ISOLATION AND PARTIAL CHARACTERIZATION OF A MERCURY-BINDING NONHISTONE PROTEIN COMPONENT FROM RAT KIDNEY NUCLEI

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Summary A nonhistone protein component (NHP_{Ins}) firmly bound to DNA of rat kidney nuclei has been isolated and partially characterized. In vivo studies show that this protein specifically incorporates 35 to 45 times more ^{203}Hg than any other nuclear protein fractions. No difference in the ratio of NHP_{Ins} to DNA between normal and mercury-poisoned rat kidney nuclei was observed. NHP_{Ins} protein gives a single major band by sodium dodecyl sulfate gel electrophoresis. Electrophoretic pattern as well as amino acid composition of this protein isolated from both normal and mercury-poisoned rats are also found to be similar. Cysteine content is 1.3 to 1.4 mole per cent.

Introduction Recent work from several laboratories has shown that nonhistone protein fractions present in the nuclei play a major role in controlling genetic transcription (1-3) as well as in maintaining its structure (3-9). Some progress has been made in fractionating and isolating individual nonhistone protein fractions to study their specific roles in gene regulation (2,3,10,11). Heavy metals like mercury compounds have been found to produce chromosomal abnormalities and induce genetic effects (12). Mercury compounds are also shown to alter chromosomal structure and affect mitotic apparatus (13-15). Recently Choie and Richter (16) and others (17) have reported the formation of lead-bound intranuclear inclusions by lead poisoning and proposed that they might result from degradation and restructuring of nonhistone nucleoproteins. Although some work has been done on the effects of heavy metals on chromatin apparatus or on mitosis, very little is known about the isolation and characterization of chromosomal fractions associated with heavy metals. The present work was undertaken to isolate and study the mercury-binding nuclear proteins.

Materials and Methods Female Sprague-Dawley rats (170-200 g) were injected intravenously with ^{203}Hg-labeled methyl mercury or mercuric chloride (1 mg/kg body weight). The radioactive ^{203}Hg-methyl mercury (specific activity 3 mc/mg Hg) and mercuric chloride (2 mc/mg Hg) were obtained from New England Nulcear Corporation. Five to six hours after

injection rats were killed, the kidneys were removed, and nuclei were isolated at pH 5.8 (18). The nuclear protein fractions and DNA were isolated by the method of Chanda (19). After washing the nuclei in 0.14 M NaCl, the residue was solubilized in 0.6 M NaCl--6 M urea--0.05 M $NaHSO_3$ solution (pH 7.6). The solution was then dialyzed overnight at 0-4 C to bring down the concentration of NaCl to 0.14 M and then centrifuged. The supernatant is called soluble nonhistone protein fraction (NHP_{Sol}). After histones were removed from the residue with 0.2 N HCl, the material was suspended in an appropriate buffer and treated with a mixture of DNAase and RNAase or else with 5% trichloroacetic acid at 95 C in order to remove nucleic acids. After removing lipids from the residue, it was dissolved in 0.2 M phosphate buffer (pH 7.4) containing 6 M urea, 0.05 M $NaHSO_3$ and 10% sodium dodecyl sulfate. This fraction is called insoluble nonhistone protein (NHP_{Ins}). DNA was estimated by the Dische-Schneider technique as described previously (18). Protein was estimated by the method of Lowry et al. (20) and sodium dodecyl sulfate gel electrophoresis was performed for 12 hours according to the method of Teng et al. (2) using a current of 3 mA/tube. Densitometric bracing of stained gels was done by the use of a Gilford instrument and resolved by DuPont curve analyzer. The radioactivity in the different nuclear fractions, in DNA hydrolysates, and in gel slices (2 mm each) was counted in a Packard scintillation spectrophotometer equipped with an NaI detector with a counting efficiency of 40%. The amino acid analysis of insoluble nonhistone protein (NHP_{Ins}) component was done by the technique of Moore and Stein (21).

Results Table 1 gives the different nonhistone proteins to DNA ratios isolated from both normal and mercury-injected rat kidney nuclei. The ratios of individual protein fractions to DNA remained unaltered after mercury administration. Approximately 18% of the total NHP was present as insoluble nonhistone protein component (NHP_{Ins}). The ratios of NHP_{Ins} to DNA were 0.43 and 0.44 for normal and mercury-poisoned nuclei. The binding of mercury to different nuclear fractions are given in Table 2. The incorporation of mercury into NHP_{Ins} fraction was highest (12,800 cpm per mg protein); DNA and other soluble NHP fractions gave only 150 and 340 counts per mg DNA or mg protein. Histones and 0.14 M NaCl soluble extracts also showed very small binding of mercury.

