The Binding of Various Mercurial Compounds to Serum Proteins

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Interaction of mercurial compounds with proteins and enzymes is generally considered the biochemical basis of their toxicological effects. The toxicity of mercuric ion is associated with the high affinity for thiol group of protein molecules. The binding characteristics of mercuric ion to native and chemically modified human serum albumin (Perkin, 1961), bovine serum albumin (Katz and Weisgerber, 1969; Katz and Samitz, 1973) and human hemoglobin (Barltrop and Smith, 1973) have been reported. Very little is known regarding the binding of organomercurial compounds to soluble proteins. Because the blood is responsible for the distribution of the ingested mercurial compounds throughout the body, knowledge of their binding characteristics to serum proteins is essential to the understanding of their behavior in mammalian system. This paper reports the binding of mercuric, aryl and alkyl mercury compounds to three serum proteins, and also to serum albumin from six mammalian species in order to gain better understanding of the pharmacodynamic behaviors of these compounds and their toxic effect to biological systems.

Materials and Methods

Methylmercuric chloride (MMC), ethylmercuric chloride (EMC) and phenylmercuric acetate (PMA) labeled with ^{203}Hg were prepared by an exchange reaction according to previously described procedures (Norseth and Clarkson, 1970; Fang and Fallin, 1973; Rao, Fallin, and Fang, 1966). The initial specific activity of these compounds was between one to two mCi/mM. The organomercurial compounds were first dissolved in absolute ethanol at a concentration of 1 mg/ml, and then further diluted to appropriate concentrations in Krebs-Ringer phosphate buffer, pH 7.4. The buffer contained in millimoles per liter approximately the following: Na^+, 170; K^+, 5; Ca^{2+}, 1; Mg^{2+}, 1; Cl^-, 122; HPO_4^{2-}, 24; and SO_4^{2-}, 1; giving an ionic strength of 0.182.

All proteins (human, horse, ovine, porcine, rabbit and bovine albumins fraction V powder; bovine hemoglobin, type 1 and bovine γ

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globulin, Cohn fraction II) were purchased from Sigma Chemical Company, and were dissolved in Krebs-Ringer phosphate buffer, pH 7.4, at a concentration of one or two mg/ml.

Radioactivity was either determined by liquid scintillation counting procedure using a solution described by Prockop and Ebert (1963) and a Packard Tricarb Model 314-X spectrometer, or a γ-scintillation spectrometer (Technical Associates Model SM-10) equipped with 2" well NaI(Tl) detector.

The binding of mercury to various proteins was studied using the equilibrium dialysis technique. Several series of six dialysis cells (1.0 ml capacity) fitted with a sheet of semipermeable membrane. The membrane (visking dialysis tubing) was treated with successive washes of 0.1 N HCl; 0.1 N NaOH, and finally boiling, distilled water to reduce the mercury binding (Barltrop and Smith, 1973). To the right side of the dialysis cells, a measured volume of proteins solution was introduced and to the left side of the cells an equal volume of mercurial solution in the same buffer was added. The concentration of mercurial solution varied from 1 x 10^{-6}M to 1 x 10^{-4}M. Generally, six different concentrations of each mercury compound were run at the same time. The filled dialysis cells were stoppered to prevent evaporation and allowed to stand for 72 hours at 3°C to insure that equilibrium was established. (Preliminary study showed that equilibrium was generally established within 48 hours.) After this time, duplicate 0.25 ml samples were removed from each side of cells and assayed for radioactivity. The binding of mercury compound to protein was evaluated from the difference in radioactivity between the right and left cells across the membrane. The results are presented as the number of moles of mercury compound bound per mole of protein. Scatchard equation ($R/A = nk - Rk$) was used to calculate the affinity and capacity values, where R = moles bound per mole of protein, A = the unbound concentration of mercury compounds (m), n = number of binding sites, and k = association constants (1949).

Results and Discussion

The results of these binding measurements between Hg^{2+}, PMA, EMC or MMC to bovine serum albumin, hemoglobin and γ-globulin are presented in Figures 1, 2, 3 and 4. At a free Hg^{2+} concentration of 3.5 x 10^{-5}M, the binding of Hg^{2+} was approximately two moles of Hg^{2+} per mole of bovine serum albumin or hemoglobin, and one mole per mole of γ-globulin. Scatchard plots strongly indicate two independent classes of binding sites for Hg^{2+} in bovine albumin and hemoglobin as suggested by a biphasic binding curve. The higher affinity portion of the binding curves extrapolates to give the number of the binding site to be 1 and 1.5 respectively for bovine serum albumin and hemoglobin. The total available binding sites are four for albumin and three for hemoglobin. Katz and Samitz (1973) studied the binding of Hg^{2+} to bovine albumin at pH 2 reported that Hg^{2+} binds to at least three sites per one molecule of protein. The primary site is thiol group which binds one mercury ion per molecule of albumin. This difference in total binding sites of bovine albumin as observed between our experiment and

