Specific Ability of Sulfur-Ligands on Removal of ^{203}Hg -Labeled Organomercury from Hemoglobin in Comparison with Nitrogen-Ligands

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Removal of ^{203}Hg -labeled organomercurials, bound to sulfhydryl groups of hemoglobin, by various chelating agents was investigated by the use of equilibrium dialysis. Organomercurials employed were chlormerodrin, methylmercury, ethylmercury and phenylmercury compounds. The higher and more specific effects of the sulfur-ligands such as penicillamine and glutathione on removal of organomercurials were found as compared with those of the nitrogen-ligands such as EDTA, glycine and polymethylenediamines. The linear correlation was observed between the degree of organomercury elimination from hemoglobin and the stability constant ($\log K_1$) of 1:1 organomercury complex in both the sulfur- and nitrogen-ligand systems, and at the same value of $\log K_1$, the elimination-effect of sulfur-ligands was extremely greater than that of nitrogen-ligands. The relationship between the average percentage of removal and the Taft's polar substituent constant of organic moiety of the metal was also linear among the organomercury compounds other than chlormerodrin. The average removal percentage by sulfur-ligands increased in the order, ethylmercury > methylmercury > phenylmercury, while that by nitrogen-ligands was not different among organomercurials investigated. In addition, the direct ligand-exchange reaction between hemoglobin-SH and the ligand coordinating-atom (S or N) against organomercurials rather than S_N reaction via the ternary complex, hemoglobin-S-RHg-ligand, is postulated.

1. Introduction

The pollution of environment caused by short-chain alkyl mercurials, which damage brain and nervous system seriously, has become a great menace to living organisms. Organomercury is transported in the body by blood, where organomercury is kept for long period in high concentration bound to sulfhydryl group of hemoglobin¹⁾ and this binding retards the entry of organomercury into brain²⁾. The diffusion of the short-chain alkyl mercurials from the red blood cell is thought to be the rate-determining step in the transport of mercury from blood to tissues, at least with regard to uptake by brain. In this transport, diffusible complexes of mercury released from red blood cell into plasma play an important role³⁾. The

diffusible complex has shown to be similar to that in bile which is probably a complex of organomercury with a thiol-containing dipeptide⁴⁾. Mechanism of the transport of organomercury, however, is hardly clarified. Furthermore, the interaction of several radioactive scanning agents containing radioactive mercury, such as chlormerodrin and neohydrin, with reticulocyte has become a problem in recent years in connection with the toxicity of mercury and exposure to radiant energy⁵⁾. Although penicillamine and other thiol compounds are frequently used as antidote against the organomercury-poisoning, their detoxicating mechanism remains almost unknown.

We have already reported that chelating agents containing sulfhydryl group were more effective in removal of inorganic mer-

cury and ethylmercury compounds bound to hemoglobin than nitrogen-containing agents such as methionine and EDTA. A ternary complex, hemoglobin-S-mercury-cysteine, was found to be an important intermediate in the removal of inorganic mercury by cysteine⁶⁾. These results had been expected from the facts that a low molecular ternary complex, sulfur ligand-mercury-sulfur ligand complex, is formed, whereas a similar ternary complex, sulfur ligand-mercury-nitrogen ligand complex, is not formed⁷⁾. The reaction mechanism for ethylmercury elimination from hemoglobin was not discussed. We have showed also from pH titration study that in the presence of excess ligands, the 1:1 organomercury complex with ligand is formed and the 1:2 complex is not observed⁸⁾. The stability constant of the organomercury complex decreased in the order, methylmercury-sulfur ligand > phenylmercury-nitrogen ligand $\geq$ phenylmercury-sulfur ligand > methylmercury-nitrogen ligand $\gg$ methylmercury-and phenylmercury-oxygen ligands. In the paper, the ability of various ligands in removal of various organomercurials from hemoglobin is investigated by the equilibrium dialysis method, and the correlation between the stability constant of organomercury complexes and the elimination ability of these ligands is discussed.

2. Experimental

Penicillamine, cysteine, glutathione, 2,3-dimercapto-1-propanol(BAL), thiomalic acid, thiolactic acid, N-acetylcysteine, thioglycol, glycine, S-methylcysteine, ethylenediamine-N,N,N',N'-tetraacetic acid disodium salt (EDTA) and polymethylenediamine dichloride $\text{NH}_2(\text{CH}_2)_n\text{NH}_2 \cdot 2\text{HCl}$ (abbreviated as N2, N3, N4, N5, N6 and N12 for $n=2, 3, 4, 5, 6$ and 12 hereafter) were commercial reagent grade materials and purified whenever necessary. Radio alkyl-mercury compounds, namely ^{203}Hg -methylmercuric chloride (MeHg), ^{203}Hg -ethylmercuric chloride (EtHg) and ^{203}Hg -phenylmercuric acetate (PhHg), and ^{203}Hg chlormerodrin (ChHg) were purchased from The Radiochemical Centre, England and

Daichi Radioisotope Laboratories, Japan. Bovine hemoglobin was obtained from the Mann Research Laboratories and almost 100 % purity was confirmed by electrophoresis. Michaelis buffer (1/30M potassium phosphate-1/30M sodium phosphate) and 0.05M borate buffer were employed for pH 6.9 and pH 9.4, respectively.

