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The Chemical Forms of Mercury in Human Hair: A Study using X-ray Absorption Spectroscopy

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Abstract

Human hair is frequently used as a bio-indicator of mercury exposure. We have used X-ray absorption spectroscopy to examine the chemical forms of mercury in human hair samples taken from individuals with high fish consumption and concomitant exposure to methylmercury. The mercury is found to be predominantly methylmercury cysteine or closely related species, comprising approximately 80% of the total mercury with the remainder an inorganic thiolate-coordinated mercuric species. No appreciable role was found for selenium in coordinating mercury in hair.

Keywords

human hair; mercury; X-ray absorption spectroscopy; Extended X-ray absorption fine structure; X-ray absorption near-edge spectra

Introduction

Fish is the major dietary source of potentially neurotoxic methylmercury species in human populations. Predatory marine fish such as swordfish and shark contain sufficiently high levels of methylmercury species that consumers are currently advised to eat these fish less frequently than once a month, and not at all if pregnant [1]. The precise nature of the methylmercury coordination in marine fish was only recently discovered to be an aliphatic thiolate, similar to the methylmercury-cysteine complex, using *in situ* X-ray absorption spectroscopy (XAS) [2]. More recently, it has been shown that this chemical form of mercury is not modified by digestion with simulated gastric fluid [3]. Mercury is excreted from the human body via a number of different routes. A fraction of the mercury in fish consumed as food probably cycles through the enterohepatic circulation, finally exiting in feces via sloughing of intestinal lumen cells, reducing the mercury dose absorbed into the body [4,5,6]. In agreement with this, Berntssen and coworkers [7] have reported that rats fed high-mercury fish show higher fecal excretion and lower tissue accumulation of mercury than rats consuming fish to which methylmercury chloride was added artificially. While such mechanisms may eliminate some of the mercury ingested, there is continued concern

that what remains could influence neurodevelopment. Another route of mercury excretion is via deposition of the metal into hair as it grows, and mercury may be excreted in mammals and birds by this route [8], although the fraction eliminated via this route has been estimated to be less than 10% [9].

Mercury levels in hair are a frequently used marker of mercury exposure in humans, and when the hair is long and analyzed segmentally it can recapitulate a history of exposure [10]. The levels of mercury in newly formed hair appear to be directly proportional to the concentrations found in blood and brain [11]. The hair to blood ratio in humans is approximately 250:1 [12,13], although most animal studies indicate considerably lower ratios [12]. The high value appears to be specific to humans; in agreement with this, a ratio of 217:1 has recently been reported for human scalp hair transplanted into athymic nude mice [14]. It has been suggested that mercury enters hair and brain by similar mechanisms, and consequentially that hair can be considered to be a biological marker which is directly correlated with brain mercury exposure [11,15].

Three large studies have been reported on the effects of dietary mercury on human populations. One study is located in the Faeroe Islands in the North Atlantic [16], a second in the Seychelles Islands in the Indian Ocean [17] and the third in New Zealand [18]. The Faeroes study is of a population with high dietary mercury primarily from pilot whale, but also from fish. The primary source of exposure in the New Zealand study was shark prepared as “fish and chips”. The Seychelles study is of a population that consumes large quantities of fish that have mercury levels similar to those in oceanic fish consumed in North America. The studies reach different conclusions regarding the hazards of mercury exposure from fish consumption. While the combined conclusions of these studies are still the subject of debate, all three used human hair as one indicator of mercury exposure, with blood and in the Faeroe Islands chord blood as additional indicators. X-ray absorption spectroscopy (XAS) is unique in that it is capable of determining the chemical form of any heavy element *in situ*, with no sample pre-treatment required. Here, we use XAS to investigate the chemical nature of the mercury deposited in hair samples taken from three individuals with high dietary mercury due to a high-fish diet in the Seychelles islands.

