*. *Teratology* **17**:83–92 (1978).
15. Hoffman, D. J., and J. M. Moore. Teratogenic effects of external egg applications of methyl mercury in the mallard, *Anas platyrhynchos*. *Teratology* **20**:453–462 (1979).
16. Kelman, B. J., S. E. Steinmetz, B. K. Walter, and L. B. Sasser. Absorption of methylmercury by the fetal guinea pig during mid to late gestation. *Teratology* **21**:161–165 (1980).
17. Harper, K., R. Burns, and R. P. Erickson. Genetic aspects of the effects of methylmercury in mice: the incidence of cleft palate and concentrations of adenosine-3′:5′-cyclic monophosphate in tongue and palatal shelf. *Teratology* **23**:397–401 (1981).
18. Otsuki, L. G., and D. W. Gruenwedel. Methylmercury-chromosome interactions. I. Thermal denaturation of calf thymus chromatin in presence of CH_3HgOH. *Z. Naturforsch.* **35c**:605–610 (1980).
19. Kunkel, G. R., M. Mehrabian, and H. G. Martinson. Contact-site crosslinking agents. *Mol. Cell. Biochem.* **34**:3–13 (1981).
20. Thomas, J. O., and R. D. Kornberg. The study of histone-histone associations by chemical cross-linking. *Methods Cell Biol.* **18**:429–440 (1978).
21. Gruenwedel, D. W., and N. Davidson. Complexing and denaturation of DNA by methylmercuric hydroxide. I. Spectrophotometric studies. *J. Mol. Biol.* **21**:129–144 (1966).
22. Gruenwedel, D. W., and B. L. Fordan. Effects of methylmercury(II) on the viability of HeLa S3 cells. *Toxicol. Appl. Pharmacol.* **46**:249–256 (1978).
23. Blobel, G., and V. R. Potter. Nuclei from rat liver: isolation method that combines purity with high yield. *Science* (*Wash. D. C.*) **154**:1662–1665 (1966).
24. Garrard, W. T., and R. Hancock. Preparation of chromatin from animal tissues and cultured cells. *Methods Cell Biol.* **17**:27–50 (1978).
25. Shapiro, H. S. DNA content of chordate cell nuclei, in *Cell Biology* (P. L. Altman and D. Dittmer Katz, eds.), Vol. 1. Federation of American Societies for Experimental Biology, Bethesda, Md., 367–378 (1976).
26. Todd, R. D., and W. T. Garrard. Two-dimensional electrophoretic analysis of polynucleosomes. *J. Biol. Chem.* **252**:4729–4738 (1977).
27. Hoffman, P., and R. Chalkley. Procedures for minimizing protease activity during isolation of nuclei, chromatin, and the histones. *Methods Cell Biol.* **17**:1–12 (1978).
28. Panyim, S., and R. Chalkley. High resolution acrylamide gel electrophoresis of histones. *Arch. Biochem. Biophys.* **130**:337–346 (1969).
29. Martinson, H. G., R. J. True, and J. B. E. Burch. Specific histone-histone contacts are ruptured when nucleosomes unfold at low ionic strength. *Biochemistry* **18**:1082–1089 (1979).
30. Hardison, R., and R. Chalkley. Polyacrylamide gel electrophoretic fractionation of histones. *Methods Cell Biol.* **17**:235–251 (1978).
31. Martinson, H. G., R. True, C. K. Lau, and M. Mehrabian. Preliminary location of multiple contact sites between histones 2A, 2B, and 4. *Biochemistry* **18**:1075–1082 (1979).
32. Van Lente, F., J. F. Jackson, and H. Weintraub. Identification of specific crosslinked histones after treatment of chromatin with formaldehyde. *Cell* **5**:45–50 (1975).

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