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Studies on the Sulfur-containing Chelating Agents. XLV.[1] Kinetics of Ligand Exchange Reaction of Mercury-Monothiodibenzoylmethane Complex with Some Thiol Compounds by Stripping Solvent Extraction

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The kinetics of ligand exchange reaction of mercury-monothiodibenzoylmethane complex with various thiol compounds (THIOL) is studied by stripping solvent extraction method. Mercaptoethylamine (MEA), penicillamine (Pen), cysteine (Cys), glutathione (GSH), α-mercaptopropionic acid (α-MPA), β-mercaptopropionic acid (β-MPA), N-acetylcysteine (NAC) and thiomalic acid (TMA) are used as thiol compounds. The reaction rate is determined by the measurements of the radioactivity of ^{203}Hg in the aqueous phase. Thiol compounds were classified into two types based on their reaction rates, and in these reactions the formations of the mixed ligand complexes are considered to be involved. The observed reaction rates at physiological pH are distinctly higher in the thiols with amino group than in other thiols.

The analysis of the reaction of mercury with thiol compounds may be of significance for the interpretation of the behavior of mercury in the analytical procedure as well as in the biological system. This paper deals with a kinetic study of the reaction of mercury with various water-soluble thiol compounds (abbreviated as THIOL hereafter), which have been used as the analytical reagents[3] or the antidotes[4] for the heavy metal poisonings. We adopted the solvent-extraction method based on its usefulness indicated in the previous papers.[1,5] Monothiodibenzoylmethane (abbreviated as SBB hereafter) which is regarded as the most advantageous extraction reagent,[6–8] for mercury among various monothio-β-diketones[9] was chosen as a reagent for the solvent extraction. The kinetics of the ligand exchange reaction of Hg-SBB complex with THIOL was investigated by the stripping solvent extraction method.

Experimental

Materials——Reagent grade Hg $(OAc)_2$ was used to prepare approximately 1.0×10^{-3} M solution, which was standardized by EDTA titration. When this solution was diluted to proper concentration of mercury, trace amount of radioactive ^{203}Hg from The Radio Chemical Center in the form of Hg $(OAc)_2$ was added. SBB was synthesized and purified by the method reported previously.[10] Reagent grade cysteine, penicillamine, mercaptoethylamine, thiomalic acid, N-acetylcysteine, α-mercaptopropionic acid, β-mercaptopro-

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[1] Part XLIV: N. Nakanishi, A. Yokoyama, and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo) 23, 1677 (1975).

[2] Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto.*

[3] H. Tanaka, Y. Sugiura, K. Seki, K. Sakaguchi, and A. Yokoyama, *Bunsekikagaku*, **17**, 1309 (1968).

[4] E.D. Weinberg, "Metal-Binding in Medicine," Lippincott, Philadelphia, 1960; Y. Sugiura and H. Tanaka, *Radioisotopes*, **19**, 7 (1970).

[5] P.R. Subbaraman, M.C. Sister, and H. Freiser, *Anal. Chem.*, **41**, 1878 (1969); I.P. Alimarin, Y.A. Zolotov, and V.A. Bodnya, *Pure. Appl. Chem.*, **25**, 667 (1971); T. Sekine and Y. Komatsu, *Bunsekikagaku*, **23**, 222 (1974).

[6] A. Yokoyama, N. Nakanishi, and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **20**, 1856 (1972).

[7] H. Tanaka, N. Nakanishi, Y. Sugiura, and A. Yokoyama, *Bunsekikagaku*, **17**, 1428 (1968).

[8] E. Uhlemann, B. Schuknecht, K.D. Busse, and V. Pohl, *Anal. Chim. Acta*, **56**, 185 (1971).

[9] M. Cox and J. Darken, *Coord. Chem. Rev.*, **7**, 29 (1971); S.E. Livingstone, *Coord. Chem. Rev.*, **7**, 59 (1971).

[10] A. Yokoyama, S. Kawanishi, M. Chikuma, and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **15**, 540 (1967).

pionic acid and glutathione shown in Chart 1 were chosen as THIOL for this study because they have high affinity to mercury and are insoluble in $CHCl_3$. Their aqueous solutions were prepared freshly at each experiment. Acetate buffer solution was used and the ionic strength of the solution was adjusted to 0.1 by $NaNO_3$. After being shaken with water, $CHCl_3$ was distilled and saturated with buffer solution before use. Water was purified by distillation and passing through an ion exchange column.

