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Action of Thiol-containing Chelating Agents in the Removal of Mercury Bound to Hemoglobin

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Introduction

The mercurial compounds are of toxicological importance [1], especially as environmental pollutants. They are able to inactivate thiol-containing enzymes even in low concentrations, and thus interfere with cellular metabolisms and functions.

Thiol-compounds have a high affinity for mercury and provide the basis of therapeutic treatment for mercury poisoning with thiol-containing ligands [2]. According to Pearson's theory of hard and soft acids and bases, the coordination of sulfur atom in the ligand toward mercury ions should be favored, since thiols and thiolate anions belong to soft bases and mercurous and mercuric ions are soft acids [3].

In this paper, we have investigated the removal of Hg(II) ions bound to hemoglobin by various thiol-containing chelating agents, especially cysteine, 2-mercaptoimidazole and 2-mercaptohistamine. 2-Mercaptohistamine (2-MH) possessing a mercaptoimidazole and an amino group, has a high stability constant to Hg(II) ion in complexation [4]. As one of the reasons for the high stability of 2-MH-Hg(II) complex, it was postulated that the contribution of thiol form of 2-MH was strongly favorable in the acid dissociation process [5].

Materials and Methods

2-Mercaptohistamine hydrochloride (2-MH) was prepared by the method of Fraser [6] and was used after recrystallization from 80% acetic acid, mp. 241–2° (lit. mp. 244–5° [6]). 2-Mercaptoimidazole (MI), recrystallized from water, L-ergothioneine and glutathione (reduced form) were obtained from Koch-Light Laboratories Ltd., Sigma Chemical Company and Kohjin Co Ltd., respectively. α-Mercaptopropionylglycine was a gift from Santen Seiyaku Co. Other chelating agents used in this experiment were obtained from Nakarai Chemicals Ltd. Bovine hemoglobin was obtained from Sigma Chemical Company. The seamless cellulose tubing was obtained from Visking Company.

A typical removal experiment was carried out as follows: the seamless cellulose tubing (8/32) containing the solution (2 ml) of 3 m*M* $HgCl_2$ and 0.3 m*M* bovine hemoglobin was dialyzed against 50 ml of 1/15 *M* phosphate buffer, pH 6.90, containing 50 times as much chelating agent (6 m*M*) as the amount of $HgCl_2$ with mechanical stirring. All experiments were carried out at 22°. The amount of Hg(II) ions removed from protein into buffer solution was determined by analyzing 10 μl of the outer solution with a Hitachi Zeeman Effect Mercury Analyzer 501. The percentage of mercury removed from hemoglobin was calculated. Other detailed experimental conditions are in the legends to figures.

Results and Discussion

The number of binding sites of Hg(II) ions on hemoglobin is considered to be two at pH 7, and in spite of the dissociation or denaturation of hemoglobin the number is retained or increased but not decreased [7]. In our experiment the amount of Hg(II) ions removed could be neglected in the control which contained no chelating agents, hence Hg(II) ions are bound tightly to hemoglobin molecules.

In order to fix the experimental conditions the following experiments were carried out. Figure 1

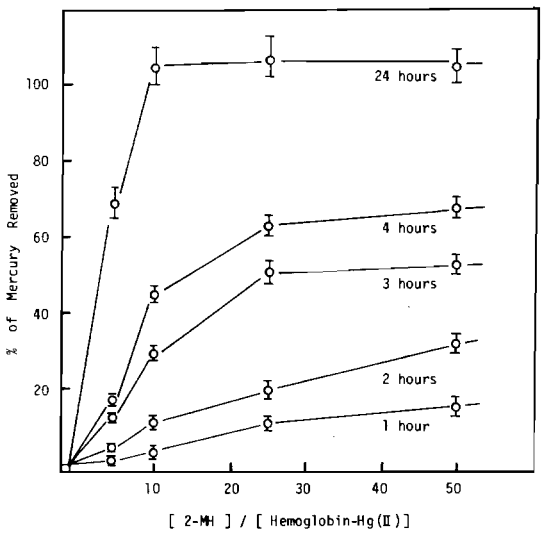

Fig. 1. Concentration and Reaction-Time Dependences of 2-Mercaptohistamine on the Removal of Mercury Bound to Hemoglobin at pH 6.90. Concentrations of hemoglobin and mercuric chloride are 0.3 m*M* and 3 m*M*, respectively. The vertical bars are the standard deviation of the mean of five determinations.

