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Thiamine Derivatives of Disulfide Type. XIV.[1] Kinetic Studies on the Reaction between Thiamine and Propyl Propane-Thiolsulfinate or -Thiolsulfonate[2]

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Kinetic studies were conducted on the reaction between thiamine and propyl propane-thiolsulfinate (PrSSOPr) or -thiolsulfonate ($PrSSO_2Pr$) to give thiamine propyl disulfide (B_1SSPr). The folllowing mechanisms were suggested for the reaction with PrSSOPr.

$$B_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B_1SH \text{ (thiol-type thiamine)} \quad (1)$$

$$B_1SH + PrSSOPr \xrightarrow{k_5} B_1SSPr + (PrSOH) \quad (2)$$

$$2(PrSOH) \xrightarrow[\text{fast}]{k_6} PrSSOPr + H_2O \quad (3)$$

The reaction with $PrSSO_2Pr$ was more complicated, since the hydrolysis of $PrSSO_2Pr$ was not neglected, as shown in the following equations.

$$B_1SH + PrSSO_2Pr \xrightarrow{k_3} B_1SSPr + PrSO_2H \quad (4)$$

$$PrSSO_2Pr + H_2O \xrightarrow{k_4} (PrSOH) + PrSO_2H \quad (5)$$

The rate of conversion to thiol-type thiamine was found to be the rate limiting step and k_1 determined as 1.66×10^{-4} sec^{-1} at pH 8 and 37°. The validity of the discussion was supported by the agreement between the experimental data and the simulation curves by analog computation.

Since allithiamine was discovered in the course of studies on the behavior of thiamine in the extract of *Allium sativum* L. by Fujiwara, *et al.*,[4] a number of thiamine derivatives have been prepared. Thiamine propyl disulfide (TPD) is one of those derivatives, which was synthesized by the group of Matsukawa[5] at an early stage of the long course of studies on thiamine derivatives. TPD is superior to thiamine as such properties, high stability against aneurinase, good absorption through the intestinal tract and sustainability of high blood level.[5] Because of these merits, its clinical application has been developed in our country.

In order to make clear the foundamental nature of the thiamine derivatives of disulfide type, the studies on the disulfide bond, which was presumed to be the major properties of the derivatives, have been under way be Nogami and co-workers from the kinetic and enzymatic points of view.[6] Among the various methods for TPD synthesis, there is a one which com-

1) Part XIII: H. Nogami, J. Hasegawa, and K. Aoki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2442 (1971).
2) This work was presented at the 24th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1967.
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4) M. Fujiwara. and H. Watanabe, *Proc. Japan Acad.*, **28**, 156 (1952).
5) T. Matsukawa, S. Yurugi, H. Kawasaki, and Suzuoki, *Takeda Kenkyusho Nenpo*, **12**, 1 (1952).
6) *a*) H. Nogami, J. Hasegawa, and K. Noda, *Chem. Pharm. Bull.* (Tokyo), **17**, 219 (1969); *b*) H. Nogami, J. Hasegawa, and N. Ikari, *ibid.*, **15**, 685, 693 (1967); *c*) H. Nogami, J. Hasegawa, T. Suzuki, and K. Hirata, *ibid.*, **16**, 1272 (1968) *etc.*

prises the reaction between thiamine and propyl propanethiolsulfinate (PrSSOPr) or propyl propanethiolsulfonate ($PrSSO_2Pr$).[7] These reactions have received attention since they are similar to the formation of allithiamine from thiamine and allicin (AlSSOAl; Al=-CH_2-CH $=CH_2$) in the extract of Allium sativum L. and from thiamine and pseudo allicin ($AlSSO_2Al$).

The mechanisms of these reactions, however, remain uncleared except that thiamine may be converted to the thiol-type form as the active species. One of the reasons why the reaction mechanism was not studied might be due to that the purification of PrSSOPr or $PrSSO_2Pr$ had been difficult, therefore, no kinetic study has been conducted. The other reason might be that the reaction mechanism was very complicated. The purification of PrSSOPr and $PrSSO_2Pr$ was accomplished and their physical and chemical properties have been reported.[8] The following mechanisms were proposed for the reactions between cysteine and these compounds.[8b]

$$CySH + PrSSOPr \longrightarrow CySSPr + (PrSOH)$$
$$2(PrSOH) \xrightarrow{\text{fast}} PrSSOPr + H_2O \quad \text{and}$$
$$CySH + PrSSO_2Pr \longrightarrow CySSPr + PrSO_2H$$

It was presumed that the thiol-type thiamine formed by the opening of the thiazolium ring might react with the oxides in a similar manner as cysteine does. During the course of investigation on thiolsulfonate,[8a] it was found that $PrSSO_2Pr$ was hydrolyzed into PrSSOPr and $PrSO_2H$. Since the hydrolysis was considered to be the parallel reaction to the synthesis of TPD, the kinetic study was conducted on the hydroysis and the following mechanism was postulated.[1]

$$PrSSO_2Pr + H_2O \longrightarrow PrSO_2H + (PrSOH)$$
$$2(PrSOH) \xrightarrow{\text{fast}} PrSSOPr + H_2O$$

The present study has been planned to conduct the basic research for the chemistry of thiamine and thiamine derivatives in physiological conditions.