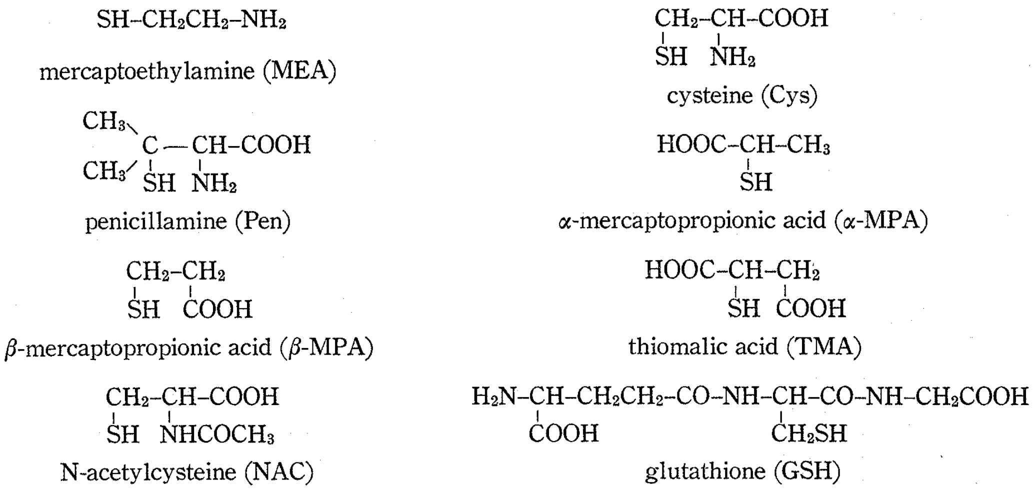

Chart 1. Structure of Thiol Compounds

Apparatus——All solutions were prepared in 500 ml three necked flask and stirred vigorously by Yamato Labo, Stirrer with Tokyo Rika Kikai Vacuum Stirrer at 25.0°±0.5°. The flask was dipped in water bath with thermostat. Measurements of radioactivity were made with a Fujitsu well-type scintillation counter Model ATS-121 for a period of time nesessary to obtain total counts of at least 10000. A Hitachi-Horiba Model F-5 type pH meter was used for pH measurements.

Procedure——An aliquot of 75 ml of the solution containing radioactive Hg $(OAc)_2$ and buffer solution was stirred with a 100 ml aliquot of $CHCl_3$ solution of SBB in the three necked flask for 10 min. Hg is completely extracted into $CHCl_3$ phase in the form of Hg $(SBB)_2$. A 2 ml portion of $CHCl_3$ phase was taken into a plastic tube for the counting of the radioactivity, $\{A_{Hg}\}_T$. Then 25 ml aliquot of THIOL solution was added to the flask and the solution was stirred. At regular time intervals, 3 ml of the aqueous phase was taken and allowed to stand for 10 min, and its 2 ml portion was taken into a plastic tube for the counting of the radioactivity $\{A_{Hg}\}_a$, and the stirring of the solution was continued.

The concentration of mercury in the $CHCl_3$ phase was expressed as equation (1).

$$[Hg]_0 = \frac{\{A_{Hg}\}_T - \{A_{Hg}\}_a}{\{A_{Hg}\}_T}\{Hg\}_T \qquad (1)$$

$[Hg]_0$: mercury concentration in the organic phase
$[Hg]_T$: total mercury concentration in the aqueous and organic phases.

The relation of the stirring speed and the radioactivity in the aqueous phase was checked and the stirring speed was set up so as to obtain constant extraction rate.

Results

Dependence on the Concentration of Mercury in Organic Phase

The reaction is first order with respect to the concentration of mercury-SBB complex in the organic phase. Fig. 1 gives an example of the results. A pseudo first order rate constants, k_{obs}, is determined from the slope of the line.

Dependence on pH

Pseudo first order rate constants were determined at pH 3—5. As shown in Fig. 2, in the cases of MEA, Pen, and Cys, k_{obs} *vs.* $1/[H^+]$ plots give straight lines which intercept the k_{obs} axis through the origin. Whereas, in the case of GSH, the similar plots give the straight line which doesn't intercept through the origin. In the cases of α-MPA, β-MPA, NAC and TMA, as shown in Fig. 3, k_{obs} *vs.* $1/[H^+]$ plots are parallel to $1/[H^+]$ axis. In the case of TMA, however, k_{obs} value distinctly changes at about pH 4.