Materials and Methods

X-ray Absorption Spectroscopy

X-ray absorption spectroscopic (XAS) measurements were conducted at the Stanford Synchrotron Radiation Lightsource (SSRL) with the SPEAR storage ring containing between 90 and 100 mA at 3.0 GeV. Mercury L_{III}-edge and selenium K-edge data were collected on the structural molecular biology XAS beamline 9-3 operating with a wiggler field of 2 T and employing a Si(220) double-crystal monochromator. Beamline 9-3 is equipped with a rhodium-coated vertically collimating mirror upstream of the monochromator, and a downstream bent-cylindrical focusing mirror (also rhodium-coated). Harmonic rejection was accomplished by setting the cutoff angle of the mirrors to reject energies above 15 keV. To minimize radiation damage samples were maintained at a temperature of approximately 10K in a liquid helium flow cryostat (Oxford Instruments, Abingdon, UK). X-ray absorption spectra were measured as the Se K $\alpha_{1,2}$ or Hg L $\alpha_{1,2}$ fluorescence excitation spectra using a 30-element germanium array detector [19] with analog electronics (Canberra Corporation, Meriden CT, USA) employing an amplifier shaping time of 0.125 μ sec. To avoid problems with non-linearity of the detector due to high count-rates, X-ray filters (made of elemental As for Se, and Ga₂O₃ for Hg) were used to preferentially absorb scattered radiation. Silver Soller-slits (EXAFS Co., Pinoche NV) were optimally positioned between the sample and the detector to reduce filter fluorescence registered by the detector. Incident and transmitted XAS intensities were measured using

nitrogen-filled ionization chambers. The mercury spectra were energy-calibrated with reference to L_{III}-edge spectrum of Hg-Sn amalgam foil measured simultaneously with the data, the lowest energy inflection of which was assumed to be 12285.0 eV. The selenium spectra were similarly energy calibrated with reference to the lowest energy inflection of hexagonal elemental selenium which was assumed to be at 12658.0 eV. For near-edge spectra six sweeps were averaged, each of 25 minutes duration, whereas for the EXAFS data set thirty-six individual sweeps were averaged, each of 35 minutes duration (totaling 21 hours). Given the limited amount of synchrotron beamtime available the time required to collect individual data sets precludes the collection of data on large numbers of samples.

XAS data were processed using standard techniques and employing the EXAFSPAK program suite [20]. Near-edge spectra were fitted to linear combinations of standard spectra using the EXAFSPAK program DATFIT using the criteria previously described [3]. EXAFS oscillations were analyzed using *ab initio* theoretical phase and amplitude functions calculated using the program FEFF v7.02 [21,22].

Sample Preparation

Samples of scalp hair approximately 10 cm in length were obtained from individuals in the republic of Seychelles, and were stored at room temperature prior to X-ray absorption spectroscopy. The hair was loosely packed into acrylic XAS sample cuvettes, which were frozen in liquid nitrogen immediately prior to insertion in the liquid helium cryostat for XAS measurements.

Results

X-ray absorption spectra arise from photo-excitation of a core electron, a 1s electron for a K-edge, or a 2p_{3/2} electron for an L_{III} edge. The spectrum can be arbitrarily divided into two overlapping regions – the near-edge spectrum which is the structured region within approximately 50 eV of the absorption edge, and the extended X-ray absorption fine structure (EXAFS) which comprises oscillations on the high-energy side of the absorption edge and which can be accurately interpreted in terms of a local radial structure [23,24]. Nomenclature of near-edge spectra spectrum is confused [23] but this region is often referred to as the X-ray absorption near-edge fine structure or XANES. The structure in near-edge spectra is due to transitions from the core level (1s for a K-edge) to unoccupied molecular orbitals of the system. Intense transitions are Laporte-allowed $\Delta l = \pm 1$, and thus for K and L_{III} edges are to levels with a considerable *p* and *d* orbital character, respectively. Near-edge spectra are therefore sensitive to electronic structure, and give a fingerprint of the chemical species of the metal or metalloid concerned. The advantage of the near-edge region of the spectrum is that it can be quickly collected with good signal to noise. In contrast, EXAFS is more difficult to collect with adequate signal to noise, and is not always practical on dilute samples. A unique benefit of XAS, both near-edge and EXAFS, is that it requires no pre-treatment or extraction and thus provides a tool that can probe chemical species *in situ*.