Experimental

Materials——PrSSOr and $PrSSO_2Pr$: The same as reported in the preceeding paper.[8a]

Thiamine–HCl, TPD and Thiothiamine: Supplied from Takeda Chemical Industries, Ltd.

Other chemicals and solvents were used those of reagent grade.

Thin-Layer Chromatography——Thiamine and Its related Compounds: Silica gel (Wako gel B-5, Wako Pure Chemical Ind., Ltd.) plates of 250 μ thickness and benzene: MeOH (7: 3 v/v) solvent system were used Spots were detected by using UV-lamp, Dragendorff reagent or cysteine treatment following ferricyan reagent.

PrSSOPr and the related Compounds: According to the previous report,[8a] silica gel plates, heptane: dioxane (9: 1 v/v) (or benzene) for the development, and HCl–iodide–starch reagent, *etc.* for the detection were used.

Determination of Thiamine——Thiamine was converted to thiochrome by the usual method using BrCN and absorption at 268 mμ was measured spectrophotometrically using Hitachi Perkin Elmer model 139 or Hitachi model 124. TPD was determined reducing to thiamine by cysteine at pH 7.

Determination of PrSSOPr in the Reaction Sloutions of Thiamine and $PrSSO_2Pr$——10 ml of the reaction solution withdrawn was mixed with 10 ml of hexane (Spectrophotosol, Wako Pure Chemical Ind., Ltd.) and shaken immediately. The hexane layer was washed with 1 ml of 0.1 N HCl and dried by 1 g of Na_2SO_4. The absorbance at 258 mμ was measured. PrSSOPr shows λ_{max} at 258 mμ (ε=2000) in hexane solution but $PrSSO_2$-Pr has little absorbance at this wave length. Control solution treated with the same procedure was used.

7) T. Matsukawa and S. Yurugi, *Yakugaku Zasshi*, **72**, 1616 (1952); T. Matsukawa and H. Kawasaki, *ibid.*, **73**, 216 (1953).

8) *a*) H. Nogami, J. Hasegawa, and K. Aoki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2472 (1971); *b*) *Idem*, *ibid.*, **19**, 2433 (1971).

Kinetic Run——Thiamine aqueous solution and PrSSOPr (or $PrSSO_2Pr$) ethanolic solution were to a pre-incubated at 37°±0.1° phosphate buffer solution. The final reaction mixture was pH 8.05, $\mu=0.18$ and 2% in EtOH concentration. Samples were withdrawn into 0.5 N HCl to stop the reaction. Fig. 1 illustrates the result of experiment when the both of concentrations of thiamine and PrSSOPr were 2×10^{-4} M. No difference at the decrease of thiamine was observed between in air and in nitrogen atmosphere. Fig. 1 also shows that all of loss of thiamine can be recovered as thiamine after the reduction with cysteine. The production of thiamine disulfide was neglected when $PrSSO_2Pr$ or at least more than one molar equivalent of PrSSOPr was reacted with thiamine, as mentioned later (see Fig. 3). Therefore, all the decrease of thiamine can be regarded as the production of TPD.

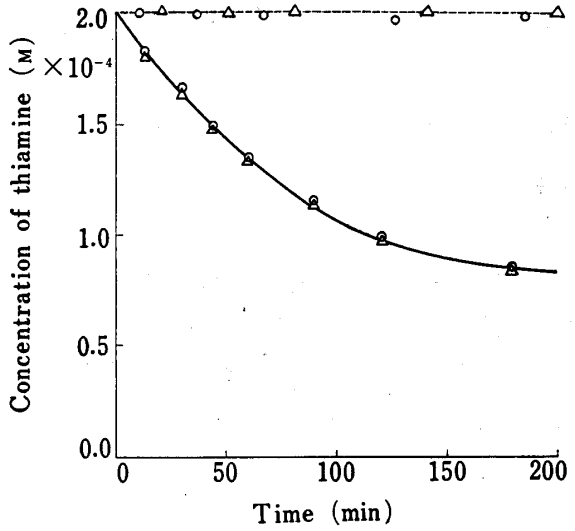

Fig. 1. A Plot of the Concentration of Thiamine *versus* Time for the Reaction of Thiamine (2×10^{-4}M) and PrSSOPr (2×10^{-4}M) at pH 8 and 37°