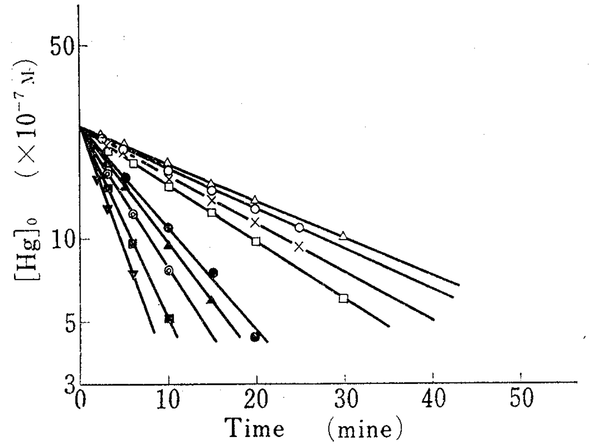

Fig. 1. Relationship between log $[Hg]_0$ and Reaction Time

△: TMA $=2.5\times10^{-5}$M, pH 4.82
○: GSH $=5.0\times10^{-5}$M, pH 4.67
×: MEA $=5.0\times10^{-5}$M, pH 3.97
□: Pen $=5.0\times10^{-5}$M, pH 4.10
●: Cys $=1.0\times10^{-4}$M, pH 5.44
▲: NAC $=5.0\times10^{-5}$M, pH 3.90
⊙: β-MPA $=2.5\times10^{-5}$M, pH 4.65
■: TMA $=2.5\times10^{-5}$M, pH 3.52
▼: α-MPA $=2.5\times10^{-5}$M, pH 4.86
($Hg_T=2.5\times10^{-6}$M, $SBB_T=2.0\times10^{-4}$M)

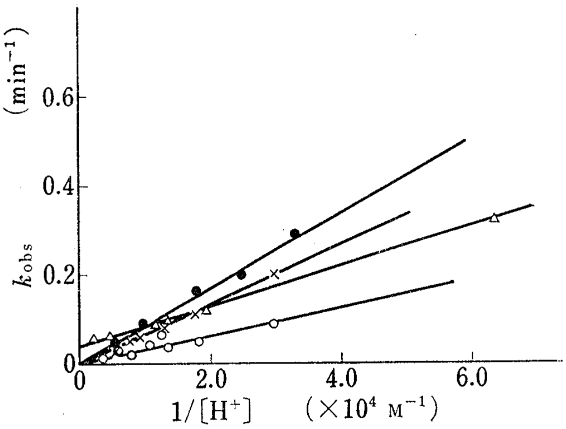

Fig. 2. Relationship between k_{obs} and $1/[H^+]$

●: MEA $=1.0\times10^{-4}$M ×: Pen $=1.0\times10^{-4}$M
○: Cys $=1.0\times10^{-4}$M △: GSH $=1.0\times10^{-4}$M
($Hg_T=2.5\times10^{-6}$M, $SBB_T=2.0\times10^{-4}$M)

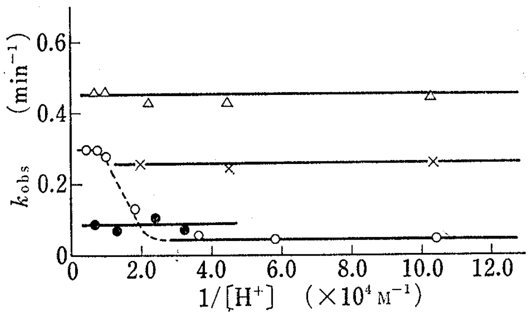

Fig. 3. Relationship between k_{obs} and $1/[H^+]$

△: α-MPA $=5.0\times10^{-5}$M ●: NAC $=5.0\times10^{-5}$M
×: β-MPA $=5.0\times10^{-5}$M ○: TMA $=5.0\times10^{-5}$M
($Hg_T=2.5\times10^{-6}$M, $SBB_T=2.0\times10^{-4}$M)

Dependence on the Concentration of THIOL in the Aqueous Phase

As shown in Fig. 4, k_{obs} *vs.* $[THIOL]_a$ plots give the straight line which intercepts the origin.

Dependence on the Concentration of SBB in the Organic Phase

As shown in Fig. 5, k_{obs} *vs.* $[SBB]_0$ plots are parallel to the $[SBB]_0$ axis.

Discussion

From above results, in the range of pH 3 to 5, the rate of the decrease in the concentration of mercury-SBB complex in the organic phase are divided into two types, the case of GSH being excluded.

For α-MPA, β-MPA, NAC and TMA (type A), the rate is expressed as equation (2).

$$-\frac{d[Hg(SBB)_2]_0}{dt} = k_2[Hg(SBB)_2]_0[THIOL]_a$$

$$= k_2'P_c[Hg(SBB)_2]_a[THIOL]_a \qquad (2)$$

For Cys, Pen, and MEA (type B), the rate is expressed as equation (3).

$$-\frac{d[Hg(SBB)_2]_0}{dt} = k_1[Hg(SBB)_2]_0[THIOL]_a/[H^+]$$

$$= k_1'P_c[Hg(SBB)_2]_a[THIOL]_a/[H^+] \qquad (3)$$

In these equations, subscripts o and a indicate the organic and aqueous phases, respectively.