Figure 1 shows the Hg L_{III} near-edge spectra of three hair samples from different individuals. The spectra of individual samples are very similar, with differences between them being smaller than the signal-to-noise of the spectra (demonstrated by the difference spectra shown in Figure 1). Principal component analysis [23] (not illustrated) confirms that no additional components can be resolved from differences between the three spectra of Figure 1. Because collection of EXAFS requires extensive signal averaging for dilute samples, a single representative hair sample was selected for study by EXAFS spectroscopy. Figure 2 shows the Hg L_{III} EXAFS and the results of a curve-fitting analysis, together with the EXAFS Fourier transforms. The signal to noise ratio of the data precluded analysis

beyond $k=11 \text{ \AA}^{-1}$, but nevertheless two distinct types of backscatterer are clearly observed as two peaks in the EXAFS Fourier transform (Figure 2). Consistent with the two peaks observed in the Fourier transform, quantitative EXAFS curve-fitting analysis indicates the presence of two distinct backscatterers, one Hg—C at $2.06 \text{ \AA}$, and one Hg—S at $2.36 \text{ \AA}$ (Table 1). We note that minor components are very hard to detect in EXAFS data, especially if the data has low signal to noise content.

More information on the chemical composition can be obtained from analysis of the near-edge portion of the spectra [23]. Figure 3 shows the least-squares fit of the Hg L_{III} near-edge data of the sample of Figure 2. The edge-fitting procedure was applied for two and three component fits, but in the latter the third component was found to be not statistically significant in any of the cases tested, being rejected by the algorithm [23]. A major component of methylmercury-cysteine was consistently required for an adequate fit, and essentially equivalent best fits were obtained for different minor components corresponding to mercury coordinated to aliphatic thiolates. For this minor component, compounds with three and with four thiolate donors ($[\text{Hg}(\text{SR})_3]^-$ and $[\text{Hg}(\text{SR})_4]^{2-}$, respectively) gave essentially equivalent fits. As expected, linear combination fitting of the data from all three hair samples gave essentially indistinguishable fits. These results are summarized in Table 2. The near-edge analyses are in excellent agreement with the results of conventional analysis of Seychellois hair [11], which indicated that ~80% of the mercury is present as methylmercury forms and ~20% as inorganic forms.

The presence of 23% $[\text{Hg}(\text{SR})_3]^-$ in the hair might be expected to affect the EXAFS data. An alternative fit is presented in Table 1, with 77% of interactions expected for methyl mercury bound to sulfur (0.77 Hg—C and 0.77 Hg—S) plus 23% of the longer Hg—S interaction corresponding to the inorganic species determined by fitting the near-edge spectra ($0.23 \times 3 \text{ Hg—S}$). As discussed above, Hg—S bond-lengths of 2.34 and $2.44 \text{ \AA}$ are expected for two and three coordinate mercury, respectively. The EXAFS resolution ΔR can be defined as the smallest difference in inter-atomic distance that can be discriminated for similar backscatterers, and to a first approximation this is given by $\Delta R \approx \pi/2k$ where k is the extent of the data in $\text{\AA}^{-1}$. We note that our EXAFS data lacks the k -range to properly resolve the bond-lengths of two and three coordinate components, but nevertheless the fit shown in Table 1 indicates that the EXAFS data are consistent with the near-edge analysis, with somewhat smaller Debye-Waller factors for the short Hg—S components, and larger estimated standard deviations for all parameters. Alternatively, the use of higher effective Hg—S coordination numbers, and slightly lower effective Hg—C coordination numbers (Table 1) derived from the near-edge analysis also gives a very slight improvement in the fit. The improvement in the fit errors for both alternative fits is marginal and while we can conclude that the EXAFS data are fully consistent with the near-edge analysis, our data are not of sufficient quality to answer detailed questions on coordination numbers. Nevertheless, the observation of significant Hg—C EXAFS clearly indicates that the majority of the mercury present is methylmercury with sulfur donors completing the metal coordination environment.

Discussion

The X-ray absorption spectroscopy (XAS) data presented herein provide clear evidence concerning the chemical speciation of mercury in human hair. Our data are in agreement with and extend the results from conventional chemical analysis, which showed Seychellois hair to contain approximately 80% of the total mercury as methylmercury forms with the remainder in inorganic forms [11]. Different proportions of methylmercury and inorganic forms are found in other populations [25,26]. The EXAFS-derived bond-lengths are in excellent agreement with those reported for the methylmercury complex with cysteine.