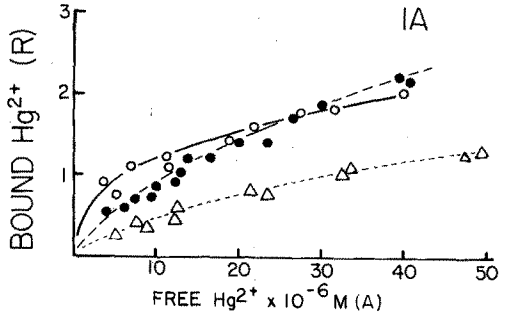

Figure 1. A. In vitro binding of $^{203}Hg^{2+}$ to bovine serum proteins. B. The Scatchard plot for the determination of association constant and the number of binding sites. ●---● albumin, O——O hemoglobin, Δ---Δ γ-globulin. R = moles bound per mole of protein.

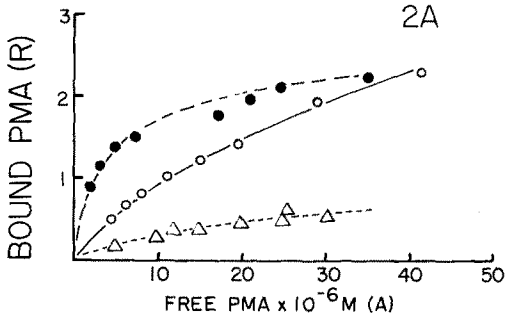

Figure 2. A. In vitro binding of ^{203}Hg-PMA to bovine serum proteins. B. The Scatchard plot. All symbols are similar as in Figure 1.

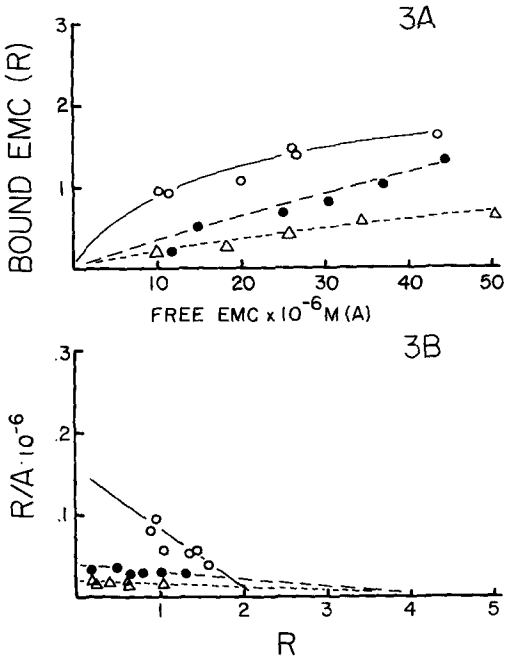

Figure 3. A. In vitro binding of ^{203}Hg-EMC to bovine serum proteins. B. The Scatchard plot. All symbols are similar as in Figure 1.

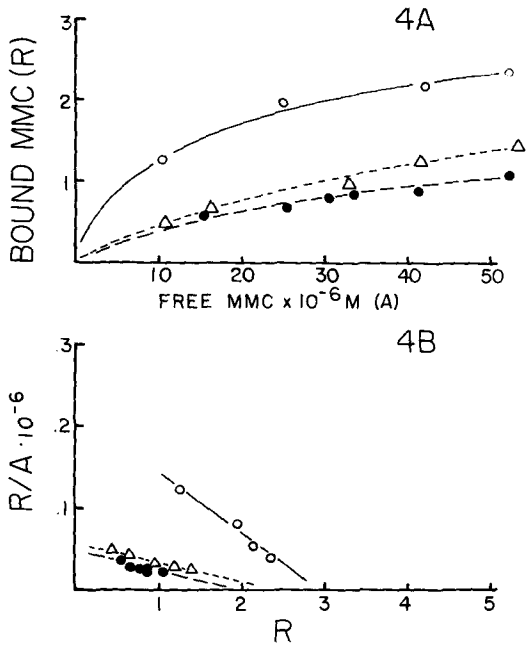

Figure 4. A. In vitro binding of ^{203}Hg-MMC to bovine serum proteins. B. The Scatchard plot. All symbols are similar as in Figure 1.

that of Katz and Samitz (1973) may be due to pH difference which effects on the structure of the protein or on the association of subunits (Webb, 1966). The value obtained for the number of Hg^{2+} binding to bovine hemoglobin molecule is in good agreement with those obtained by others (Ingram, 1955). However, the biphasic binding curve observed with bovine hemoglobin differs from that reported by Barltrap and Smith (1973) who used human hemoglobin. There were three low affinity binding sites in each γ-globulin molecule. The linearity of the plot in Figure 1B indicates that the binding sites are all in a single class.