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TABLE I. The Ability of Various Chelating Agents in Removal of Mercury Bound to Hemoglobin at pH 6.90.[a]

Chelating Agents	% of Mercury Removed					
	1 hr	2 hr	3 hr	4 hr	5 hr	24 hr
Buffer	0	0	0	0	0	0
2-Mercaptoimidazole	42.93 ± 3.40	50.09 ± 1.89	66.17 ± 2.24	95.15 ± 10.52	114 ± 8.56	119 ± 6.7
L-Cysteine	42.22 ± 0.58	91.58 ± 3.87	98.70 ± 3.44	98.99 ± 1.87	99.48 ± 3.42	98.48 ± 3.42
2-Mercaptohistamine	15.21 ± 1.06	32.89 ± 1.84	52.62 ± 1.77	69.11 ± 4.23	77.19 ± 3.81	101.48 ± 2.72
DL-Penicillamine	35.98 ± 2.28	47.76 ± 2.89	61.94 ± 1.81	94.28 ± 4.32	97.39 ± 9.04	88.15 ± 3.02
Thiosalicylic acid	7.27 ± 0.08	18.54 ± 0.72	27.65 ± 0.50	38.82 ± 1.37	55.20 ± 1.14	39.18 ± 2.18
α-Mercaptopropionylglycine	18.00 ± 0.90	48.72 ± 1.81	68.10 ± 1.04	85.38 ± 6.97	92.31 ± 5.79	119 ± 1.7
L-Ergothioneine	12.77 ± 0.55	25.37 ± 0.47	35.91 ± 1.22	63.81 ± 4.43	64.37 ± 2.44	79.26 ± 2.28
Glutathione	14.68 ± 0.45	28.89 ± 1.77	39.01 ± 2.10	42.52 ± 1.64	62.33 ± 1.71	85.33 ± 10.64
EDTA	4.32 ± 0.30	4.93 ± 0.33	8.85 ± 0.49	11.47 ± 1.07	12.42 ± 0.33	29.60 ± 0.92
Glycine	0	0	0.69	0.46	0.46	0.97 ± 0.23
Histamine	1.73 ± 0.33	2.33 ± 0.49	5.20 ± 0.19	7.80 ± 0.18	9.03 ± 0.16	24.81 ± 0.50
Imidazole	0.92	4.31	6.15	7.69	8.92	43.69 ± 3.03

[a] Each datum is the average and standard deviation of five determinations in mercury analyses.

shows that complete removal was obtained when the molar ratio of 2-MH to Hg(II) ions bound to hemoglobin and the incubation times were over 10 and 24 hours, respectively. In addition, pH dependency was observed, namely, the removal of Hg(II) was increased with raising the pH of the solution but decreased at near pH 8, with the optimal pH region being at physiological pH value (Fig. 2).

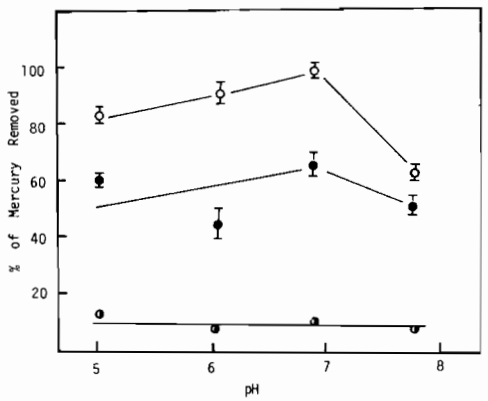

Fig. 2. pH Dependence on the Removal of Mercury Bound to Hemoglobin by 2-mercaptohistamine. Reaction time: ○ 1 hr, ● 4 hr, ◑ 24 hr. Concentrations of hemoglobin, mercuric chloride and 2-mercaptohistamine are 0.3 m*M*, 3 m*M* and 6 m*M*, respectively. The vertical bars are the standard deviation of the mean of five determinations.