dotted line: after the treatment by cysteine
○: air, △: under nitrogen stream

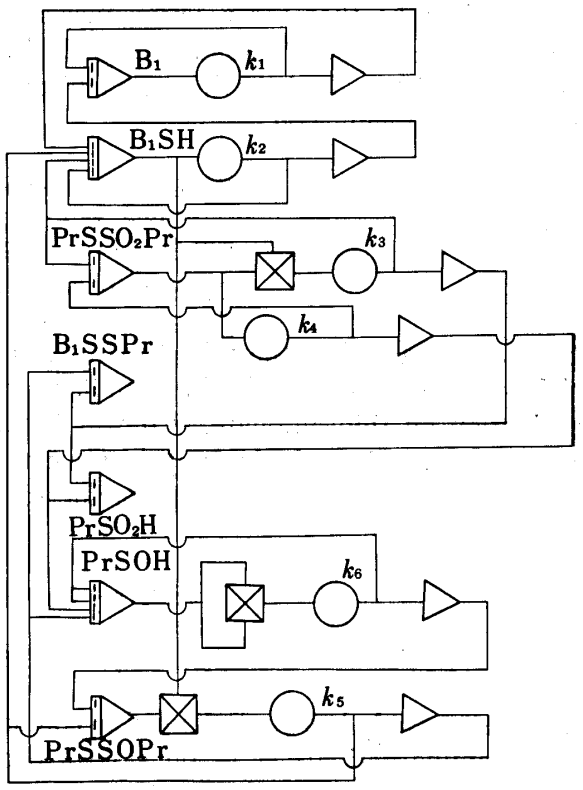

Fig. 2. Analog Computer Program for the Reaction of Thiamine and $PrSSO_2Pr$

Analog Computation——Hitachi ALM-502T and Mitsubishi MELCOM-EA 7307 were used. The procedure was referred to the reports of Garrett and Suzuki, *et al.*[9] The blockdiagram used for the analysis (Chart 2) is shown in Fig. 2.

Calculation

Let assume the following situation:

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B, \quad B + C \xrightarrow{k_5} D + E, \quad 2E \xrightarrow[\text{fast}]{k_6} C$$

In the reactions, k_6 is extremely large, the initial concentrations of A and C are expressed by a and b, and the concentrations of D and intermediate B are represented by x and y at time t, respectively.

Thus, the concentrations of A and C at time t are expressed as

$$A = (a-x-y) \text{ and } C = (b-x/2)$$

The rates, dx/dt and dy/dt are represented by Eq. 1) and 2)

$$dx/dt = k_5(b-x/2)y \tag{1}$$

$$dy/dt = k_1(a-x-y) - k_2y - k_5(b-x/2)y \tag{2}$$

9) E.R. Garrett, T. Suzuki, and D.J. Weber, *J. Am. Chem. Soc.*, **86**, 4460 (1964); E.R. Garrett, P.B. Chemburkar, and T. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **13**, 1113 (1965).

If $k_1 \ll k_2+k_5\ (b-x/2)$ is assumed, the steady state assumption, $dy/dt=0$, holds for y (y is extremely low) when $dy/dt=0$ is assumed to Eq. 2), Eq.3) is given

$$y = \frac{k_1(a-x)}{k_1+k_2+k_5(b-x/2)} \tag{3}$$

Eq. 1) can be written by Eq. 4)

$$\frac{dx}{dt} = \frac{k_1k_5(a-x)(b-x/2)}{k_1+k_2+k_5(b-x/2)} \tag{4}$$

Integration of $\frac{k_1+k_2}{k_1k_5(a-x)(b-x/2)}dx + \frac{1}{k_1(a-x)}dx = dt$

yields Eq. 5) or 6).

$$\frac{2(k_1+k_2)}{k_1k_5(2b-a)} \ln \frac{a(2b-x)}{2b(a-x)} - \frac{1}{k_1} \ln \frac{(a-x)}{a} = t \qquad (\text{at } a \neq 2b) \tag{5}$$

$$\frac{2(k_1+k_2)}{k_1k_5} \frac{x}{a(a-x)} - \frac{1}{k_1} \ln \frac{(a-x)}{a} = t \qquad (\text{at } a=2b) \tag{6}$$

When $b \gg x$ establishes, Eq. 5) becomes

$$-\frac{1}{k_1}\left[1 + \frac{2(k_1+k_2)}{k_5(2b-a)}\right] \ln \frac{(a-x)}{a} = t$$

Further, it can be simplified as Eq. 7)

$$-\frac{1}{k_1}\left[1 + \frac{2\gamma}{(2b-a)}\right] \ln \frac{(a-x)}{a} = t \tag{7}$$

where $\gamma=(k_1+k_2)/k_5$.

The apparent rate constant, k_{obs}, which can be obtained from the straight line of $\ln(a-x)$ against t in the condition of $a<b$, can be expressed by Eq. 8)

$$k_{obs} = k_1\left[\frac{1}{1+2\gamma/(2b-a)}\right] \tag{8}$$

From two different k_{obs}'s, k' and k'', which are obtained from the data in which b is changed to b' and $b''(b>a)$, k_1 and γ can be calculated using Eq. 9) and 10), respectively.

$$k_1 = \frac{\begin{vmatrix} k'(2b'-a) & -k' \\ k''(2b''-a) & -k'' \end{vmatrix}}{\begin{vmatrix} (2b'-a) & -k' \\ (2b''-a) & -k'' \end{vmatrix}} \tag{9}$$

$$\gamma = \frac{1}{2} \frac{\begin{vmatrix} (2b'-a) & k'(2b'-a) \\ (2b''-a) & k''(2b''-a) \end{vmatrix}}{\begin{vmatrix} (2b'-a) & -k' \\ (2b''-a) & -k'' \end{vmatrix}} \tag{10}$$

