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Thiamine Derivatives of Disulfide Type. XV.[1] The Ring Opening-closing Reactions of 4-Methylthiazolium Salts[2]

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The ring opening-closing reaction of the thiazolium moiety of thiamine was studied by the pH-stat method at 20° and 30°. The reaction could be represented by the following equations.

$$\text{thiazolium salt} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{pseudo-base} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{thiol form;}$$

$$k_1 = k_1'(OH^-);\ k_4 = k_4'(H^+)$$

Between pH 7 and pH 11, the rate constant k_2 was nearly equal to k_3 but it was considerably larger than k_1 and k_4 in magnitude. The negative dependency of k_1 and k_4 on the ionic strength was observed. The rate constants, k_1' and k_4', were also obtained in the analogous reactions of 3-benzyl-4-methylthiazolium chloride, 4-methyl-3-phenylethylthiazolium bromide and 3,4-dimethylthiazolium iodide.

Reference was made to the mechanism of the thiol exchange reactions between thiamine derivatives of disulfide type (IV) and thiols, and it was confirmed that the rapid ring closing reaction to the thiazolium salt hindered the formation of IV from thiol form of thiamine below neutral pH.

Although it is well known[1] that the thiazolium ring of thiamine opens slowly upon attack of hydroxide ion and closes slowly in the presence of hydrogen ion below pH 11 as shown in Chart 1, very few studies on the rates of these reactions have been reported. The nonprotonated form and the sodium salt of the thiol form have been isolated,[5] but the pseudo-base is too reactive to be isolated. The kinetic study of the ring opening-closing reaction of thiazolium moiety of thiamine has been difficult for lack of suitable analytical methods of determination of these three reaction species. For instance, spectrophotometry is not so useful in distinguishing them in the wide pH range, because the pyrimidine moiety has very strong ultraviolet absorption. Only in the pH range 10 to 10.8, Maier[6] could obtain the rate of the thiol formation from the nonprotonated form by following the increase in absorbancy at 255 mμ in the study of the yellow form of thiamine.[7] Recently Asahi[8] studied the kinetics of the formation and disappearance of the thiol form of some thiazolium salts including thiamine by means of polarography.

1) Part XIV: H. Nogami, J. Hasegawa, M. Hanano, and K. Aoki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2448 (1971).
2) The 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, Apr. 1968.
3) Location: *a*) *7-Chome, Hongo, Bunkyo-ku, Tokyo*; *b*) Present address: *Ayerst Laboratory Inc., Rouses Point, N. Y., U.S.A.*; *c*) Present address: *Faculty of Pharmaceutical Sciences, University of Chiba, Yayoi-cho, Chiba.*
4) R. R. Williams and A. E. Ruehle, *J. Am. Chem. Soc.*, **57**, 1856 (1935).
5) O. Zima and R. R. Williams, *Ber.*, **73**, 941 (1940).
6) G. D. Maier and D. E. Metzler, *J. Am. Chem. Soc.*, **79**, 4386 (1957).
7) The formation of the yellow form of thiamine is rapidly reversible at the high pH region but is hardly observed below pH 10.6 (ref. 6). Therefore the yellow form of thiamine was disregarded throughout this study.
8) T. Fushimi, Y. Hara, S. Yurugi, and Y. Asahi, *Ann. Rept. Takeda Res. Lab.*, **26**, 15 (1967); Y. Asahi and M. Nagaoka, *Chem. Pharm. Bull.* (Tokyo), **19**, 1017 (1971).

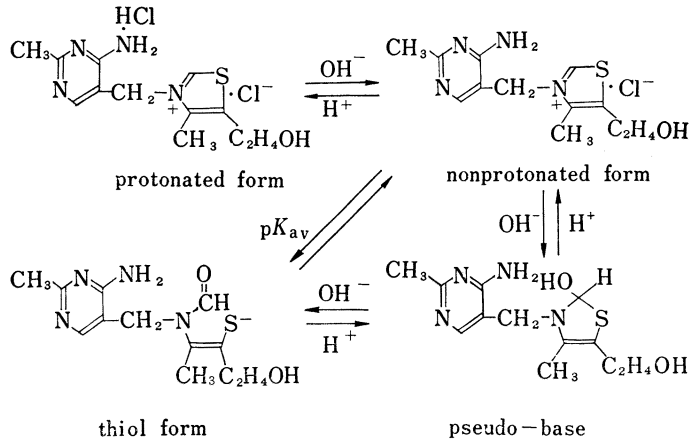


Chart 1

The present investigation was undertaken to study the kinetics of the ring opening-closing reaction of thiamine by means of the pH-stat method, taking account of the points that the reactions are the in or out reaction of hydrogen or hydroxide ion.

