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Binding of Methylmercury and Methylmercury-Thiol Complexes by Myelin Isolated from Mice of Differing Selenium Status

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The neurotoxic effects of methylmercury have been studied extensively and reviewed by Chang (1977). Following the outbreak of Minamata Disease in Nigata, mercury was localized in the glial cells of the nervous system. Further involvement of glial cells during mercury intoxication is demonstrated by their proliferation following mercury intoxication. In intact nervous tissue it has been demonstrated that, in general, as mercury intoxication progresses, an increasing amount of mercury is detected in the nerve cells, glia and nerve fibers (Chang et al., 1972). Since glial cells are responsible for producing myelin, it is not surprising that large myelinated fibers are affected to a greater extent than smaller fibers (Somjen et al., 1973) and that myelin sheaths lose their lamination (Miyakawa et al., 1970) and degenerate (Kim, 1971).

There are potential reactive sites, including sulfhydryl groups and disulfides, for mercury in myelin proteins (Braun and Brostoff, 1977). Metals, especially mercury, have a high affinity for thiol groups. Since the cysteine groups found on proteins are generally the most likely thiol groups available for binding metals, Clarkson and Magos (1966) suggested that in tissue homogenates protein thiol groups serve as binding sites. However, the mercury reactive groups in myelin are much less reactive than are the most reactive groups of other tissue (Ganser and Kirschner, 1985). The most prevalent thiol found in cells and plasma is the tripeptide glutathione. Metabolites of glutathione, such as cysteine, have been shown to modify the short-term accumulation of methylmercury in the cerebrum (Thomas and Smith, 1982). Glutathione (L-γ-glutamyl-L-cysteinyl-glycine, GSH), which is the major cellular sulfhydryl available for binding and inactivating toxic electrophilic species, is the substrate for GSH-transferases and GSH-peroxidase. Selenium, an integral part of glutathione peroxidase, has now been shown to be intimately involved in many other glutathione metabolic enzymes.

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The chemical form of methylmercury found in the body provides evidence of the intimate involvement of glutathione and glutathione metabolites with methylmercury. The involvement of glutathione in converting protein bound methylmercury to low molecular weight methylmercury-selenium compounds was shown by Iwata et al. (1981), and the role of methylmercury-thiol complexes in the metabolism and tissue deposition of methylmercury was confirmed by the co-administration of L-cysteine and methylmercury (Thomas and Smith, 1982). Refsvik and Norseth (1975) reported that the major form of methylmercury excreted in the bile is CH_3Hg-GSH. Furthermore, they also reported finding some methylmercury complexed to cysteine. Likewise, Naganuma and Imura (1979) have shown that in human red blood cells almost half of the methylmercury is bound to glutathione. Thomas and Smith (1979) presented evidence that 30% of the cerebral methylmercury is found bound to glutathione.

The effectiveness of selenium in modifying mercury metabolism has attracted increasing attention. The alleviation of chronic methylmercury toxicity by dietary selenium was first reported by Ganther et al. (1972). It was shown that the survival and growth rates of rats were greater when fed both selenium and methylmercury as compared to those fed methylmercury alone. After finding that concurrent equimolar selenite injections increased the uptake of mercury but did not alter the mercury distribution in the brain and that selenium reversed the effect of mercury upon glutathione peroxidase, Prohaska and Ganther (1977) suggested a complex of mercury and selenium may be involved in the interaction of mercury and selenium. However, considering that the protective effect of selenium is exerted within the tissue without decreasing the concentration or amount of mercury in the tissue, selenium is probably not simply chelating the bulk of brain mercury.

The purpose of the following experiments was to determine the binding characteristics of thiol-metabolites of methylmercury to myelin isolated from the central nervous system. The possible alteration of such binding by dietary selenium was also investigated.

MATERIALS AND METHODS

Methylmercury complexes of cysteine, homocysteine and glutathione (Sigma; St. Louis, MO)[1] and methylmercury chloride (^{203}Hg) (Amersham; Arlington Heights, IL) were prepared by mixing equimolar amounts of the thiol and methylmercury in 0.1 M Tris-HCl, pH 8.0. The methylmercury complex with cysteine-glycine was prepared by mixing a pH 5 solution of cysteine-glycine with a solution of methylmercury chloride (^{203}Hg) in 0.1 M Tris-HCl, pH 8.0 in equimolar proportions (Refsvik and Norseth, 1975).

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[1] Reference to trade names or products does not imply endorsement by the National Marine Fisheries Service, NOAA.

Purity of the thiol-methylmercury complexes was monitored by applying the complexes to 20x20 cm Silica Gel G Redi-Plates (Fisher Scientific; Norcross, GA). The plates were developed at room temperature in a n-propanol:25% ammonia (70:30) solvent system (Refsvik and Norseth, 1975). Visualization of methylmercury was accomplished by spraying with a 0.10% dithizone solution in chloroform (Iwata et al., 1981). To determine the extent of the complexation of the methylmercury by the thiols, each plate was divided into 1 cm sections, and the silica scraped and counted for ^{203}Hg in a Beckman 8000 gamma counter. The percent methylmercury complexed to each thiol was: glutathione 93%, homocysteine 95%, cysteine 91%, and cysteine-glycine 93%. The Rf values for the methylmercury complexes were: methylmercury 0.12, methylmercury-glutathione 0.44, methylmercury-homocysteine 0.56, methylmercury-cysteinylglycine 0.63, and methylmercury-cysteine 0.71.