On the other hand, when $\frac{1}{(2b-a)} \ln \frac{a(2b-x)}{2b(a-x)}$ ($x/a(a-x)$ in Eq. 6) and $\ln \frac{(a-x)}{a}$ are expressed as $F(t)$ and $G(t)$ in Eq. 5), respectivly, Eq. 5) and 6) can be simplified to Eq. 11).

$$\frac{1}{k_1}[2\gamma F(t) - G(t)] = t \tag{11}$$

Result and Discussion

Reaction between Thiamine and PrSSOPr

The products in the reaction between thiamine and PrSSOPr were investigated by thin-layer chromatography. As seen in the chromatograms of Fig. 3, TPD was the product except

a very little amount of thiamine disulfide (TDS), which was detected as a by-product when the amount of PrSSOPr was one quater of thiamine. On the other hand, non of PrSSOPr-related compound such as dipropyl disulfide or sulfinic acid ($PrSO_2H$) was detected at all. Thus, it can be assumed that the decrease of each reactant is proportional with the increase of TPD when the causion to avoid the formation of TDS is taken. TDS might be derived from the oxidation of thiol-type thiamine by oxygen dissolved in the system, as reported by Kawasaki, *et al.*[10] Moreover, none of the following compounds, thiochrome, thiothiamine[11] which is presumed to be the decomposed product[11c] of thiol-type thiamine and the other degradation compounds[12], were detected.

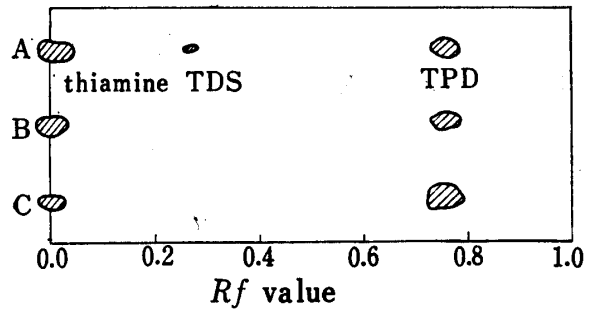

Fig. 3. Thin-Layer Chromatograms Illustrating the Reaction between Thiamine (5×10^{-3}M) and PrSSOPr (A: 1.25×10^{-3}M, B: 2.5×10^{-3}M, C: 5×10^{-3}M) at pH 8 and 37° after Four Hours

plate: silica gel, developing solution; benzene: methanol (7: 3)

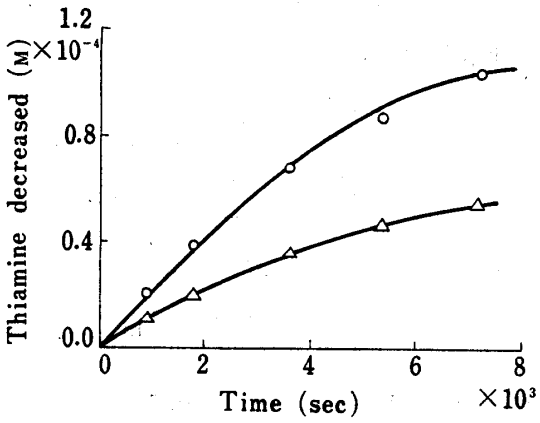

Fig. 4. Thiamine Decrease by the Reaction between Thiamine (○: 2×10^{-4}M, △: 1×10^{-4}M) and PrSSOPr (2×10^{-4}M) at pH 8 and 37°

Therefore, the reaction rate was measured from the decrease of the concentration of thiamine. The decrease of thiamine was nearly proportional to the initial thiamine concentration as shown in Fig. 4. When the initial concentration of thiamine was fixed, the rate of decrease was not directly proportional to the concentration of PrSSOPr, as seen in Fig. 5, which was different from that of second order reaction between cysteine and PrSSOPr.[8b] The situation mentioned was interpreted from the reason as follows. The active species of thiamine should be the thiol form and the convertion rate from ring-closed thiamine to thiol-type might not be fast enough to react with PrSSOPr. The stoichiometry of thiamine and PrSSOPr was not measured, but it was presumed to be 2:1 from the reaction between cysteine and PrSSOPr.[8b] From these considerations, the reaction mechanism has been suggested as Chart 1, where B_1 is thiamine, B_1SH thiol-type thiamine (more exactly it should be written as the thiol S-anion form) and B_1SSPr TPD. The mechanism that PrSOH is produced and it immediately changes to PrSSOPr was derived from the results of preceding papers.[8b,1] Although a number of investigations on the formation of thiol-type thiamine have been made,[13] the information on k_1 in physiological condition is not available. However, it has been found that the concentration of thiol-type thiamine is extremely low in neutral pH region,[12,13d] that is, $k_1 \ll k_2$ establishes. Therefore, a steady state assumption can be applied to the concentration