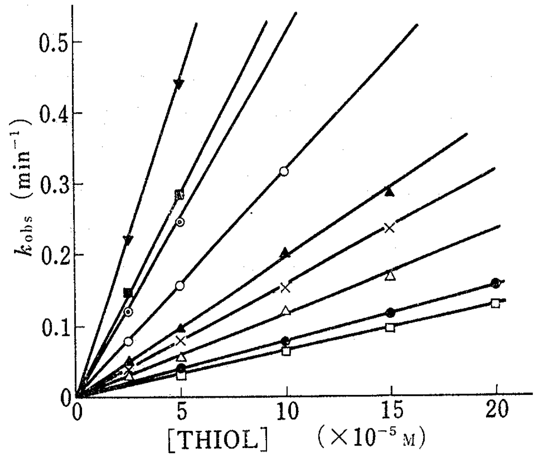

Fig. 4. Relationship between k_{obs} and [THIOL]

▼: α-MPA, pH 4.86	×: MEA, pH 4.26
■: TMA, pH 3.25	△: TMA, pH 4.82
⊙: β-MPA, pH 4.65	●: Cys, pH 4.48
○: GSH, pH 4.80	□: Pen, pH 3.85
▲: NAC, pH 3.90	

($Hg_T = 2.5\times10^{-6}$ M, $SBB_T = 2.0\times10^{-4}$ M)

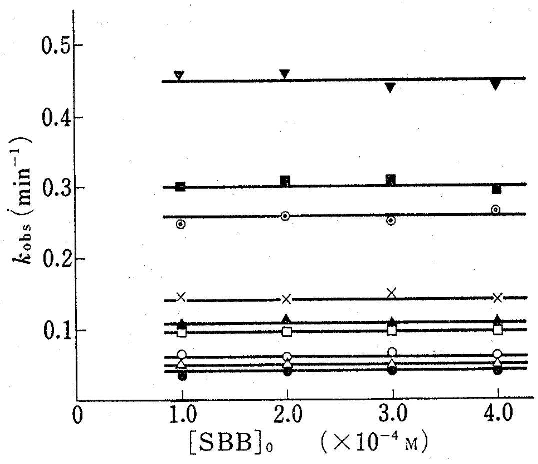

Fig. 5. Relationship between k_{obs} and $[SBB]_0$

▼: α-MPA$=5.0\times10^{-5}$ M, pH 4.80
■: TMA$=5.0\times10^{-5}$ M, pH 3.52
⊙: β-MPA$=5.0\times10^{-5}$ M, pH 4.65
×: MEA$=5.0\times10^{-5}$ M, pH 4.51
▲: NAC$=5.0\times10^{-5}$ M, pH 3.90
□: Pen$=5.0\times10^{-5}$ M, pH 4.48
○: GSH$=5.0\times10^{-5}$ M, pH 4.60
△: TMA$=5.0\times10^{-5}$ M, pH 4.20
●: Cys$=5.0\times10^{-5}$ M, pH 4.82
($Hg_T = 2.5\times10^{-6}$ M)

k_1, k_2: observed rate constant
P_c: partition coefficient of mercury-SBB complex

In the case of GSH, it is expected that the reaction similar to that observed in type B thiol compounds is involved. Further the result in Fig. 2 suggests the possibility that beside this reaction, other reaction may occur at the same time.

The rate constants determined are shown in Table I, together with pK_a values of THIOL taken from the references.[11] Based on the fact that the reaction rate was found to be independent on the concentration of SBB, the possibility that THIOL reacts with mercury after the dissociation of SBB may be excluded in all cases.