Further characterization of the mercury-bound NHP_{Ins} protein component was done by SDS gel electrophoresis and by amino acid analyses. The electrophoretic picture and the distribution of ^{203}Hg in the said protein component is shown in Fig. 1. There was only one major protein band and almost all the radioactive mercury was associated with this band.

Table 1

Ratios of Different Nonhistone Proteins to DNA in Rat Kidney Nuclei*

Fractions Analyzed	Ratio of Nuclear Protein to DNA**	
	Normal	Mercury Injected
0.14 M NaCl extracts	1.10	1.12
Soluble nonhistone protein	1.65	1.58
Insoluble nonhistone protein	0.43	0.44

* Nuclei isolated at pH 5.8.

** Each value is the average of five separate determinations.

Table 2

In vivo Incorporation of Radioactive $HgCl_2$ into Different Fractions of Rat Kidney Nuclei*

Fraction Analyzed	cpm/mg Protein or mg DNA**
DNA	150
0.14 M extract	340
Histone	310
Soluble NHP	285
Insoluble NHP	12,800

* Insoluble NHP fraction constitutes 12% of the total nonhistone nuclear protein and 80% of the total mercury of the nuclei.

** Each value is the average of five separate determinations.

Table 3 shows the amino acid composition of NHP_{Ins} protein component for normal and mercury-poisoned rats. There was practically no change in the amino acid composition after mercury administration. The protein contained 1.3 to 1.4 mole percent of cysteine and 1.7 mole percent of methionine. The acid:basic amino acid ratios were found to be 1.02 in both normal and mercury-treated protein.

Discussion The present work reports the isolation and partial characterization of a mercury-binding nonhistone protein fraction from rat kidney nuclei. In this study, ^{203}Hg-labeled mercuric chloride was injected into experimental rats in order to get high incorporation of mercury

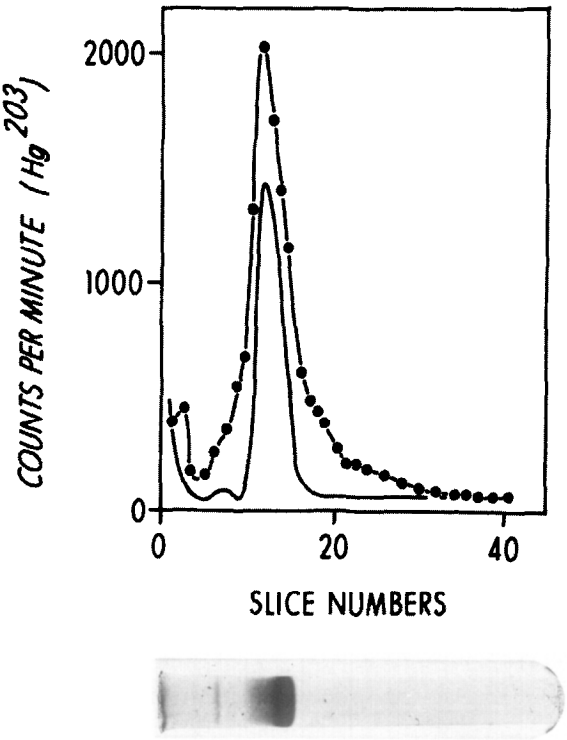

Fig. 1. SDS gel electrophoretic separation and $^{203}Hg^{++}$ incorporation pattern of NHP_{Ins} protein component isolated from rat kidney nuclei. For details see Materials and Methods. ——, densitometric tracings of the gel; ●—●, distribution pattern of $^{203}Hg^{++}$ in the gel.

into the kidney. Although similar distribution patterns of mercury into different nuclear fractions have been noticed after injection of methyl mercury, the total overall incorporation into rat kidney is low.

The purity of the isolated nuclei was checked by phase contrast microscope in each experiment. In order to minimize any proteolysis during extraction of nuclear protein fractions, 0.05 M $NaHSO_3$ was used throughout the process (22). Soluble nonhistone protein fractions (NHP_{Sol}) of both normal and mercury-poisoned rats gave approximately 12 to 14 bands by SDS gel electrophoresis, and also the 0.2 N HCl extracts in both cases showed a normal electrophoretic pattern of histones (unpublished results).