10) C. Kawasaki and T. Horio, *Vitamin*, **19**, 48 (1960).
11) *a*) T. Matsukawa and S. Yurugi, *Yakugaku Zasshi*, **72**, 1599 (1952); *b*) N. Hayashi, the 19 th Annual Meeting of Chemical Society of Japan, 1966; *c*) K. Aoki and Y. Morita, Private communication.
12) T. Higuchi and J.J. Windheuser, *J. Pharm. Sci.*, **51**, 354 (1962).
13) *a*) R.R. Williams and S.E. Ruehle, *J. Am. Chem. Soc.*, **57**, 1856 (1935); *b*) H.T. Clarke and A. Curin, *ibid.*, **57**, 1876 (1935); *c*) O. Zima and R.R. Williams, *Ber.*, **73**, 941 (1940); *d*) A. Watanabe and Y. Asahi, *Yakugaku Zasshi*, **75**, 1046, 1050 (1955); *e*) G.D. Maier and D.E. Matzler, *J. Am. Chem. Soc.*, **79**, 4386 (1957).

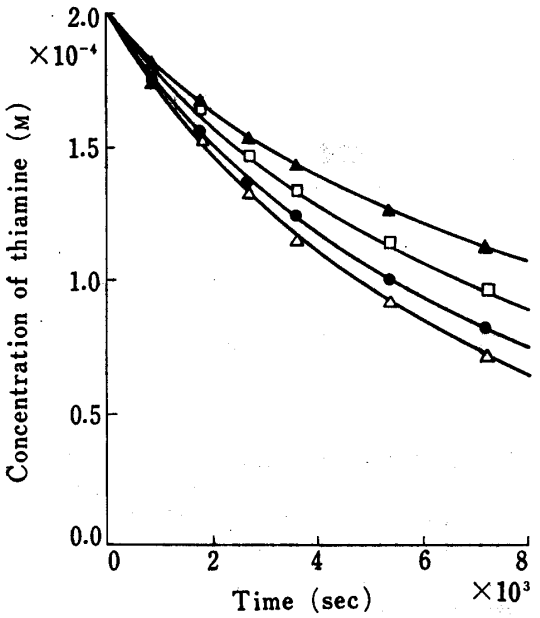

Fig. 5. A Plot of Concentration of Thiamine *versus* Time for the Reaction between Thiamine (2×10^{-4}M) and PrSSOPr (▲: 1×10^{-4}M, □: 2×10^{-4}M, ●: 4×10^{-4}M, △: 10×10^{-4}M) at pH 8 and 37°

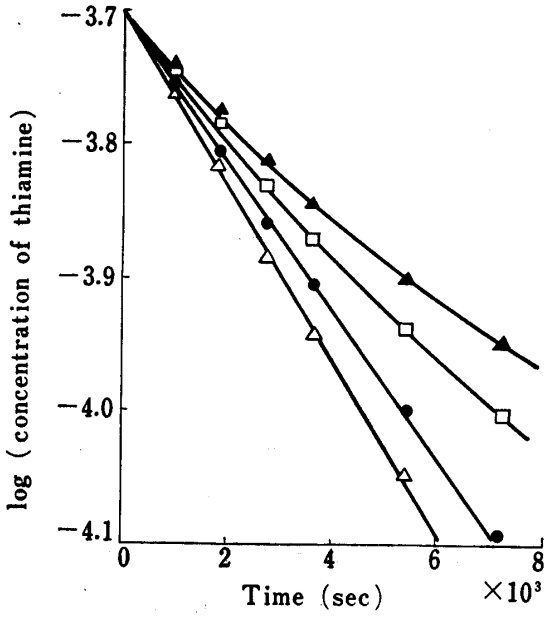

Fig. 6. A Plot of Logarithm of Concentration of Thiamine *versus* Time for the Reaction between Thiamine (2×10^{-4}M) and PrSSOPr (▲: 1×10^{-4}M, □: 2×10^{-4}M, ●: 4×10^{-4}M, △: 10×10^{-4}M) at pH 8 a nd 37°

of thiol-type thiamine in the present condition, as mentioned in the previous section. This assumption is also derived from the following discussion: the disapperance of thiamine, shown in Fig. 5, was plotted on a semilog paper, as seen in Fig. 6, where the straight lines are shown in high concentration of PrSSOPr. The straight line shows the case of either $k_1 \gg k_5$ [PrSSOPr] or $k_1 \ll k_5$[PrSSOPr]. If the case of $k_1 \gg k_5$[PrSSOPr], where k_5 is the rate determining step, would come into exist, k_{obs}, k_5[PrSSOPr] should be proportional with the concentration of PrSSOPr. But, such a fact was denied as mentioned above. Thus, the case of $k_1 \ll k_5$[PrSSOPr], where k_1 is the rate determining step and the amount of thiol-type thiamine is extremely small, can be derived. Thus, it is concluded that the case is both of k_2 and k_5 $(b\text{-}x/2) \gg k_1$.

$$B_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B_1SH$$

$$B_1SH + PrSSOPr \xrightarrow{k_5} B_1SSPr + (PrSOH)$$

$$2(PrSOH) \xrightarrow[\text{fast}]{k_6} PrSSOPr + H_2O$$

Chart 1. Proposed Mechanism for the Reaction between Thiamine and PrSSOPr

The calculation of rate constant is mentioned in the preceding section of this paper and Eq. 3) is obtained from the steady state assumption.