Values from X-ray crystallography for Hg—C and Hg—S are 2.098 and 2.352 Å [27], respectively, and from EXAFS of solutions of methylmercury-cysteine are 2.07 and 2.35 Å [28] (Table 1). Stable complexes are known with either two [Hg(SR)₂], three [Hg(SR)₃][−], or four [Hg(SR)₄]^{2−} coordination, and well-characterized examples of all three coordination modes have been identified by X-ray crystallography (e.g. [27]). A four-coordinate cysteine complex [Hg(Cysteine)₄] has recently been reported to be formed in aqueous solutions at high pH values [30]. The Hg—S bond-length is highly characteristic of the coordination [31]. The Cambridge Crystal Structure Database [32] indicates typical Hg—S bond-lengths of 2.34, 2.44 and 2.55 Å for two, three and four coordinate compounds, respectively. No coordination compounds are listed in the Cambridge Crystal Structure Database containing sulfur coordinated by four mercury atoms [32], although this structure is found in black mercuric sulfide, otherwise known as β-HgS or metacinnabar, which has the zincblende structure with tetrahedral coordination of Hg by S, and tetrahedral coordination of S by Hg, with an Hg—S bond-length of 2.55 Å [33].

Li et al. recently reported an XAS study of human hair from individuals exposed in a heavily contaminated elemental mercury mine site area [26]. Conventional analysis showed predominantly inorganic mercury [26], contrasting with methylmercury exposure from fish consumption. Li et al. report both sulfur K and mercury L_{III} near-edge and EXAFS data of human hair [26]. From the Hg EXAFS, Li et al. determined three Hg—S interactions with a 2.48 Å bond-length, consistent with mercury coordinated by three sulfurs. However, Li et al.'s analysis of the S EXAFS yielded four S—Hg interactions at 2.36 Å [26], a bond-length neither consistent with that from Hg EXAFS nor characteristic of four-coordinate sulfur. Furthermore, the 0.0084 weight % mercury content [26] contrasts with about 18 weight % sulfur in normal human hair [34], and corresponds to less than one mercury atom per 10,000 sulfur atoms. We conclude that sulfur K-edge measurements of hair cannot possibly detect mercury backscatterers. The sulfur near-edge spectra [26] strongly resemble those of cystine disulfide species [35], the form expected in extracellular environments such as hair [35]; the S EXAFS are likely due to unresolved S—C and S—S from disulfide species. Four Hg backscatterers have a much larger amplitude than do a single S or C, and distinguishing these backscatterers would normally be relatively simple. However, several factors can complicate assignment, such as if the threshold energy shift ΔE_0 is allowed to refine outside reasonable bounds [36], although ΔE_0 values are not listed by Li et al. [26]. Very large, physically unrealistic Debye-Waller factors, such as 0.025 Å² [26], severely reduce EXAFS amplitudes except at lower *k*-values. Fourier filtering with window functions, which to have been used, severely reduce amplitudes at low-*k*. These factors may have led Li et al. to erroneous conclusions from the S EXAFS.

We now return to the major question addressed here, the chemical nature of mercury in human hair. From a simple chemical point of view, the high sulfur content of hair (discussed above) might argue for sulfur donors to mercury. However, as we have discussed above, the sulfur is essentially present as oxidized disulfide cystine, and thus could not directly provide thiolate donors to Hg. The molecular toxicology of mercury is often associated with selenium [37,38], and because the Hg L_{III} near-edge spectra from compounds containing Hg—S and Hg—Se coordination can sometimes be difficult to distinguish [3], we also examined the Se K-edge spectra of hair. The relative levels of Hg and Se can be estimated from a combination of the Se K and Hg L_{III} edge jumps and the Se K α_{12} and Hg L α_{12} fluorescence intensities, correcting for fluorescence yields [39], and this indicates that the Se levels are approximately 43% of the Hg levels. Figure 4 shows the near-edge spectrum of the hair sample of figures 2 and 3, together with the analysis of the near-edge spectrum. The spectrum could be adequately analyzed without any model spectra typifying Hg—Se coordination [3] and instead could be adequately modeled using oxidized selenium species such as selenocystine [40] with a small amount of selenite (Figure 4). This together with the

Hg L_{III} near-edge and EXAFS analysis indicates that selenium plays no significant role in coordination of mercury in human hair.