The insoluble nonhistone protein component (NHP_{Ins}), which showed only one main protein band on SDS gel electrophoresis (Fig. 1), is a high molecular weight protein (M.W. >> 150,000) found by comparing the R_f value of the protein band with standard proteins. It could only be isolated after removal of DNA and DNA-protein complex either by DNAase treatment or by 5% trichloro-

Table 3

Amino Acid Composition of the Nonhistone Protein Fraction from Normal and Treated Rat Kidney Nuclei

Amino Acid	μmole Per cent*	
	Normal	Mercury Treated
Lysine	8.6	8.8
Histidine	2.8	2.9
Arginine	7.9	8.1
Aspartic acid	8.4	8.4
Threonine	4.5	4.6
Serine	4.2	4.8
Glutamic acid	11.8	11.9
Proline	4.8	4.8
Glycine	7.8	7.4
Alanine	8.0	7.8
Cysteine	1.3	1.4
Valine	6.6	6.3
Methionine	1.7	1.7
Isoleucine	5.6	5.4
Leucine	9.5	9.2
Tyrosine	2.8	3.0
Phenylalanine	3.7	3.9
Lysine/arginine	1.08	1.02
Acidic/basic	1.02	1.02

*Based on total amino acids recovered from column.

acetic acid extraction at 95 C. Although the type of linkage of this protein to DNA is not yet known, the above observation suggests a firm binding of this protein to DNA. In fact, the existence of some firmly bound nonhistone protein in chromatin structure has been reported (3-8).

The ratio of NHP_{Ins} to DNA was found to be the same in both normal and mercury-poisoned rats (Table 1). The amino acid composition as well as the electrophoretic profile of this protein component were also found to be very similar in both cases (Table 3 and Fig. 1). This protein seems to be an acidic protein with a ratio of acidic to basic amino acids of 1.02 (Table 3). The slower moving minor band, which constitutes only 1.5% of the total NHP_{Ins} by densitometric tracings, might be a multimer of NHP_{Ins} because of

their same amino acid composition (unpublished results). Analysis of NHP_{Ins} isolated from rat liver nuclei gave exactly similar results. It is interesting to note that the incorporation of ^{203}Hg in vivo into NHP_{Ins} was 35-45 times more than any other nuclear protein fraction (Table 2). About 80-85% of the total ^{203}Hg incorporated into nuclei could be accounted in this fraction. Although other nuclear proteins contain cysteine residues also, very little binding of mercury to these fractions was noted (Table 2). Strikingly, exactly the same results as those of rat kidney NHP_{Ins} component were obtained with the nuclear NHP_{Ins} protein isolated from another species, namely, monkey kidney. The amino acid composition and electrophoretic profile of NHP_{Ins} component from rat liver nuclei are also found to be identical to those of rat kidney (unpublished results). All these observations might indicate that perhaps no new protein is synthesized or intruded in the nuclei in the presence of mercury. Müller and Stöcker (23) have also found no lead-induced nonhistone protein synthesis in nuclei.

It might also be speculated that this protein is a common DNA-associated protein present in all eukaryotes and is structural in nature. The importance of the presence of some NHP protein components in chromatin structure has also been reported from other laboratories (4-9).

The amino acid composition, particularly the presence of high cysteine content (1.3 mole per cent) and absence of hydroxy amino acids in NHP_{Ins} eliminates the possibility of this protein being the structural protein collagen (24).

The state of chromatin is very important in controlling genetic transcription (3,25,26). It has been reported that in genetically inactive metaphase chromosomes, the ratio of disulfide to sulfhydryl groups of the nuclear proteins is higher than in extended interphase chromatin (3,25,26). Because of the presence of high cysteine content in NHP_{Ins} component and also of its strong association with DNA, this protein might play a vital role in controlling size and shape of chromatin which in turn might influence gene regulation or replication.

Malling et al. (12) and others (13,14) have observed chromosomal structural alteration in the presence of mercury compounds. Mercury has also been found to affect mitotic apparatus and spindle formation (15). Choie and Richter (16) have recently shown the presence of heavy metal-bound nuclear NHP fraction and that injection of heavy metals stimulates DNA synthesis.

Very little is known about the actual mechanism of action of heavy metals like mercury on genetic apparatus caused by their specific binding

to nuclear proteins. Further studies on mercury-bound NHP_{Ins} protein might be helpful in understanding the biological role of mercury or other heavy metals in chromosomal damage and also in replication and transcription.

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