Weanling female ICR mice, obtained from Sprague-Dawley (Indianapolis, IN), were maintained in a controlled environment under a 12-hour light-dark cycle. Animals were housed in polycarbonate cages containing shaved wood chips (Se < 0.1 μg/g) and fitted with stainless steel lids and polycarbonate water bottles. The mice were fed either selenium deficient (Se < 0.05 μg/g) or selenium control (Se 0.5 μg/g) diets obtained from Teklad Test Diets (Madison, WI) for 6 weeks before being used. Both selenium-deficient and selenium-control animals were healthy and gained weight equally throughout the experimental period. Diets and double-distilled deionized water were available ad libitum. The selenium status (liver selenium and blood GSH-peroxidase) of such animals has been reported (Balthrop and Braddon, 1985).

Myelin was isolated from the brains of selenium deficient and selenium control mice by the method of Norton and Poduslo (1973). Four brains were removed and homogenized in 15 ml of 0.32 M sucrose. The homogenate was spun at 44,000 x g for 60 min. The pellet was suspended in 0.32 M sucrose, layered over 0.85 M sucrose and spun for 60 min at 44,000 x g. The interfacial layer was removed and 2 volumes of H_2O were added to this layer before spinning at 44,000 x g for 60 min. The resulting pellet was washed once with H_2O and spun at 44,000 x g for 30 min. The purified myelin pellet was then brought up to 1 ml in 0.10 M Tris-HCl, pH 8.0 buffer.

The binding of the methylmercury-thiol complexes to mouse brain myelin was determined by incubating mouse brain myelin (0.46 mg protein) at 37° for 30 min in 100 mM Tris-HCl, pH 8.0 (1 ml) containing 3.2×10^{-4} to 3.2×10^{-6} M (nine concentrations in triplicate) CH_3Hg-thiol complexes. After incubation in a shaking water bath the tubes were centrifuged at 44,000 g for 60 min at 4°. The supernatant and pellet were separated and the tube drained and wiped free of supernatant. The radioactivities of the whole pellet and 0.5 ml of the supernatant were counted. The results were analyzed by use of Scatchard plots.

RESULTS AND DISCUSSION

The brains from both selenium control and selenium deficient mice showed no gross abnormalities and the protein content of the isolated myelin preparations were similar, 10.3 and 9.2 mg/ml, respectively.

The binding of uncomplexed methylmercury to the myelin proved to be uninterpretable using Scatchard analysis (Fig. 1). The binding of uncomplexed methylmercury to myelin yielded a Scatchard plot with two nearly horizontal lines; one line was formed at a high bound/free by the binding of up to 150 nmoles methylmercury/mg protein while the other was formed at a lower bound/free by the binding of greater than 150 nmoles methylmercury/mg protein.

On the other hand, Scatchard analyses of the binding of thiol complexes of methylmercury to myelin gave typical plots (Fig. 2) for data representing binding to more than one site. These data can be interpreted to represent two methylmercury binding sites on myelin, a high and a low affinity site. For the high affinity site the binding constants (k) for the thiol complexes ranged from 2.10×10^8 M^{-1} to 4.47×10^8 M^{-1} (Table 1). Whereas, for the low affinity site the binding constants ranged from 6.6×10^6 M^{-1} to 2.67×10^7 M^{-1} (Table 2). The data suggest that there is a major difference in the way the thiol-methylmercury complexes bind myelin when compared to uncomplexed methylmercury; however, there apparently are little if any differences when comparing the complexes to each other. Furthermore, the selenium status of the animal appears to have no influence on the binding constants (Tables 1 & 2).

The complexation in the brain of methylmercury to glutathione was demonstrated by the work of Thomas and Smith (1979). They showed that a glutathione-methylmercury complex appeared in the brain within minutes and accounted for almost one third of the cerebral methylmercury. Because this complex was not found in the plasma and only a trace was found in the erythrocytes they concluded that the formation of this methylmercury-thiol complex occurred in the brain. Our data suggest that glutathione and its metabolites, when complexed to methylmercury, have a high affinity binding site located on myelin found in the brain. These sites are likely to be the thiol groups located on the myelin proteolipid protein. On the other hand, uncomplexed methylmercury had less affinity for the myelin and appeared to bind in a nonspecific manner.