While fish are the major source of mercury exposure in the Seychelles, dental amalgam is an alternative potential source of the mercury in hair. The amalgam status of the individuals from which hair was sampled is unknown, but this is unlikely to be a major factor because it is well established that there is no correlation between hair mercury levels and numbers of dental amalgams [41]. Any large-scale influence of amalgam on our results is therefore unlikely, but mobilization of mercury from dental amalgam and subsequent methylation by microflora (oral and gut) cannot rigorously be excluded as an additional potential source of some of the methylmercury found in hair.

Previous work indicates that methylmercury cysteine is actively transported as a substrate for human L-type large neutral amino acid transporter (LAT), and that this is responsible for its uptake into cells [5]. We have shown that this cannot be due to a methionine-specific molecular mimicry as previously assumed, but rather due to a non-specific neutral amino acid activity [28]. Assuming that this is correct then our results show that no major changes in mercury coordination occur on accumulation into hair. As discussed above, remarkable levels of mercury concentration occur in human hair, at around 250 times blood levels [12,13]. A similarly remarkable concentration of methylmercury cysteine has recently been observed in zebrafish larvae in the rapidly proliferating single layer of cells comprising the lens epithelia [42]. Recent work using autoradiography of human scalp hair transplanted into athymic nude mice has shown that most of the mercury taken up is concentrated in the hair shafts and in the keratogenous zone of the hair follicle [14]. Both hair keratinocytes and lens epithelial cells are engaged in significant protein synthesis. Given that large neutral amino acid transporter expression is known to be related to protein synthesis, the explanation for both of these accumulations may be as simple as increased methylmercury-cysteine transport due to increased transporter levels. Furthermore, it has been shown that the LAT 1 isoform of the transporter is selectively expressed at the blood brain barrier [43] suggesting a role for the transporter in brain accumulation of methylmercury cysteine. Transport of inorganic mercury and methylmercury species are likely to be different. Thus, recent work on sea urchin embryos indicates that inorganic mercury species are exported by MRP/ABCC-type transporters whereas methylmercury species are not [44]. The presence of inorganic mercury species in hair could potentially arise from two sources, either a mechanism exists for directly incorporating inorganic mercury into hair, or a fraction of the methylmercury in hair is demethylated subsequent to incorporation. The latter could arise via coordination of mercury by more than one thiolate, which would increase the negative charge on the methyl group [45] and promoting C—Hg protonolysis to yield methane and inorganic mercury. Further work is required to determine which of these two mechanisms is in effect.

Concluding remarks

We have shown that the predominant chemical form of mercury in human hair strongly resembles methylmercury cysteine with Hg—C and Hg—S bond-lengths of 2.06 and 2.36 Å, respectively. The analysis of the near-edge spectra agrees with that of the EXAFS, and also indicates approximately 20% of inorganic mercury that is a high-nuclearity aliphatic thiolate complex, probably either [Hg(SR)₃][−] or [Hg(SR)₄]^{2−}. No involvement of selenium in coordination of mercury is indicated from analysis of Hg L_{III}-edge and Se K-edge XAS. Because the mechanisms of uptake in brain and hair are thought to be related, our finding that methylmercury cysteine is incorporated into hair may be relevant to the mechanism of neurotoxicity of methylmercury species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EXAFS	Extended X-ray absorption fine structure
XAS	X-ray absorption spectroscopy
LAT	L-type large neutral amino acid transporter

Fig. 1. Comparison of Hg L_{III} near-edge spectra of hair samples from three individuals. The differences between adjacent spectra are shown beneath illustrating that the three samples have chemical compositions that are indistinguishable by analysis of near-edge spectra.

Fig. 2. Mercury L_{III} EXAFS Fourier transforms of hair sample *a* (Figure 1) with Hg—S phase-correction. The inset shows the raw k^3 weighted EXAFS data. Solid lines (—) show the experimental data, while broken lines (-----) show the best fit.

Fig. 3.