The binding of PMA was also approximately two moles of PMA per mole of bovine serum albumin or hemoglobin at 3.5×10^{-5}M PMA (Figure 2A). In contrast to Hg^{2+} there was only a single class, low affinity binding site for PMA in the bovine hemoglobin. However, the total binding capacity in each molecule of hemoglobin was almost twice as much for PMA as for Hg^{2+}. In bovine serum albumin, two classes of binding sites were evident for the binding of PMA, with a total of four available sites per molecule of protein (Figure 2B). There were also three low affinity binding sites for PMA in each γ-globulin molecule. Very little differences in the affinity of binding and the total capacity between the binding of Hg^{2+} and PMA to γ-globulin were observed.

Both MMC and EMC showed only weak binding to either bovine serum albumin or γ-globulin (Figures 3 and 4). The binding capacity was three moles of alkyl mercury per mole of protein. On the other hand, the binding affinity of aklyl mercury to hemoglobin was much higher. This could be the reason for a high cell:plasma ratio when alkyl mercury was fed to rat and a low cell:plasma ratio when inorganic mercury was used (Ulfvarson, 1962; Takeda et al., 1968).

When the binding of these mercury compounds to serum albumins from six mammalian species were compared, it was evident that species differences existed. Both human and horse serum albumins showed to have only a single class of binding site for Hg^{2+}, while the other four species having two classes. Human serum albumin has the lowest binding affinity to mercury compounds. The bindings of MMC and EMC to serum albumins showed to have only low affinity sites. The total available binding sites varied between one to three moles per molecule of albumin. The association constant, k, for the binding of different mercury compounds to the specific sites of each individual serum proteins are listed in Table 1. A large difference in association constant between bovine albumin and albumin of other species was noted. Additional experiment was carried out with another bovine albumin sample. However, similar results were observed. It is assumed that protein structures and properties from different species may play an important role in determining the binding characteristics to various mercurial compounds.

Both MMC and EMC displayed only low affinity binding with asciation constant ranging from 2,500 in human and porcine albumins to 152,000 in hemoglobin. The binding of Hg^{2+} to most serum proteins were biphasic with the exception of human and horse albumins.

TABLE 1

In Vitro Binding of Mercury Compounds to Serum Proteins

Serum Proteins	Binding Site	Hg^{2+}		PMA		EMC		MMC	
		k	B	k	B	k	B	k	B
Bovine albumin	1	390,000	1.0	460,000	2.0	10,600	3.0	9,000	4.0
	2	26,000	2.0	53,000	1.8	--	--	--	--
Human albumin	1	15,000	4.0	100,000	1.8	2,500	2.0	3,000	2.0
Horse albumin	1	19,000	5.0	160,000	2.4	17,600	1.5	12,000	1.7
Ovine albumin	1	35,000	3.0	390,000	1.7	59,000	1.0	7,700	2.5
	2	17,000	2.0	--	--				
Porcine albumin	1	33,000	3.0	170,000	1.7	16,000	0.9	2,500	3.0
	2	13,000	2.0	--	--	--	--	--	--
Rabbit albumin	1	91,000	2.1	180,000	1.4	78,000	0.9	37,000	1.4
	2	27,000	2.2	--	--	32,000	1.5	5,000	1.6
Bovine hemoglobin	1	485,000	1.4	31,200	4.0	152,000	1.4	152,000	1.1
	2	53,000	1.6	--	--	--	--	--	--
Bovine γ-globulin	1	16,600	3.0	10,000	1.0	20,000	2.4	5,000	4.0

k = association constant, B = binding capacity

The first association constant ranged from 485,000 for the binding of hemoglobin to 15,000 for the binding of human serum albumin. The average binding affinity for Hg^{2+} to these serum proteins is as follows: hemoglobin $\geq$ albumin > γ-globulin; for PMA, albumin > hemoglobin > γ-globulin; and for MMC and EMC, hemoglobin > albumin > γ-globulin. This observation is in agreement with previous findings that the binding of soluble proteins varied with the forms of mercury (Ellis and Fang, 1971; Fang, 1973). At present we do not have the values of blood/brain or blood/fetus ratio from these species after exposure to various mercurial compounds, the findings of Casterline and Williams (1972) and Mansour et al. (1973) in rats revealed the relative ease of transfer of methylmercury across the placenta than did the inorganic mercury suggesting that their different behaviors may closely relate to their differences in protein binding.

Summary

Binding study of ^{203}Hg-labeled Hg^{2+}, PMA, MMC and EMC to serum albumin of six mammalian species, bovine hemoglobin and bovine γ-globulin is presented. Both MMC and EMC bound only weakly to serum albumin and γ-globulin and more strongly to hemoglobin; Hg^{2+} bound very strongly to both albumin and hemoglobin and weakly to γ-globulin; and PMA bound most strongly to albumin, next to hemoglobin and the least, to γ-globulin. The available binding sites varied from one to five per molecule of protein. Human serum albumin has the lowest association constants with all four mercurial compounds, indicating that it was not as tightly bound to mercurial compounds as found with serum albumins from other species.

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