The values of k_1 and γ, $(k_1+k_2)/k_5$, were calculated from the apparent rates in PrSSOPr concentrations of 4×10^{-4}M and 10×10^{-4}M, as shown in Fig. 6. These were,

$$k_1 = 1.52\times10^{-4}\ \text{sec}^{-1}, \quad \gamma = 7.2\times10^{-5}\ \text{mole liter}^{-1}.$$

The validity of the discussion was proved as follows. When 2×10^{-4}M of thiamine was reacted with 1×10^{-4}M or 2×10^{-4}M of PrSSOPr, the consumed thiamine at selected time could be obtained from Fig. 5. In another words, the every term in Eq. 11) is known, therefore, $t_{calcd.}$ may be obtained. If the discussion in previous section is correct, $t_{calcd.}$ should agree with the time when the decrease of thiamine was determined, $t_{exptd.}$ The result is shown in Fig. 7, where a nice agreement is found. Therefore, it might be concluded that the reaction scheme shown in Chart 1 is experimentally supported.

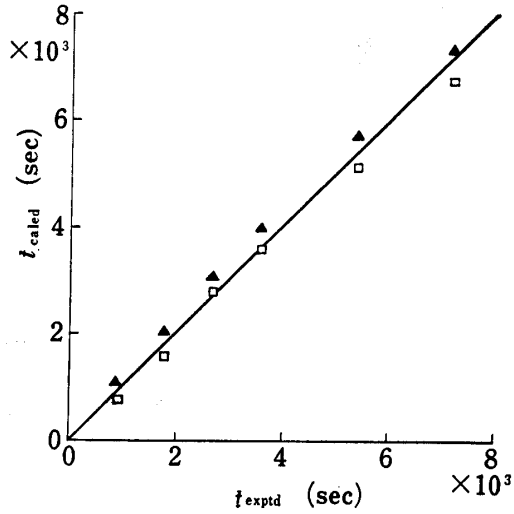

Fig. 7. Figure Showing Comparison of t_{caled} (Using the Values of $k_1=1.52\times10^{-4}$ sec^{-1} and $\gamma=7.2\times10^{-5}$ mole liter^{-1} by Eq. 11) with t_{exptd}
PrSSOPr; ▲: 1×10^{-4}M, □: 2×10^{-4}M

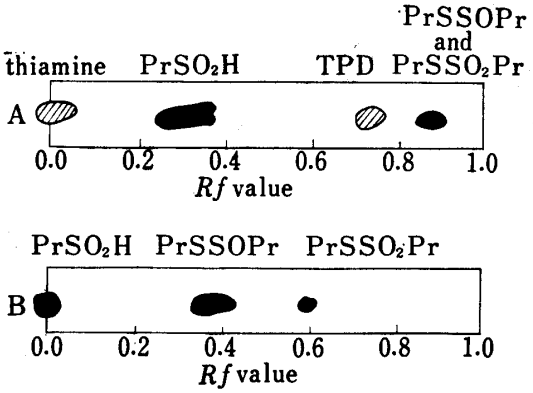

Fig. 8. Thin-Layer Chromatograms Illustrating the Reaction between Thiamine (5×10^{-3}M) and $PrSSO_2Pr$ (5×10^{-3}M) at pH 8 and 37° after One Hour
●: detected by Dragendorf reagent
●: detected by HCl–iodine–starch reagent
A: developing solution; benzene: methanol (7: 3)
B: developing solution; benzene: heptane: dioxane (50: 45: 5)

Reaction between Thiamine and $PrSSO_2Pr$

The compounds detected from the reaction between thiamine and $PrSSO_2Pr$ were $PrSO_2H$, PrSSOPr and TPD as shown in the chromatograms of Fig. 8. None of other compounds related to thiamine were detected except a trace of thiochrome or TPD, the major product expected. PrSSOPr and one part of $PrSO_2H$ might be given by the hydrolysis of $PrSSO_2Pr$ which was studied in the preceding paper.[1]

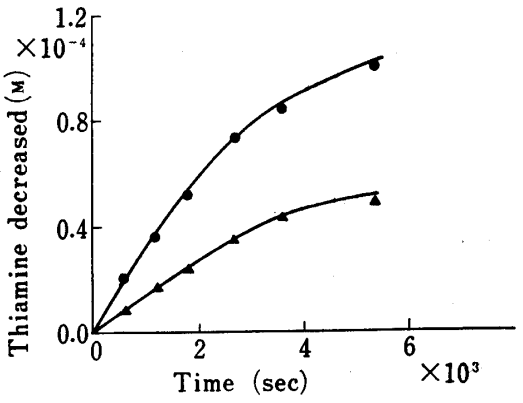

Fig. 9. Thiamine Decrease by the Reaction between Thiamine (●: 2×10^{-4}M, ▲: 1×10^{-4}M) and $PrSSO_2Pr$ (2×10^{-4}M) at pH 8 and 37°