From these results, all removal experiments were carried out at amounts of chelating agents 50 times that of Hg(II) ions bound to hemoglobin at pH 6.90 against 1/15 *M* phosphate buffer.

Table I shows clearly that the ability of such thiol-containing chelating agents as 2-MH, MI, L-cysteine, DL-penicillamine, α-mercaptopropionylglycine, L-ergothioneine, glutathione and thiosalicylic acid were superior to non-sulfur containing chelating agents in both the removal rate and the amount of Hg(II) ions removed. Only thiosalicylic acid showed relatively low activity, probably owing to its low solubility in buffer solution.

Among all chelating agents tested in this experiment, the effect of the ligands was decreased in the order of SNN ≈ SNO > SO > NO ≈ NN for the donor set of chelating agents. This result agrees well with the theoretical consideration that sulfur atom as a soft base is easy to bind with Hg(II) ions as a soft acid according to the rule of Pearson's HSAB (Hard and Soft Acids and Bases) theory [3]. This theory is based on the strength or tightness of the complex formed between metal ion and chelating agent. The thiol-containing chelating agents used in our experiment have high stability with Hg(II) in complexation, ranging about 8–40 as $\log K_1$ values [4, 10–13]. However, EDTA and glycine have also relatively high stability with Hg(II) ion (EDTA: $\log K_1$ = 22.1 [8], glycine: $\log K_1$ = 9.44 [9]). Therefore, we could not choose the stability constants of the chelates as a main parameter affecting the removal of mercury ions from protein, because the stability constants reported hitherto had a variety of values depending on the method applied. A similar result was observed on the removal of copper ion from plasma and ceruloplasmin, in which the effects of the chelating agents roughly paralleled their copper binding ability [14]. The authors, therefore, suggested the presence of some other mechanisms involving chelation.

As another factor affecting the removal of Hg(II) ions from hemoglobin bound Hg(II), we suppose herein the effect of molecular weight of chelating agents: the aliphatic thiol-containing chelating agents of low molecular weight could remove Hg(II) ions with relatively better efficiency than the higher molecule such as glutathione in early reaction times (Fig.

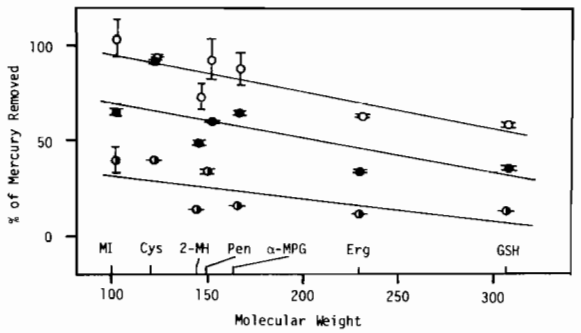

Fig. 3. Relationship between Removal Effect and Molecular Weight of Aliphatic Thiol-containing Chelating Agents. Data are obtained from Table I. Reaction time: ○ 1hr, ● 3 hr, ◑ 5 hr. Abbreviations in this figure include: MI, mercaptoimidazole; Cys, cysteine; 2-MH, 2-mercaptohistamine; Pen, penicillamine; α-MPG, α-mercaptopropionylglycine; Erg, ergothioneine; GSH, glutathione.

3). But non-sulfur containing chelating agents failed to show this tendency.

On the basis of these data, it was indicated that a compound containing -SH and -NH_2 or -COOH group of molecular weight below 200 promotes the removal of mercury ions from protein. 2-Mercaptohistamine having a donor set of sulfur and nitrogen and a molecular weight of 144 showed a relatively low effectiveness in removal of Hg(II) ions at short reaction times; however, after 24 hours it removed Hg(II) ions completely. Therefore, it can be proposed that this compound can be a new antidote for mercury poisoning. 2-Mercaptohistamine had no acute toxicity when it was injected i.p. at the dose of 3.6 mg/mouse, suggesting a possibility as a tool of medication.

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