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ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · AUGUST 2000

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Lead Interaction with Human Protamine (HP2) as a Mechanism of Male Reproductive Toxicity

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Received February 1, 2000

During spermatogenesis, histones are replaced by protamines, which condense and protect sperm DNA. In humans, zinc contributes to sperm chromatin stability and binds to protamine P2 (HP2). Chemical interactions with nuclear protamines, which prevent normal sperm chromatin condensation, may induce changes in the sperm genome and thus affect fertility and offspring development. Since lead has a high affinity for zinc-containing proteins, we investigated lead interactions with HP2 as a novel mechanism of its toxicity to sperm. UV/vis and CD spectroscopy results indicated that HP2 binds Pb^{2+} at two different sites, causing a conformational change in the protein. They also provided evidence that thiol groups are primarily involved in Zn^{2+} and Pb^{2+} binding to HP2 and that HP2 may have additional binding sites for Pb^{2+} not related to Zn^{2+}. HP2 affinities for Pb^{2+} and Zn^{2+} were very similar, suggesting that Pb^{2+} can compete with or replace Zn^{2+} in HP2 in vivo. This interaction of lead with HP2 resulted in a dose-dependent decrease in the extent of HP2−DNA binding, although lead interaction with DNA also contributed to this effect. Therefore, the ability of lead to decrease the level of HP2−DNA interaction may result in alterations to sperm chromatin condensation, and thus in reduced fertility.

Introduction

Although the deleterious effects of lead exposure on the male reproductive system have been known for decades (*1*), the mechanisms of lead actions are not clear. Some epidemiological studies suggest that lead has a direct effect on testis, resulting in decreased production of sperm or altered sperm function (*2*, *3*), whereas other studies suggest that lead acts on the hypothalamic−pituitary axis, causing hormonal imbalance (*4*, *5*). Only a few reports have examined the direct effects of lead on sperm chromatin stabilization (*6*−*8*). An increased number of spermatozoa with unstable chromatin have been reported in men occupationally exposed to lead (*7*), whereas chronic exposure alters sperm chromatin structure in the absence of effects on endocrine function and traditional measures of semen quality in monkeys (*8*). An alteration in sperm chromatin could cause a variety of adverse reproductive outcomes and infertility. During the later stages of mammalian spermatogenesis, chromatin structure is completely reorganized through a series of sequential steps that remove the nucleosomal histones and replace them with small, arginine-rich protamines (*9*). The tyrosine-containing protamine (P1)[1] is present in sperm nuclei of all mammalian species, while the His-containing protamine (P2) has been found in only a few species, including humans (*10*). Human P2 (HP2) is a zinc-containing protein, whose structure has not yet been completely elucidated (*11*, *12*). Some cases of human male infertility have been associated with an altered P1/P2 ratio. A reduced HP2 content (*13*, *14*), the absence of HP2 (*15*), and the presence of its precursors in human sperm heads (*16*) have been reported in infertile men, in the presence of normal levels of HP1. Moreover, the HP2 affinity for DNA appears to be considerably weaker in infertile men (*17*). Therefore, HP2 may serve an important role in sperm chromatin organization and fertility. Stabilization of sperm chromatin occurs with the formation of disulfide bonds between the Cys residues of protamines as spermatozoa pass through the epididymis (*18*). The stability of condensed sperm chromatin is normally maintained during ejaculation by

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[1] Abbreviations: P1, protamine 1; P2, protamine 2; HP2, human protamine 2; HP1, human protamine 1; DTT, dithiothreitol; CM, carboxymethyl; EMSA, electrophoresis mobility shift assay; CT, charge transfer.

10.1021/tx000017v CCC: $19.00
Published on Web 06/27/2000

prostatic zinc, which is sequestered by spermatozoa and binds to the free thiols of Cys in protamines (*19*).

Zinc is known to be an important component of protein molecules, including more than 300 enzymes and almost 1000 proteins involved in gene expression (*20*). The ability of some metal ions to substitute for zinc in the DNA binding domain of some zinc finger has been reported, suggesting that this mechanism may be relevant to the cellular toxicity and/or carcinogenicity of these metals (*21*). Lead has a high affinity for several zinc-binding proteins, and lead interaction with them represents a fundamental mechanism underlying its toxicity (*22*). For these reasons, the aims of this study were to examine (i) the ability of lead to bind to HP2, (ii) the ability of lead to change the HP2 conformation, and (iii) whether this interaction affects the HP2−DNA binding relevant to lead toxicity.