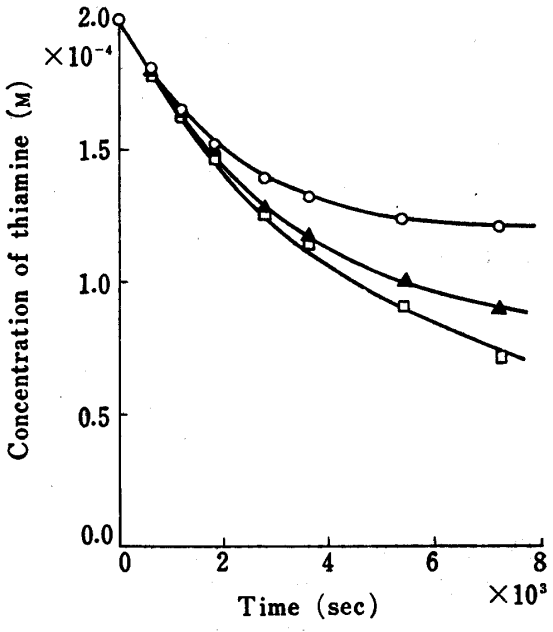

Fig. 10. A Plot of Concentration of Thiamine *versus* Time for the Reaction between Thiamine (2×10^{-4}M) and $PrSSO_2Pr$ (○: 1×10^{-4}M, ▲: 2×10^{-4}M, □: 4×10^{-4}M) at pH 8 and 37°

Although the stoichiometry of the reaction between thiamine and $PrSSO_2Pr$ was not studied, it was postulated to be 1:1 from the reaction between cysteine and $PrSSO_2Pr$.[8b]

The reaction rate was measured from the decrease of the concentration of thiamine, and given in Fig. 9 at constant $PrSSO_2Pr$ concentration and in Fig. 10 at constant thiamine concentration. As seen in Fig. 9, the decrease is proportional to the initial concentration of thiamine. However, the decrease is not relate to $PrSSO_2Pr$ as seen in Fig. 10. Therefore, it should be concluded that the reaction differs from the second order reaction between cysteine and $PrSSO_2Pr$[8b] in mechanism.

It is reasonable to consider that the reaction is devided into the following two phases. The initial reaction phase which was independent on the concentration of $PrSSO_2Pr$ and the slow reaction one which was depend on the concentration of $PrSSO_2Pr$, was observed. It was assumed that the thiol-type thiamine formed immediately reacted with $PrSSO_2Pr$ because the reactivity of $PrSSO_2Pr$ was significantly high in the first stage. In another words, this indicates that the thiol-type thiamine production is rate determinant. The difference between the patterns of Fig. 10 and Fig. 5 may support such an interpretation mentioned.

On the other hand, highly reactive $PrSSO_2Pr$[8b] may be vanished from the system by the reaction with thiamine and/or by the hydrolysis. PrSSOPr is formed by the hydrolysis of $PrSSO_2Pr$. The reactivity of PrSSOPr is much less than that of $PrSSO_2Pr$,[8b] therefore, the slow phase may be due to the reaction between thiamine and PrSSOPr.

The reaction mechanism suggested is given in Chart 2, where B_1 is thiamine, B_1SH thiol-type thiamine, and B_1SSPr TPD. The value for k_1 can be obtained from the first order plots of the initial phase in Fig. 10. The value was 1.66×10^{-4} sec^{-1} and agreed well with 1.52×10^{-4} sec^{-1} which was calculated from the reaction between thiamine and PrSSOPr.

The validity of the kinetic model given in Chart 2 was examined further by analog computation under the following conditions.

The value for k_1 was determined to be 1.66×10^{-4} sec^{-1} as mentioned previously. The one for k_4 was determined spectrophotometrically to be 5.2×10^{-4} sec^{-1} in the preceding paper.[1] The one for k_2 was calculated as follows from pK_{a_2} (av.) at 37°=9.2 given by Higuchi, *et al.*[12]

$$\frac{k_1}{k_2} = \left(\frac{Ka_2(av.)}{[H^+]}\right)^2 = \left(\frac{6.3\times10^{-10}}{8.9\times10^{-9}}\right)^2 \fallingdotseq 5.0\times10^{-3}$$