Although many intermediates or transition states have been proposed in these reactions,[9] only the concentration of the consumed hydrogen or hydroxide ion can be followed by the pH-stat method. Therefore the ring opening-closing reactions of thiazolium salts can be shown as

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} C \qquad \text{Eq. (1)}$$

where A, B, and C represent thiazolium salt, pseudo-base, and thiol form, respectively. The results analyzed with Eq. (1) were satisfactory to provide a background for the consideration of the reactions of thiamine and related compounds.

Experimental[10]

Materials——Thiamine chloride hydrochloride was of commercial origin and was used after twice recrystallization from 95% EtOH, mp 247°. 4-Methylthiazole was prepared according to the method of Schwarz,[11] bp 56° (45 mmHg). *Anal.* Calcd. for C_4H_5NS: C, 48.45; H, 5.08; N, 14.13. Found: C, 47.57; H, 5.03; N, 14.02. Picrate, mp 180—180.5°. Following thiazolium salts were prepared according to the method of Bahner:[12]——3-Benzyl-4-methylthiazolium chloride (I),[13] mp 188°. *Anal.* Calcd. for $C_{11}H_{12}NSCl$: C, 58.53; H, 5.36; N, 6.21. Found: C, 58.24; H, 5.39; N, 6.19.——4-Methyl-3-phenylethylthiazolium bromide (II),[14] mp 190.5°. *Anal.* Calcd. for $C_{12}H_{14}NSBr$: C, 50.71; H, 4.97; N, 4.93. Found: C, 50.64; H, 5.13; N, 4.91.——3,4-Dimethylthiazolium iodide (III),[15] mp 120°. *Anal.* Calcd. for C_5H_8NSI: C, 24.91; H, 3.34; N, 5.81. Found: C, 25.12; H, 3.43; N, 5.93.

Kinetics——Apparatus for the pH-stat method were Radiometer (Copenhagen) Type TTTlc and equipments. The ionic strength of the solution was adjusted with NaCl. A typical kinetic run was as follows. Twenty ml of the solution of thiazolium salt (2.5×10^{-3}M) was placed in a thermostated vessel and nitrogen gas was bubbled moderately. After the solution was reached a desired temperature, the machine was set to start the reaction. The reaction was followed by the addition of the titrant (0.25N NaOH) automatically.

9) A. Watanabe, *Bitamin*, **18**, 267 (1957).
10) All melting points are not corrected.
11) G. Schwarz, "Organic Syntheses," Vol. 25, ed. by W.E. Bachmann, John Wiley and Sons, Inc., New York, 1945, p. 35.
12) C.T. Bahner, D. Pickens, and D.B. Bales, *J. Am. Chem. Soc.*, **70**, 1652 (1948).
13) P. Sykes, and A.R. Todd, *J. Chem. Soc.*, **1951**, 535.
14) J.E. Downes and P. Sykes, *J. Chem. Soc.*, **1960**, 963.
15) G.E. Lienhard, *J. Am. Chem. Soc.*, **88**, 5642 (1966).

At the beginning, though the amount was very small, the titrant was consumed rapidly in order to adjust the solution at a given pH. Then a slow titration was followed. This was recorded on the chart paper as a sharp bending point. From the tangent of this point, an initial velocity was obtained. As the total amount of the titrant was arranged to be less than 0.5 ml in every kinetic run, the volume change of the solution was ignored.

The nonprotonated thiamine was prepared by adding exactly one equivalent of NaOH to the solution of thiamine chloride hydrochloride pre-equilibrated at a given temperature. The pH of the solution made in this way was always about 6.80 at 30°. Therefore most of the nonprotonated thiamine at 30° were prepared conveniently by setting the machine at pH 6.80 before the reaction was initiated.

The solution of the thiol form of thiamine, (B_1S^-), was prepared by adding exactly 3 equivalents of NaOH to the solution of thiamine chloride hydrochloride in every kinetic run and the solution was left for a long time, usually 40 min or more, to attain the equilibrium while nitrogen gas was bubbled.

The thiol forms of other thiazolium salts were prepared similarly to the case of thiamine except that 2 equivalents of NaOH were added.