Linear combination analysis of mercury L_{III} X-ray absorption near-edge spectra of hair. The open circles show the experimental hair spectrum (○), the solid line (—) shows the best fit, and the broken lines show the spectra of individual components, methylmercury cysteine (-----) 77±3%, and [Hg(S^TButyl)₃]⁻ (.....) 23±3% (errors are estimated standard deviations obtained from the diagonal elements of the covariance matrix).

Fig. 4.

Linear combination analysis of selenium K X-ray absorption near-edge spectra of hair. The open circles show the experimental hair spectrum (○), the solid line (—) shows the best fit, and the broken lines show the spectra of individual components, selenocystine (-----) $91\pm4\%$, and selenite (.....) $9\pm3\%$ (errors are estimated standard deviations obtained from the diagonal elements of the covariance matrix).

Table 1

Structural parameters for mercury^a in hair compared with those of L-cysteinato(methyl)mercury(II) in both crystalline and solution state.

	Hg—C			Hg—S		
	N ^b	R	σ ²	N ^b	R	σ ²
Hair EXAFS	1	2.06(1)	0.003(2)	1	2.356(4)	0.0023(5)
						-16.5 ^c
	0.77 ^d	2.03(1)	0.003(2)	1.46	2.357(4)	0.0051(5)
						-16.5 ^c
	0.77 ^e	2.05(1)	0.002(2)	0.77	2.33(1)	0.002(1)
				0.69	2.42(1)	0.004(2)
L-Cys-HgCH ₃ crystallography [27]	1	2.098	—	1	2.352	—
L-Cys-HgCH ₃ EXAFS [28]	1	2.070(3)	0.0020(2)	1	2.351(2)	0.0029(1)
						-16.5(5)
						0.29

^a Coordination numbers *N*, interatomic distances *R* (Å), Debye-Waller factors *σ*² (the mean-square deviations in interatomic distance) (Å²), and the threshold energy shifts Δ*E*₀ (eV). The values in parentheses are the estimated standard deviations obtained from the diagonal elements of the covariance matrix. The fit-error function *F* is defined by

$$F = \sqrt{\sum k^6 \left(\chi(k)_{\text{calcd}} - \chi(k)_{\text{expt}} \right)^2 / \sum \chi(k)_{\text{expt}}^2}$$

where *χ*(*k*) are the EXAFS oscillations and *k* is the photo-electron wave number given by $k = \sqrt{\frac{2m_e}{\hbar^2} (E - E_0)}$.

^b The values for the coordination numbers are not refined and are not refined due to high mutual correlation with the Debye-Waller factor in the refinements.

^c Because of the limited *k*-range the Δ*E*₀ value was constrained to the value obtained from fitting the EXAFS data of a large number of methylmercury model compounds with *k*-range 16 Å⁻¹ (not illustrated).

^d Alternative fit based on the results of the near-edge analysis, with 77% of a two coordinate CH₃HgS— species, and 23% of a three coordinate [Hg(SR)₃][−] species, assuming unresolved Hg—S bond-lengths.

^e Alternative fit based on the results of the near-edge analysis, with 77% of a two coordinate CH₃HgS— species, and 23% of a three coordinate [Hg(SR)₃][−] species, with the refinement starting from the typical Hg—S bond-lengths for two and three coordinate species.

Table 2Linear combination analysis of Hg L_{III} near-edge spectra of human hair

<i>Sample</i>	% CH ₃ Hg(Cys)	% [Hg(SR) ₃] ⁻	<i>f</i> (×10 ⁴) ^a
<i>a</i>	77(3)	23(3)	1.35
<i>b</i>	80(3)	20(3)	1.37
<i>c</i>	81(3)	19(3)	1.36

^aThe Fit-Error *f* is defined as $f = \frac{1}{n} \sum (A_{obsd} - A_{calc})^2$ where *A* is the normalized X-ray absorbance and *n* is the number of data points within range. We estimate from Fourier filtering that the contribution to *f* from high-frequency noise is close to 0.8×10^{-4} in all three cases. When only a single component was used to fit the data then CH₃Hg(Cys) provided the best fits with *f* increased by 0.47×10^{-4} , 0.46×10^{-4} and 0.49×10^{-4} for *a*, *b* and *c*, respectively. (One and two component fit residuals are shown in Supplementary Material, Fig. S1.)