Experimental Procedures

Chemicals. Carboxymethyl (CM)-cellulose for protamine purification was purchased from Whatman International Ltd. Bio-Rad Laboratories (Richmond, VA) provided electrophoresis reagents and the protein assay kit. Lead chloride and zinc chloride were purchased from Aldrich Chemical Co. (Milwaukee, WI). Salmon protamine (salmine), histone-free, was obtained from Sigma Chemical Co. (St. Louis, MO). [α-^{32}P]dCTP (3.0 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). All other chemicals used in the study were high grade and from commercial sources.

Isolation of Human Protamines. Protamine purification was carried out according to published protocols with some modifications (*10*, *17*). Human HP2 was extracted by solubilizing spermatozoa collected from the ejaculate of fertile donors in 10 mM Tris-HCl (pH 8.0) and 10 mM DTT, and removal of the acid-insoluble material by 0.25 N HCl. The University of Maryland Review Board approved all procedures. Total sperm basic proteins were precipitated with 20% trichloroacetic acid and purified by ion-exchange chromatography onto a CM-cellulose column using a linear gradient of guanidine-HCl (from 0.5 to 1.6 M) in 50 mM lithium acetate buffer (pH 5.0). To minimize oxidation, 5 mM DTT was included in the elution buffer. The purities of individual fractions (1 mL) were analyzed by acid−urea polyacrylamide electrophoresis (*23*) after desalting in 10 mM Tris-HCl (pH 7.0). The purities of protamine fractions were confirmed by Western blotting using the monoclonal antibodies Hup1b and Hup2b to HP1 and HP2, respectively (kindly provided by R. Balhorn, Livermore, CA) (data not shown). The protein concentration was determined via the method of Bradford using the Bio-Rad protein assay kit.

Metals Binding to HP2 by Spectrophotometric Analysis. Optical absorption spectra between 200 and 700 nm were recorded in a 1 cm cuvette using a DU Series 600 Beckman spectrophotometer. Desalted fractions from CM-cellulose chromatography were immediately buffered in 10 mM Tris-HCl (pH 7.0) to a final concentration of 50 μM, and increasing molar equivalents of Zn^{2+} (as $ZnCl_2$) or Pb^{2+} (as $PbCl_2$) were added sequentially at 5 min intervals. Sample solutions in a final volume of 50 μL were degassed with nitrogen prior to data collection. To evaluate the participation of Cys residues in HP2−metal binding, the sperm nuclei were incubated with 20 nM iodoacetamide in 10 mM Tris-HCl (pH 8.0) and 10 mM DTT for 1 h at 37 °C, prior to the purification step. Metal titrations were performed in duplicate and replicated using HP2 from two different preparations.

Conformational Analysis of HP2 by Circular Dichroism. CD spectra were measured according to Gatewood et al. (*12*). Spectra were recorded at 0.2 nm intervals from 190 to 250 nm. HP2 samples (17 μM) were dissolved in 10 mM Tris-HF (pH 7.5) containing 0.1 mM DTT and titrated with 0.5−13 molar equiv of Zn^{2+} or Pb^{2+}. The modulation of HP2 structure was compared as a function of adding 0−10 molar equiv of Zn^{2+}, or 0−13 molar equiv of Pb^{2+}, using 203 nm as the wavelength at which the greatest negative transition occurred ($\Delta\epsilon^{203}$). These CD experiments were duplicated using the same HP2 preparation isolated for UV/vis spectral analysis. Data were analyzed for secondary structure with DICROPROT version 2.4 software.

Estimation of the Extent of HP2−DNA Binding by the Electrophoresis Mobility Shift Assay (EMSA). The method of Bianchi et al. (*24*) was used with some modifications. Briefly, a 435 bp DNA fragment was used after nick-translation labeling with [α-^{32}P]dCTP (Amersham). Pure HP2 was diluted in 10 mM Tris-HCl binding buffer (pH 7.5), containing 5 mM $MgCl_2$, 50 mM NaCl, and 10% (v/v) glycerol, and incubated with various concentrations of Pb^{2+} or Zn^{2+} (0.02−100 μM) for 20 min. Then the HP2 was complexed with the ^{32}P-labeled DNA fragment (5 nM) at various protein to DNA ratios (protein dilutions were prepared fresh), in a final volume of 10 μL, at room temperature for 30 min. Samples were subjected to electrophoresis on a 6% polyacrylamide gel. The DNA fragments were visualized by autoradiography, and the amount of free and HP2-bound DNA was determined by densitometry using a MicroScan 1000 gel analyzer. Experiments carried out with salmon protamine were conducted like those with HP2, but the Pb^{2+} concentrations were 1−20 μM. The characterization of the effect of lead on HP2−DNA binding was carried out by following different incubation protocols. The P2−Pb standard protocol is described above. In the P2−DNA protocol, HP2 was incubated with DNA for 30 min and then with 0.1 or 10 μM Pb^{2+} for 20 min, followed by electrophoresis. In the Pb−DNA protocol, 0.1 or 10 μM Pb^{2+} was incubated with DNA for 30 min and then with HP2 for 20 min, followed by electrophoresis.