Result

Ring Opening Reaction

Reaction species A in the ring opening reaction of thiazolium moiety of thiamine was the nonprotonated form. If an equilibrium between A and B is established rapidly, a considerable amount of sodium hydroxide must be consumed immediately after the reaction is started from A, but it was found around pH 9 that very small amount of base was rapidly consumed at the first stage, which was necessary to adjust the solution at a given pH, and then rather slow consumption of base began. Thus there was no rapid equilibrium between A and B at the beginning of the reaction from A. That the consumption of base reflected the ring opening reaction was supported by such analogous compound as 3-benzyl-4-methylthiazolium chloride (I), 4-methyl-3-phenylethylthiazolium bromide (II) and 3,4-dimethylthiazolium iodide (III). These analogous compounds, which consume base only when their thiazolium rings are opened, showed a similar manner of consumption of base to thiamine. By means of initial velocities, the slow reaction was found to be first order with respect to A in concentrations ranging from 1×10^{-3} to 1×10^{-2} M. The rate constants, k_1, were obtained from initial velocities as first order ones. The logarithmic plot of k_1 as a function of pH is shown in Fig. 1. The k_1 could be represented as Eq. (2), where k'_1 is the second order rate constant and (OH^-) is the concentration of hydroxide ion, respectively. The values of

$$k_1 = k_1'(OH^-) \qquad \text{Eq. (2)}$$

k'_1 of thiamine are listed in Table I together with those of other thiazolium salts. Though the data were limited in number, the negative dependency of k_1 on the ionic strength was observed, as was to be expected[16] in the reaction of positively and negatively charged groups, namely, thiazolium salt and hydroxide ion.

Ring Closing Reaction

The reaction species C in the ring closing reaction was the thiol form of thiamine (B_1S^-). The titrant, hydrochloric acid, was consumed in a similar manner to sodium hydroxide of the ring opening reaction. Thus there was no rapid equilibrium at the beginning of the reaction from C. The first order rate constants k were obtained from initial velocities similarly to k_1. As is shown in Fig. 2, k_4 could be represented as $k_4=k'_4(H^+)$, where (H^+) is the concentration of hydrogen ion. The values of k'_4 are listed in Table I.

Apparent Velocities toward Equilibrium States

The reaction reached an equilibrium after a definite consumption of base or acid depending on the reaction condition. Ordinary semilog plots were used to obtain the apparent

16) *a*) J.J. Windheuser and T. Higuchi, *J. Pharm. Sci.*, **51**, 354 (1962); *b*) E.S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Company, Inc., New York, 1960, p. 186.

first order rate constants to reach the equilibrium states. Typical plots are shown in Fig. 3, where the asymptotic amount of base or acid is taken to be 100%. In the ring opening reaction between pH 9 and pH 11, linear lines were obtained and the reaction was found to be first order. However, in the ring closing reaction between pH 7 and pH 9, slightly curved lines were obtained. The apparent first order rate constants, k_{obs}, were obtained either from

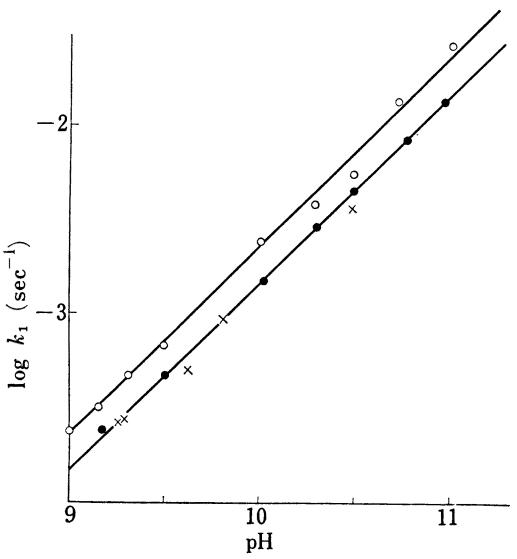


Fig. 1. The pH-Rate Profile for the Ring Opening Reaction of Thiamine at 20°
ionic strength: ○—○ 0.1; ●—● 0.3; ×—× 0.5.

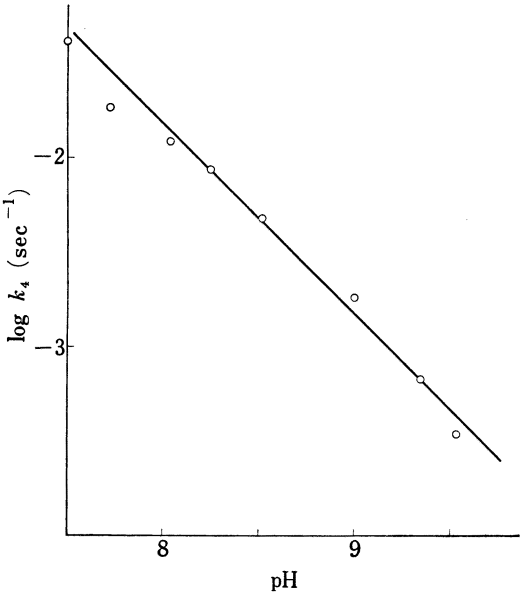
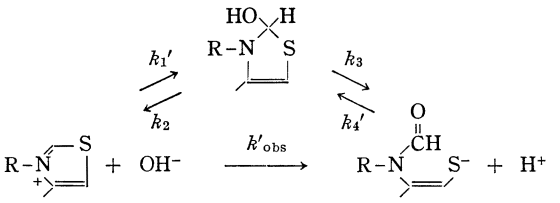