After 10 ml of $5.0 \times 10^{-5}\text{M}$ hemoglobin solution containing $5.0 \times 10^{-5}\text{M}$ organomercury- ^{203}Hg (specific activity: 73 mCi/g) was poured into Visking tube, equilibrium dialyses were carried out at 22° with 50 ml of the outer buffer solution containing $1.0 \times 10^{-3}\text{M}$ ligand. Radioactivity of ^{203}Hg in outer solution was measured by well type NaI scintillation counter. The percentage of each organomercury compound removed from hemoglobin by various ligands was calculated as follows:

$$\frac{(\text{Activity in the presence of ligand}) - (\text{Activity in the complete removal of metal})}{(\text{Activity in the absence of ligand}) - (\text{Activity in the absence of ligand})} \times 100$$

3. Results and Discussion

Organomercury compounds used were found to bind to hemoglobin almost quantitatively in the absence of ligand. Percentage of organomercury removed from hemoglobin by cysteine, reached to constant value at the ten-fold molar concentration of the ligand in 15 hours dialyses. Therefore, the twenty-fold concentration of ligand to the metal and the dialysis time of 24 hours were employed in the following experiments.

Removal of organomercury bound to hemoglobin by sulfur- or nitrogen-ligands was investigated at pH 6.9 and 9.4, and the results are presented in Table 1. All the sulfur-ligands used were remarkably effective in removal of organomercury. In contrast, the nitrogen-ligands involving no sulfhydryl group had little effect. This observation agrees with the results in the case of inorganic mercury reported previously⁶⁾. At pH 9.4, the removal of methylmercury compound was increased by the nitrogen-ligands as compared with that at pH 6.9, but not by the sulfur-ligands. The pH effect seen in the nitrogen-ligand system is attributed to

Table 1 Percentage* of organomercury removed from hemoglobin by ligands

Ligand		pH 6.9				pH 9.4
		MeHg	EtHg	PhHg	ChHg	MeHg
S, N, O	Penicillamine	83	85	83	79	78
	Cysteine	80	103	72	81	91
	Glutathione	97	90	84	86	81
S, S, O	BAL	71	67	34	62	
S, O	Thiomalic acid	98	98	95	86	101
	Thiolactic acid	100	91	100	93	
	N-Acetylcysteine	90	95	91	80	89
	Thioglycol	93	99	93	88	
N, O	Glycine	3	0	1	0	13
	S-Methylcysteine	5	1	2	1	14
N, N	EDTA	5	1	2	0	20
	N 2	5	2	1	0	12
	N 3	3	1	0	0	14
	N 4	1	1	3	4	21
	N 5	5	0	1	2	14
	N 6	4	2	1	0	14
	N 12	1	2	1	1	16

* maximal value within 24 hours after dialyses.

the optimal condition for complex formation in the alkaline region. The unexpected small effect of BAL may be due to its chemical instability in aqueous solution.

Fig. 1 represents a plot of the removed percentage of methyl- and phenylmercuries by various ligands against the stability constants of the 1:1 organomercury-ligand complexes ($\log K_1$). The linear correlation—linear increase of removed percentage with increase of $\log K_1$ —was found in both the sulfur- and nitrogen-ligand systems at pH 6.9 and pH 9.4. This fact suggests the same type of the reaction in the sulfur- and nitrogen-ligand systems, respectively. The much greater elimination-effect of sulfur-ligands than that of nitrogen-ligands as seen in Table 1 don't seem to be due to the larger stability constant of the former's organomercury complexes, because at the same value of $\log K_1$, the effect of sulfur-ligands was extremely higher than that of nitrogen-ligands.

Fig. 2 shows a relationship between the average percentage of organomercury removal

Fig. 1 Correlation between organomercury removal (%) and stability constant of organomercury complex ($\log K_1$).

I (○—○) : MeHg, sulfur-ligand, pH 6.9
 II (●---●) : MeHg, sulfur-ligand, pH 9.4
 III (△····△) : PhHg, sulfur-ligand, pH 6.9
 IV (○—○) : MeHg, nitrogen-ligand, pH 6.9
 V (●---●) : MeHg, nitrogen-ligand, pH 9.4
 VI (△····△) : PhHg, nitrogen-ligand, pH 6.9
 $K_1 = \frac{[MLH]}{[M][LH]}$ except for N2, N3 and EDTA complexes, where
 $K_1 = \frac{[ML]}{[M][L]}$. M and LH_2 represent metal and diprotonated ligand, respectively.