Statistical Analysis. Data were tested by one-way analysis of variance (ANOVA). When significant differences were indicated, the experimental and control data were compared using the Tukey's test. The confidence intervals at 95% were determined by performing statistical analysis of data expressed as a percentage of control. A p value of <0.05 was used to determine statistical significance.

Results

Lead Interactions with HP2. The UV/vis absorbance spectra of pure HP2 in the presence of an increasing number of molar equivalents of Zn^{2+} and Pb^{2+} showed that both metals bind to HP2 (Figure 1). Absorbance increased upon Zn^{2+} or Pb^{2+} addition. No specific charge transfer (CT) bands were detected for Zn^{2+} below 220 nm (Figure 1A). A CT band at 360 nm characteristic of thiol−Pb^{2+} coordination (*25*) was clearly observed (Figure 1B). To test the participation of Cys residues in HP2−metal binding, thiol groups were blocked during the isolation procedure by incubating the sperm nuclei with iodoacetamide. The effect on the HP2 spectra observed with Zn^{2+} was completely abolished by acetylation of Cys residues; however, the effect with Pb^{2+} additions was only slightly affected by acetylation (Figure 1C). Furthermore, the purification of HP2 in the absence of DTT (when Cys residues were likely to be oxidized) showed that Zn^{2+} failed to produce a change in HP2 spectra, while the addition of Pb^{2+} still resulted in an apparent alteration in the spectra, although one that was greatly reduced in magnitude (data not shown).

Conformational Analysis of HP2. CD spectroscopy was used to monitor conformational variations of HP2 elicited by binding of zinc or lead ions and to estimate the stoichiometry and affinity of binding. Panels A and B of Figure 2 show selected CD spectra resulting from titration of HP2 with Zn^{2+} (0.5−10 molar equiv) and Pb^{2+} (0.5−13 molar equiv), respectively. Figure 2C provides a