Thus, the rate determining step of the reaction is assumed to be involved in either the process of the formation of the mixed ligand complex, or that of the dissociation of SBB from the mixed ligand complex. In any event, the involvement of the formation of the mixed

Table I. Rate Constants and Acid Dissociation Constants for Thiol Compounds

	log k_1	log k_2	pK_{a_3}	pK_{a_2}	pK_{a_1}
MEA	−1.08			10.53	8.27
Pen	−1.18		10.31	8.32	2.5
Cys	−1.51		10.51	8.60	2.00
α-MPA		3.97		10.43	3.89
β-MPA		3.69		10.38	4.27
TMA[a)]		3.78	10.08	4.61	3.34
TMA[b)]		3.11	10.08	4.61	3.34
NAC		3.27		9.37	3.32
GSH	−1.35	2.56	9.65	8.75	3.59

($Hg_T = 2.5\times10^{-6}$ M, SBB$= 2.0\times10^{-4}$ M)
a) at pH <4 *b*) at pH>4

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11) L.G. Sillen and A.E. Martell, "Stability Constants of Metal-Ion Complexes," Suppl. 1, the Chemical Society, London, 1971.

ligand complex in the reaction is approved. The formation of the mixed ligand complexes has been confirmed in the course of the ligand exchange reaction in various metal complex systems.[12-14]

As mentioned above, THIOL was divided into two types (A and B), based on the difference in the manner of the dependence of the reaction rate on the concentration of hydrogen ion. In THIOL of type A, the reaction rate is independent on pH except in the case of TMA. The pK_a value of each thiol compound being referred, the first proton of THIOL is dissociated probably from the carboxyl group, under the present experimental conditions at pH 4—5. In TMA, the reaction rate was found to be reduced in accord with the proton dissociation of the second carboxyl group at higher pH region.

In THIOL of type B, which contains amino group, inverse first order was confirmed with respect to the concentration of hydrogen ion. In the case of MEA, the equation (3) can be expressed as equation (4). Thus the first proton dissociation from RSH is assumed to be involved in the reaction.

$$-\frac{d[Hg(SBB)_2]_0}{dt} = \frac{k_1'P_c}{K_{a_1}}[RSH][Hg(SBB)_2]_a \qquad (4)$$

Here

$$K_{a_1} = \frac{[RSH][H^+]}{[RSH_2^+]} \qquad (RSH_2^+: NH_3^+CH_2CH_2SH)$$

In the cases of Pen and Cys, the equations similar to equation (4) can be derived, but K_{a2} (the second proton dissociation constant) should be taken into account, because the first proton has already dissociated under the pH range in this experiment. In Pen and Cys, the first proton dissociation is supposed to be attributed to the dissociation from carboxyl group.[15] The fact that the consistent values of log k_1/K_{a1} and log k_1/K_{a2} are obtained in the cases of Pen (7.14), Cys (7.09) and MEA (7.19) proposes that the reaction mechanism in these thiols is similar to one another. Thus it may be assumed that in Pen and Cys the amino and mercapto groups contribute to the reaction with mercury independently of carboxyl group. The complexicity of proton dissociation equilibria involving the species such as neutral and zwitter ionic forms makes difficult to confirm which species is mainly concerned with the reaction. It is immediately recognized from the Table I that the similar result is obtained in GSH (log $k_1/K_{a2}=7.40$), which contains mercapto and amino groups far apart from each other. This result is comparable with that in the case of type B. In THIOL of type A, the reaction rates in β-MPA and NAC are observed to be smaller than in α-MPA, and the distance between mercapto and carboxyl groups and the steric hindrance may be regarded as the main factors which decrease the reaction rates in β-MPA and NAC. Accordingly, the results in Table I reveal that the different mechanism is considered in the ligand exchange reaction between the cases of thiol compounds with and without amino groups. There still remain unsolved problems which include the reason why two types of reactions are independently observed only in the case of GSH. However, it should be emphasized in the present study that the observed reaction rate at physiological pH is distinctly higher in thiol compounds with amino group such as Pen, Cys and GSH which are essentially related to the behavior of mercury in the biological system than in other thiol compounds which do not contain amino group in their molecules.

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12) D.W. Margerum and T.J. Bydaleck, *J. Amer. Chem. Soc.*, **83**, 1791 (1961); D.W. Margerum, B.A. Zabin, and D.L. Janes, *Inorg. Chem.*, **5**, 250 (1966); D.L. Janes and D.W. Margerum, *Inorg. Chem.*, **5**, 1135 (1966); R.A. Libby, and D.W. Margerum, *Biochem.*, **4**, 619 (1965).

13) J.L. Sudmeier and C.N. Reilley, *Inorg. Chem.*, **5**, 1047 (1966); J.D. Carr, K. Torrance, C.J. Cruz, and C.N. Reilley, *Anal. Chem.*, **39**, 1358 (1967).

14) J.L. Sudmeier and J.J. Pesek, *Anal. Biochem.*, **41**, 39 (1971); J.L. Sudmeier and J.J. Pesek, *Inorg. Chem.*, **10**, 860 (1971).

15) Y. Sugiura, A. Yokoyama, and H. Tanaka, *Chem. Pharm. Bull.*, (Tokyo), **18**, 693 (1970).