Fig. 2 Correlation between organomercury removal (%)^{a)} and Taft's polar substituent constant of metal's organic part (σ^*).

VII : sulfur-ligand; VIII : nitrogen-ligand
 ○ : MeHg, pH 6.9; ● : MeHg, pH 9.4;
 △ : EtHg, pH 6.9; ▲ : PhHg, pH 6.9;
 × : CHg, pH 6.9

a) average value in sulfur- and nitrogen-ligand systems, respectively.

al and the Taft's polar substituent constant (σ^*) of organic moiety of the metal^{8,9)}. Recently, it was found that $\log K_1$ value increases linearly with the increase and decrease of σ^* values in the organomercury complexes with the nitrogen- and sulfur-ligands, respectively⁹⁾. Therefore, σ^* values can be used as a measure of the relative stability constant of 1:1 organomercury-ligand complex among various organomercury compounds. The relation between the average percentage of removal and σ^* value in the sulfur- and nitrogen-ligand systems, respectively, was linear among the organomercury compounds except chlormerodrin, in which its steric hindrance and the weak interaction of its polar group with hemoglobin may be responsible in the decrease of the removal. The correlation indicates the same type of the reaction among organomercurials. The average percentage of organomercury removed by sulfur-ligands increased in the order, ethylmercury > methylmercury > phenylmercury, while that by nitrogen-ligands was not different among organomercurials.

From the results of the pH titration, we

have already showed that organomercury such as methyl- and phenylmercuries don't form 1:2 complexes with the sulfur- and nitrogen-ligands⁹⁾. This excludes the possibility of the formation of ternary complex, hemoglobin-organomercury-ligand complex, as the intermediate in removal of organomercury from hemoglobin by these ligands. Furthermore, it has been reported that in ligand exchange of the complex (MeHgCN) with the complex (MeHgSCH₂COO), S_{N2} mechanism via the transition state (NC-MeHg-SCH₂COO) seems to be inadequate to account for the fast rates and so that the direct exchange mechanism between MeHgCN and the mercaptoacetate complex is reasonable¹⁰⁾.

From the above-mentioned results, in removal of organomercury bound to hemoglobin by the sulfur- and nitrogen-ligands, it is reasonable to assume the direct ligand-exchange reaction between hemoglobin-SH and ligand coordinating-atom (S or N) against organomercury rather than S_{N2} reaction via the ternary hemoglobin-S-organomercury-ligand complex as an intermediate, in contrast with the case of removal of inorganic mercury compound.

The difference in the removal mechanism between organic and inorganic mercuries seems to be reasonable because the stability constant of 1:1 organomercury complex, particularly with the sulfur-ligands, is much smaller than that of the corresponding inorganic mercury complex¹¹⁾. The higher effect of the sulfur-ligands compared with that of the nitrogen-ligands on removal of organomercury, can be attributed to the greater facility of direct exchange reaction between

hemoglobin-SH and the sulfur-ligand, which contain the sulfur-donor as the same coordinating-atom to organomercury. The stability constant of organomercury complex is considered to participate not in the key step—direct exchange step—but in stabilization after the key step. These results may give an useful information on the mechanism of the transport of organomercury from blood to tissues and of detoxicating action of sulfur-containing ligand against organomercury-poisoning.

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要 旨

ヘモグロビン結合 ^{203}Hg -有機水銀の除去反応：窒素含有配位子との比較における硫黄含有配位子の特異的効果

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ヘモグロビンのSH基に結合した ^{203}Hg -有機水銀の、種々のキレート剤による除去反応を平衡透析法によって検討した。有機水銀化合物として、クロルメロドリン、メチル水銀、エチル水銀、フェニル水銀化合物を用いた。EDTA、グリシン、ポリメチレンジアミンなどの NH_2 含有配位子とくらべて、ペニシラミン、グルタチオンなどのSH含有配位子はきわめて高く、かつ特異的な有機水銀除去効果を示した。有機水銀除去率と1:1有機水銀—配位子錯体の安定度定数との間の比例的な関係がSH配位子群と NH_2 配位子群のおのおのにおいて認められ、さらに有機水銀錯体の安定度定数が同じ場合、SH配位子の除去効果は NH_2 配位子のそれよりも極端に大きかった。また、SH配位子群と NH_2 配位子群のおのおのにおける、平均除去率と各金属の有機部分の極置換基定数との間にも直線的関係が見られた。SN配位子の場合の平均除去率は、エチル水銀>メチル水銀>フェニル水銀の順に増加したが、 NH_2 配位子の場合は有機水銀によって違いはなかった。これらの結果に基づいてヘモグロビン-SHと配位子原子(SまたはN)との間で配位子が有機水銀に対して直接交換される機構が推定された。