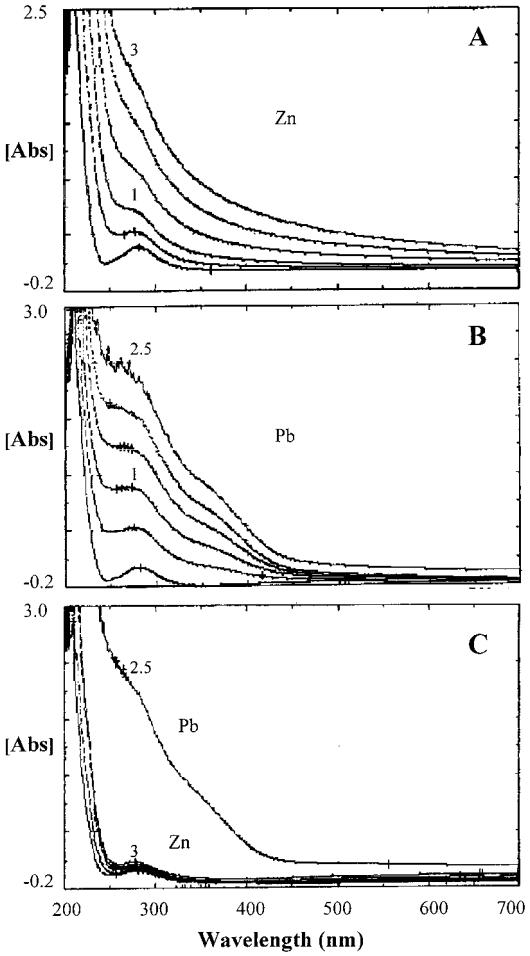

Figure 1. Ultraviolet absorption spectra of protamine HP2 after metal additions. Typical spectra of HP2 (50 μM) with 0, 0.5, 1.0, 1.5, 2.0, and 3.0 molar equiv of Zn^{2+} (A) or with 0, 0.5, 1.0, 1.5, 2.0, and 2.5 molar equiv of Pb^{2+} (B). Spectra of HP2 acetylated with iodoacetamide titrated first with 0, 0.5, 1.0, 2.0, and 3.0 molar equiv of Zn^{2+} and then with 2.5 molar equiv of Pb^{2+} (C). Metals were added directly to HP2 in a 1 cm cuvette, and each sample was degassed with nitrogen for all measurements. Numbers indicate molar equivalents of the metal. This experiment was repeated twice with similar results from two different HP2 isolations.

comparison of the binding profiles of Zn^{2+} and Pb^{2+} monitored at 203 nm. These profiles suggest the existence of two separate binding sites for each metal. Although the effects of Zn^{2+} and Pb^{2+} were qualitatively similar, they differed in terms of the amount of metal ion required to elicit an equivalent spectral change. The estimates for binding constants were obtained by using the computational methods of Bal et al. (*26*). It was assumed that both Zn^{2+} and Pb^{2+} formed 1:1 and 2:1 complexes with HP2, and that the formation of the 2:1 complex started after the 1:1 complex had been formed. This assumption was based on the shapes of titration curves, exhibiting a clear "step" for either metal ion, analogous to the alkali titration of bifunctional acids. The binding constants K_1 and K_2 correspond to appropriate complex formation reactions: $M + L \rightarrow ML$ (K_1) and $M + ML \rightarrow M_2L$ (K_2), where M is Zn^{2+} or Pb^{2+} and L is HP2. Values of $\Delta\epsilon^{203}$ were assigned to 100% formation of particular complex forms: $L \sim -70$, $ML \sim -43$, and $M_2L \sim -37$. The data were then fitted to the model curves, calculated with a range of values for K_1 and K_2, and the best fits were selected. Due to the simplistic character of the binding model, excluding, for instance, cooperativity phenomena,