$$B_1 \underset{k_2}{\overset{k_1}{\rightleftarrows}} B_1SH$$

$$B_1SH + PrSSO_2Pr \xrightarrow{k_3} B_1SSPr + PrSO_2H$$

$$PrSSO_2Pr + H_2O \xrightarrow{k_4} (PrSOH) + PrSO_2H$$

$$B_1SH + PrSSOPr \xrightarrow{k_5} B_1SSPr + (PrSOH)$$

$$2(PrSOH) \xrightarrow[fast]{k_6} PrSSOPr + H_2O$$

Chart 2. Proposed Mechanism for the Reaction between Thiamine and $PrSSO_2Pr$

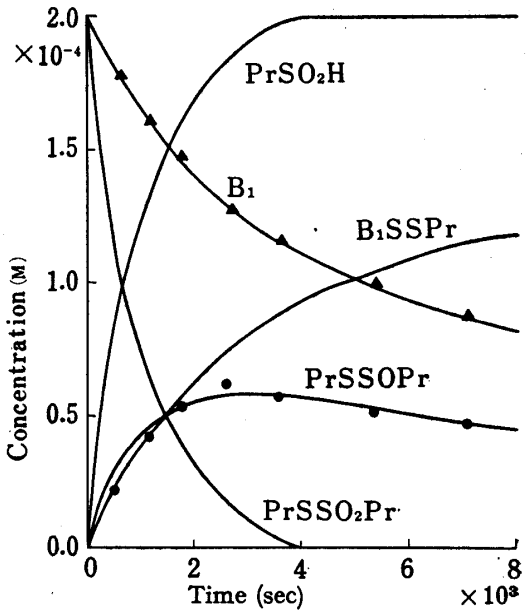

Fig. 11. Time Courses of Various Components Related to the Reaction of Thiamine (2×10^{-4}M) and $PrSSO_2Pr$ (2×10^{-4}M) at pH 8 and 37°

$K_1=1.66\times10^{-4}$ sec^{-1}, $k_2=3.3\times10^{-2}$ sec^{-1}, $k_3=1\times10^{4}$ liter mole^{-1} sec^{-1}, $k_4=5.2\times10^{-4}$ sec^{-1}, $k_5=4.6\times10^{3}$ liter mole^{-1} sec^{-1}, $k_6=1\times10^{5}$ liter mole^{-1} sec^{-1}

$$k_2 = \frac{1.66 \times 10^{-4}\ \text{sec}^{-1}}{5 \times 10^{-3}} = 3.3 \times 10^{-2}\ \text{sec}^{-1}$$

The one for k_5 was calculated from $\gamma=(k_1+k_2)/k_5=7.2\times10^{-5}$ mole liter^{-1} to be 4.6×10^2 liter mole^{-1} sec^{-1}. The value for k_3 was set a larger one than that for k_5 from the reactivity of $PrSSO_2Pr$ with cysteine,[8b] 1×10^4 liter mole^{-1} sec^{-1}. And the value for k_6 was also set an extremely large one, 1×10^5 liter mole^{-1} sec^{-1}. (Although the value for k_3 was estimated to be 10^3 times larger than that for k_5,[8b] it was set from the limitation of the analog computer used. However, it might be assumed as mentioned previously that the value of k_3 or k_6 does not affect the result as long as the value is large.)

The result is given in Fig. 11. As seen in the plots of thiamine and PrSSOPr, the change of reactants obtained experimentally agreed well with the calculated values. Therefore, it might be concluded that the reaction scheme presented in Chart 2 was valid and well supported from the experimental results mentioned.

It might be concluded as follows from the data presented in this series of study.

The oxidation of dipropyl disulfide with hydrogen peroxide was studied and the products PrSSOPr and $PrSSO_2Pr$ which can react with thiamine to result the thiamine derivative of disulfide type, TPD, were separated. The kinetic study was conducted using these purely isolated components.

The reactivity of $PrSSO_2Pr$ with thiol was a thousand times larger than that of PrSSOPr, which was larger than that of dipropyl disulfide, and the rate limiting step for the formation of TPD was proved to be the formation of thiol-type thiamine. This rate might be important for the interpretation of thiamine behavior in physiological conditions and was studied spectrophotometorically (by Maier and Metzler[13c] at higher pH, around 10.5 at 20°) or polarographically (by Watanabe and Asahi[13d]). The rate at 37° and pH 8 was determined in the present study for the first time. The more detailed studies on the ring-opening reaction will be reported in the following papers of this series.

It was reported in the preceding paper[6a] that dipropyl disulfide was formed in isolated rat intestine during the absorption process of TPD. It was also reported that the major metabolite of TPD in urine was sulfate ion,[14,15] which is the final product from various organic sulfur compounds by the sequential oxidation reactions in animal body. The one of merits of TPD, high tissue concentration of thiamine in animal or human organs, is not understandable if the thiamine detivative of disulfide type is decomposed to thiamine and dipropyl disulfide during the absorption process. The possibility of reformation of TPD through the reactions between thiamine and the oxidation products of dipropyl disulfide might be assumed for the higher tissue concentration of thiamine or its derivative. Since the rate is extremely high as reported in the present studies, it may be impossible to detect the oxidation products of dipropyl disulfide in animal tissues. It might be, however, said that the data presented in this series are useful to discuss the possibility of the hypothesis as a future project and the chemical behaviours of thiamine in physilolgical conditions.

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