Previously reported binding studies using mercury have proven difficult to interpret. Scatchard plots of the data of Clarkson and Magos (1966) gave anomalous slopes. Their data yielded positive initial slopes, and they concluded that this difficulty arose due to the low concentration of free (diffusable) mercury. In fact, the mercury may even have been present in combination with several different binding substances found in the homogenate. Other studies by Weed et al. (1962) have observed similar anomalous Scatchard curves when measuring the binding of mercury to erythro-

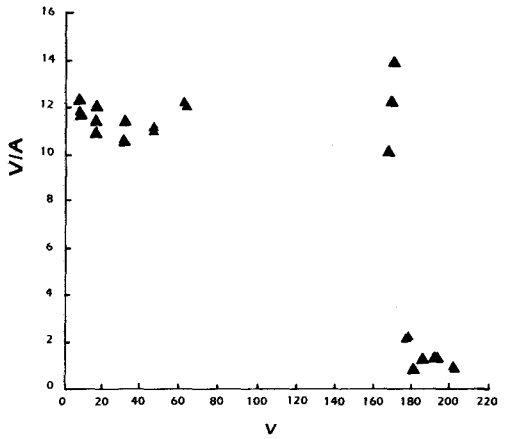

Figure 1 - Binding of methylmercury to purified brain myelin isolated from selenium fed mice.

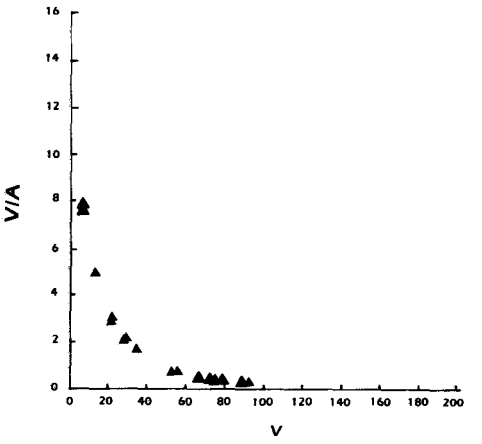

Figure 2 - Binding of a methylmercury-glutathione complex to purified brain myelin isolated from selenium fed mice. (The other methylmercury complexes gave similar results).

Table 1. Binding constants for the binding of methylmercury and methylmercury thiol complexes to the high affinity binding site on myelin.

	Se Control		Se Deficient	
	K (M^{-1})	n (nmoles/mg)	K (M^{-1})	n (nmoles/mg)
CH_3Hg	-2.60×10^6	4383	7.30×10^6	1387
CH_3Hg-GSH	3.78×10^8	26	3.06×10^8	31
CH_3Hg-CYSGLY	4.47×10^8	24	2.82×10^8	34
CH_3Hg-CYS	3.03×10^8	34	2.42×10^8	37
CH_3Hg-HOMOCYS	3.01×10^8	34	2.10×10^8	44

Table 2. Binding constants for the binding of methylmercury and methylmercury thiol complexes to the low affinity binding site on myelin.

	Se Control		Se Deficient	
	K (M^{-1})	n (nmoles/mg)	K (M^{-1})	n (nmoles/mg)
CH_3Hg	3.50×10^6	506	3.40×10^6	687
CH_3Hg-GSH	1.20×10^7	115	1.17×10^7	138
CH_3Hg-CYSGLY	1.00×10^7	148	8.50×10^6	214
CH_3Hg-CYS	6.60×10^6	226	7.90×10^6	227
CH_3Hg-HOMOCYS	2.67×10^7	88	2.13×10^7	117

cytes. They suggested that a release of mercury binding materials as the cause for such results. Fang and Fallin (1976) have reported that methylmercury binds to hemoglobin with a binding constant of 1.5×10^5. However, they found that methylmercury had only a weak ability to bind to albumin and globulin.

Other studies have investigated the binding of organometals to myelin. Lock and Aldridge (1975) reported that triethyllead and trimethyltin compete with triethyltin for the high affinity binding site on rat myelin. Their observed binding constants were approximately 6.6×10^5 M^{-1}. The binding constants we observed for the high affinity binding site for methylmercury thiol complexes ranged from 2.10×10^8 M^{-1} to 4.47×10^8 M^{-1}. However, the uncomplexed methylmercury had a high affinity binding constant in the range of -2.60×10^6 M^{-1} to 7.30×10^6 M^{-1}. Since the methylmercury-thiol complexes bind to myelin in a

different manner than uncomplexed methylmercury binds to myelin, it is possible that these mercury-thiol complexes are binding to sites normally occupied by sulfur-containing amino acids or proteins.

The effectiveness of selenium in modifying mercury metabolism has been well established. Results from previous work (Balthrop and Braddon, 1985) suggest that one of the interactions of selenium and methylmercury, which may be involved in the amelioration of methylmercury toxicity, occurs after ingestion of the metals when both are being metabolized in the liver. However, our data show that selenium in the diet does not influence the binding of methylmercury metabolites to myelin. These results lend support to the conclusions of Ganser and Kirschner (1985) that interactions of mercurials and myelin are insignificant during in vivo methylmercury intoxication. Thus, the ameliorative role of selenium in CH_3Hg intoxication cannot be explained through an effect on myelin binding of CH_3Hg.

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