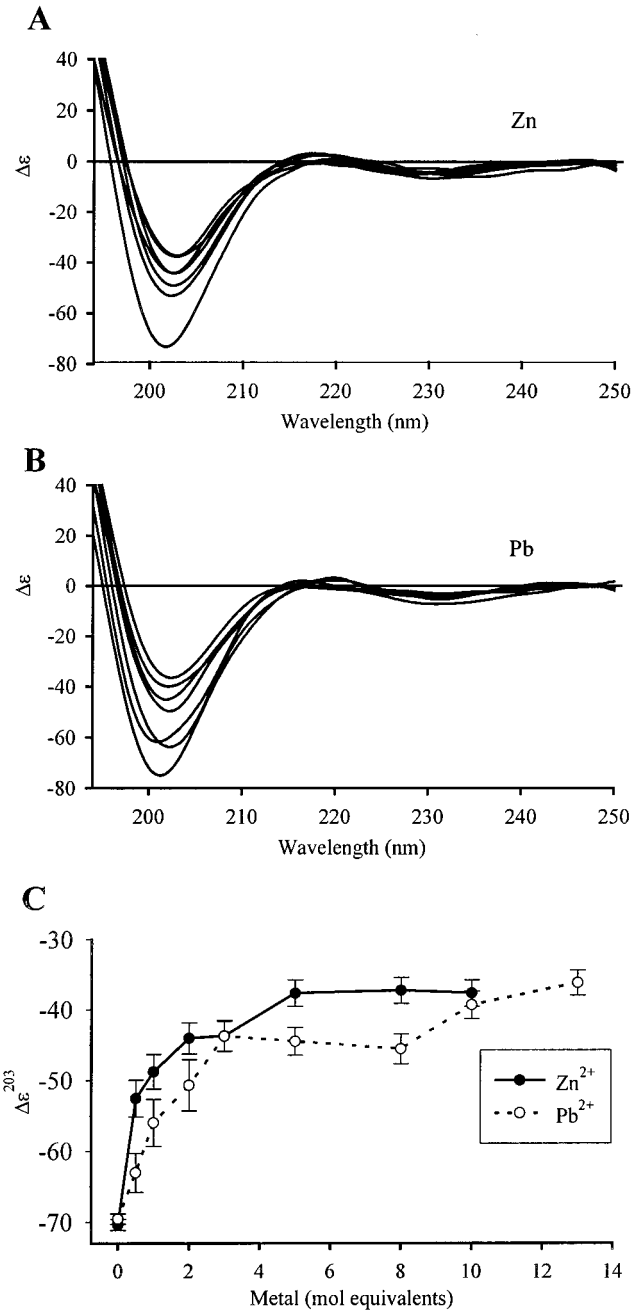

Figure 2. Conformational analysis of HP2. CD spectra of HP2 (17 μM) titrated with 0−10 molar equiv of Zn^{2+} (A) or 0−13 molar equiv of Pb^{2+} (B). The analysis was performed directly in a 1 mm cuvette, and the sample compartment was purged with nitrogen during the measurements. (C) The modulation of HP2 structure was compared as a function of the number of added equivalents of Zn^{2+}, or Pb^{2+}, as described above, using 203 nm as the wavelength at which the greatest negative transition occurred. Titrations were performed in duplicate, with the data representing the mean ± SD. Panels A and B show individual titrations, while both sets of data were used in panel C. These experiments were performed twice with similar results with the same HP2 preparation used for the data of the spectra in Figure 1. The CD spectra were analyzed for secondary structure with DICROPROT version 2.4 software by G. Deleage.

and the inherently imperfect nature of the CD data, the log K_1 and log K_2 values presented in Table 1 should be treated as only estimates. The competitive binding of Zn^{2+} and Pb^{2+} by DTT (*27*) and Tris buffer (*28*) was taken into account in the calculations of K_1 and K_2 values.

HP2 Binding to DNA. Panels A and B of Figure 3 show the curve of DNA binding after addition of increas-

Table 1. Constants for Binding of Zn^{2+} and Pb^{2+} to HP2 Calculated on the Basis of CD Titrations

metal	log $K_1 \pm$ SD	log $K_2 \pm$ SD
Zn^{2+}	7.2 ± 0.1^a	5.3 ± 0.1
Pb^{2+}	6.0 ± 0.4	4.8 ± 1.0

[a] Metal binding constants at pH 7.4 are expressed as log values. K_1 and K_2 correspond to the formation of the ML and M_2L complex, respectively (L stands for HP2). SD values correspond to the statistical spread of the data.

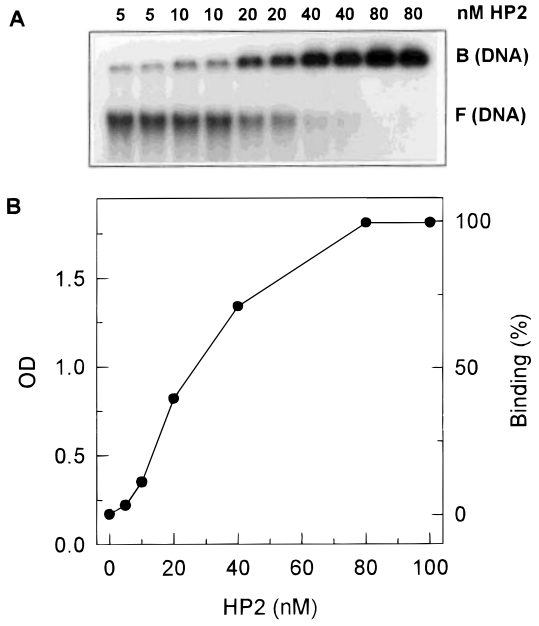

Figure 3. Estimation of the extent of HP2−DNA binding by the mobility shift assay. (A) Gel retardation shows HP2 binding to the 435 bp ^{32}P-labeled DNA probe. Protein concentrations were 5, 10, 20, 40, and 80 nM, and the DNA concentration was kept constant at 5 nM. Each concentration was performed in duplicate. B(DNA) and F(DNA) represent the bound and free DNA fractions, respectively. (B) Plot of densitometric values of the gel retardation. Mobility is expressed as optical density units and as a percentage of the bound DNA of a typical experiment. This experiment was replicated in quadruplicate, each time with a fresh stock HP2 solution from four different HP2 preparations.

ing concentrations of HP2 as determined by EMSA, where saturation was attained at 80−100 nM. A HP2 concentration of 20 nM (approximately 50% of DNA binding) was chosen for the subsequent lead treatment experiments. Lead not only binds to HP2 but also affects the interaction between HP2 and DNA. When HP2 was incubated with lead (20 nM to 100 μM Pb^{2+}), the extent of HP2−DNA binding is reduced as shown by the decrease in the fraction of HP2 bound to a DNA fragment (Figure 4A,B). Somewhat unexpectedly, DNA binding by HP2 also was sensitive to micromolar concentrations of zinc ions (Table 2). To evaluate whether lead alters HP2−DNA interactions only after the complex has been formed, or if lead binding to DNA could also decrease the extent of HP2−DNA binding, lead was added to the reaction mixture using different incubation protocols prior to EMSA (Figure 4C). These data demonstrate that the bound DNA fraction was slightly decreased when lead was added after HP2 had already been complexed with DNA (20 min DNA preincubation with HP2). This suggests a lead-induced displacement of the HP2−DNA complex. Also, when lead was allowed to interact with the DNA fragment before incubation with HP2 (20 min lead preincubation with DNA), the decrease in the extent of HP2−DNA binding was greater than when lead was

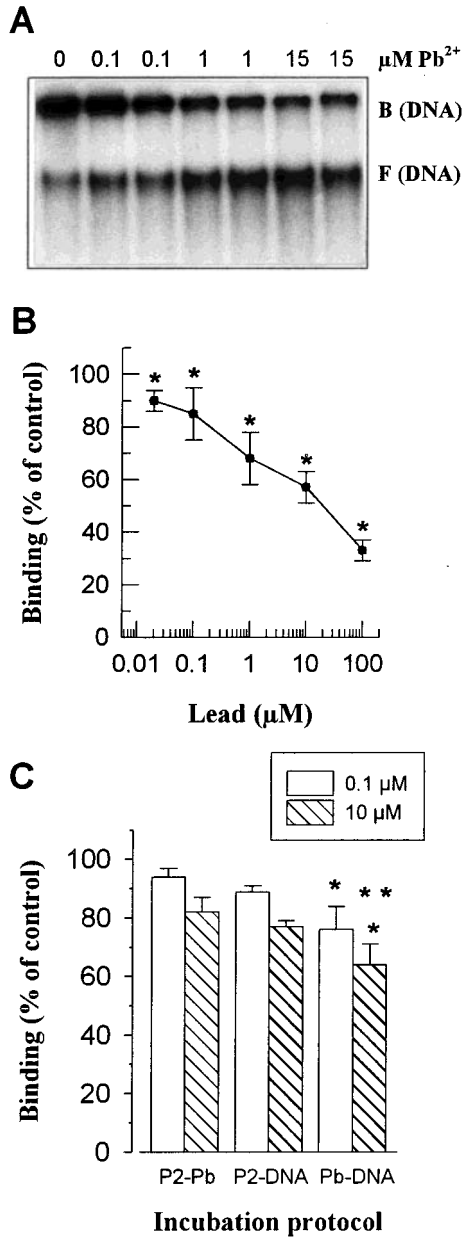

Figure 4. Gel mobility analysis of protamine HP2−DNA binding after lead incubation. (A) Lead concentrations (0.1−15 μM) were added by duplicate to the DNA binding reaction mixture by following the standard protocol (20 nM HP2 was incubated first with Pb^{2+} for 20 min and then with 5 nM DNA), and then the mixtures were subjected to electrophoresis and visualized by autoradiography. B(DNA) and F(DNA) represent the bound and free DNA fractions, respectively. (B) Different lead concentrations (20 nM to 100 μM) were added to the DNA binding reaction mixture as described above, and mobility was expressed as a percentage of the control bound DNA from image analysis. Densitometric data, expressed as a percentage of control, represent the means (±SD) of at least four experiments performed in duplicate. The asterisks denote a significant difference ($p < 0.05$) when compared with control. (C) Lead effects on HP2−DNA binding following different incubation protocols. P2−Pb represents the standard protocol described above. In the P2−DNA protocol, HP2 was incubated with DNA first for 20 min and then with 0.1 or 10 μM Pb^{2+}. In the Pb−DNA protocol, 0.1 or 10 μM Pb^{2+} was incubated with DNA first for 20 min and then with HP2. Densitometric data represent the means (±SD) of two experiments performed in duplicate. One asterisk denotes a significant difference ($p < 0.05$) when compared with the standard protocol, and two asterisks denote a significant difference ($p < 0.05$) when compared with the P2−DNA protocol.

Table 2. Electrophoretic Mobility Shift Assay of Protamines in the Presence of Metals

protamine	[lead] (μM)[a]	[zinc] (μM)	DNA binding (% of control)
human P2		0.02	81 ± 8[b]
		0.10	75 ± 3[b]
		1.0	51 ± 2[b]
		10.0	42 ± 10[b]
		100.0	21 ± 3.5[b]
salmon	1.0		74 ± 17
	10.0		66 ± 6[b]
	20.0		45 ± 2[b]

[a] Different concentrations of zinc or lead were added according to the standard protocol (Figure 3A). The results are expressed as a percentage of the binding by controls (100%) in the absence of metal (mean ± standard deviation). [b] Different from control ($p < 0.05$).

added to HP2 or to the HP2−DNA complex. The ability of lead to interact with the binding sites for HP2 on DNA was further supported by the finding that lead incubation inhibited salmon protamine−DNA binding (Table 2). Salmon protamine lacks both Cys and His residues, which are assumed to bind lead.

Discussion

Our in vitro results show that lead can interact with the zinc−protein HP2, and that lead not only binds to HP2 but also affects the interaction between HP2 and DNA. Lead binding to protamines, which would alter sperm chromatin stability and potentially affect normal chromatin condensation, has not been reported previously. The UV/vis and CD spectroscopy data showed that Pb^{2+} binds to HP2, but not to precisely the same sites that Zn^{2+} does. The UV experiments performed in the absence of DTT or after acetylation provide evidence which shows that thiol groups are primarily involved in Zn^{2+} binding and suggests that HP2 may have additional binding sites for Pb^{2+}, possibly His, since lead binds to this amino acid (*29*). In addition to its affinity for several Cys-containing proteins (*30*, *31*), lead also has a high affinity for carboxyl groups in some proteins (*32*, *33*). Further studies are needed to determine the characteristics of this non-zinc-related residue involved in lead binding to HP2.

The value obtained for the first Zn^{2+} binding to HP2 is within the range reported log *K* values (7.2−11.2) for Zn^{2+} binding to single zinc finger motifs (*34*, *35*). The lower value for the second binding site is in agreement with observations made by Roehm and Berg (*36*) for a ring finger domain. The binding constant of 10^7 is sufficient for the stabilization of HP2 function in the presence of millimolar concentrations of Zn^{2+} in the seminal fluid, and at the same time would not prevent delivery of Zn^{2+} to high-affinity zinc proteins in the oocyte. The estimates of HP2 affinities for Pb^{2+} were relatively similar to the affinities for Zn^{2+}. Thus, Pb^{2+} could potentially compete with or replace Zn^{2+} at least at one site in HP2 in vivo. Zn^{2+} or Pb^{2+} binding changed the CD spectra for HP2, by decreasing the intensity of the negative band observed near 200 nm. This may be due to a charge reduction by the binding of Zn^{2+} or Pb^{2+}, which is consistent with a compaction of the HP2 molecule and a partial alteration of the apoprotein conformation. A similar effect was observed upon decreasing the ionic charge during the pH titration of the N-terminal pentadecapeptide of HP2 (*26*). Such situations were also observed previously in Cu^{2+} complexes of oligopeptides (*37*). The highly positively charged HP2 molecule probably exists in a very extended, loosely helical, and flexible conformation of the P_{II} type (*38*), due to intramolecular electrostatic repulsion. Therefore, the HP2−Zn^{2+} complex may exist in a particular conformation which is not capable of producing a change in the CD signature. Our analysis of the CD spectra for HP2 suggests a pattern typical for an "unordered" conformation (*38*), and did not yield evidence of the formation of β-turns and β-sheets in HP2 upon Zn^{2+} binding as proposed previously by Gatewood et al. (*12*). These authors developed a model of HP2 involving the coordination of two zinc atoms to His residues which facilitate positively charged loops for DNA interaction, but the UV/vis spectra reported here suggest that Cys residues participate in Zn^{2+} binding to HP2, supporting the suggestion of Bianchi et al. (*11*). Interestingly, the changes in CD spectra of HP2 produced by Gatewood et al. (*12*) after Cd^{2+} addition are very similar to those observed here for Pb^{2+}. Ni^{2+} and Cu^{2+} are also able to bind to the N-terminal motif of HP2 (*26*), suggesting that this may be a general target for toxic metals. The interaction of lead with important target proteins is critical to understanding its toxicity (*22*). Hanas and coworkers (*39*) recently showed that lead inhibits DNA binding by the zinc fingers TFIIIA and Sp1, and in vivo exposure to lead may alter developmental gene expression and brain development through selective modulation of the transcriptional activity of Sp1 (*40*). Therefore, lead interaction with zinc-containing protamines may be a plausible mechanism of lead toxicity to male reproduction.

Incubation of pure HP2 with lead inhibited the ability of this protein to bind DNA, probably by inducing a conformational change in the protein, although the direct interaction of lead with DNA also affected DNA−HP2 binding. It is known that the polyarginine sequence of protamines binds to the external surface of the DNA helix, by interacting with DNA−phosphate residues (*41*). Since lead has affinity for phosphate groups of nucleic acids (*42*), lead may interfere with these DNA binding sites. Thus, our results demonstrate that micromolar concentrations of lead can decrease the extent of interaction between HP2 and DNA by any of three mechanisms: a direct interaction with the protein molecule, a direct interaction with DNA, or a displacement of HP2 from its DNA binding sites. The consequences of lead alteration of DNA−protamine binding would depend on the timing of exposure, such that if lead interaction occurs during spermiogenesis when protamines replace histones, a decrease in the level of condensation would be expected as protamines are synthesized and initially interact with DNA. At the same time, an increased level of chromatin condensation would result if exposure occurs during protamine disulfide bond formation within the epididymis, by the formation of S−Pb−S bridges.

The nuclear chromatin condensation−decondensation process is a time-specific one that is necessary for successful transfer of the male genome at fertilization. Chromatin decondensation begins as a progressive process first within the female genital tract by reductive cleavage of the chromatin S−S bridges (*43*) and final removal of protamines after fertilization and before chromosome pairing (*44*). Lead exposure occurring at critical stages of spermatogenesis could result in the

formation of cross-linking between lead and protamines, affecting the nuclear sperm condensation−decondensation process, thereby compromising fertility. It has been reported that semen lead concentrations can reach micromolar levels in occupationally exposed individuals (*45*, *46*), and lead has been shown to accumulate in epididymis and prostate after long-term exposure in animal studies (*47*). Furthermore, lead can accumulate in sperm heads (unpublished results), and a recent report showed that lead exposure of sperm in vitro results in an efflux of nuclear zinc (*48*). In addition, in vivo experiments in rats have shown a competition between lead and zinc in reproductive organs (*49*).

A change imposed on the chromatin structure of sperm DNA may increase the risk of DNA damage, similar to that demonstrated by chemical binding to sulfhydryl groups in protamines (*50*). It has been reported that human sperm with abnormal chromatin structure may be more vulnerable to DNA damage (*51*, *52*). An impaired structural stability of the chromatin may increase the vulnerability of the paternal genome, thereby altering normal embryo development. Thus, besides the direct toxic effects of lead on the fertility of an exposed male, any alteration in sperm chromatin structure may adversely affect the offspring, if fertilization occurs (*53*). Although the paternally mediated effects of lead on subsequent development and reproduction of offspring have not been well studied, results from our group have shown that low paternal doses of lead below the level of those affecting male fertility caused alterations in protein synthesis of two-cell embryo cultures, suggesting heritable effects of paternal exposure since lead caused effects that can be expressed in the offspring (*54*).

In summary, the decrease in the extent of HP2−DNA binding from lead exposure observed in this study could cause a perturbation of normal chromatin condensation in maturing spermatids and early spermatozoa, which may result in direct damage to the sperm, but also in direct delivery of the toxicant to the zygote. This alteration in the chromatin structure would result in poor quality mature sperm, and may in part explain epidemiological observations of lead toxicity to male reproduction. Therefore, chromatin stability alterations could have important implications for male fertility in both humans and other species, and could be a potential mechanism for infertility or other adverse reproductive outcomes following lead exposure at concentrations similar to those known to have deleterious physiological effects.

Acknowledgment. We thank Dr. M. Young for helpful discussions and valuable advice for this study, Dr. P. Smith for help with CD measurements, and Mr. J. Strong for assistance with the fluorescence analysis. We also thank Drs. M. Cebrián-García and F. Navarro-García for discussion and critical reading of the manuscript and Dr. K. S. Kasprzak for useful advice. B.Q.-V. was the recipient of a postdoctoral Pan American Fellowship (NCI /NIH-USA and CONACyT-Mexico). This work was supported by The Bressler Foundation (to L.D.A.) and in part by a grant from The Polish Committee of Scientific Research (KBN) (6P04A02413) to W.B.

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