Fig. 2. The pH-Rate Profile for the Ring Closing Reaction of Thiamine at 30°, $\mu=0.3$

TABLE I. Rate Constants for the Ring Opening-closing Reactions of Thiamine and 4-Methylthiazolium Salts



Compound R	μ [a]	Temp. (°C)	k'_1 M^{-1}sec^{-1}	k'_{obs} [b] M^{-1}sec^{-1}	k'_{obs}/k'_1	k'_4 M^{-1}sec^{-1}
Thiamine	0.1	20	34.5	13.6	0.39	
	0.1	30	54.8	29.6	0.54	
	0.3	20	21.6	10.7	0.49	
I C_6H_5-CH_2-	0.3	30	53.4	27.0	0.50	1.53×10^6
	0.1	20	15.1	7.8	0.52	
	0.1	30	39.2	19.8	0.51	0.63×10^6
II C_6H_5-C_2H_4-	0.1	20	9.6	4.7	0.49	
	0.1	30	18.6	10.0	0.54	1.18×10^6
III CH_3-	0.1	20	8.6	4.0	0.48	
	0.1	30	11.5	8.4	0.73	0.83×10^6

a) ionic strength
b) apparent rate constant for the ring opening reaction

Table II. The Influence of the Ionic Strength on Rate Constants for the Ring Closing Reaction of Thiamine at pH 8.2, at 30°

Rate constant sec^{-1}	Ionic strength			
	0.05	0.1	0.3	0.5
$k_4 \times 10^3$	10.02	9.42	9.06	8.85
$k_{obs} \times 10^3$	5.75	5.53	5.35	5.15

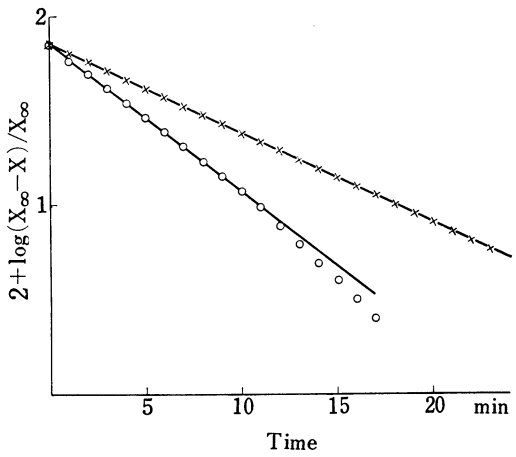


Fig. 3. The First Order Plots of Thiamine for the Apparent Velocities toward Equilibrium States at 30°, $\mu=0.3$

—×—: ring opening reaction at pH 9.61
—○—: ring closing reaction at pH 8.51
X is the consumed amount of NaOH in the ring opening reaction and that of HCl in the ring closing reaction. Zero time were appropriately chosen after the reactions proceeded to some extent.

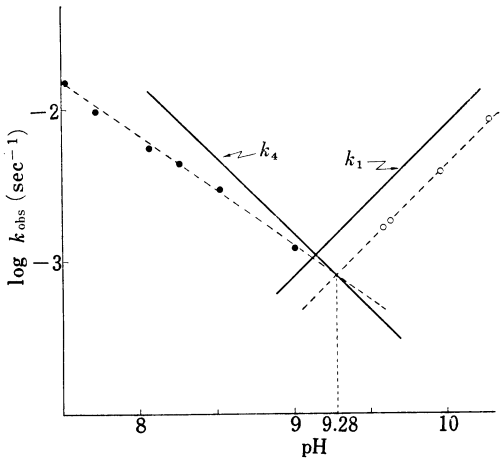


Fig. 4. Apparent Rate Constants of Thiamine toward Equilibrium States at 30°, $\mu=0.3$

···○···: ring opening reaction
···●···: ring closing reaction
Solid lines are k_1 and k_4, respectively.

the slopes of these plots[17] or by the method of Guggenheim.[18] The values of k_{obs} agreed well with those obtained previously,[1,6,8] and they were found to be a little smaller than those of corresponding k_1 and k_4 at the same pH as is shown in Fig. 4. In preliminary experiments the dependency of k_{obs} on the ionic strength was found to be similar to that of corresponding k_1 and k_4. The second order rate constants, k'_{obs}, were obtained in the ring opening reaction by Eq. (3).

$$k_{obs} = k_{obs}'(OH^-) \qquad \text{Eq. (3)}$$

As shown in Fig. 4, the pH of the intersecting point of k_{obs} is 9.28 at 30°, where the apparent rate constant of the ring opening reaction is equal to that of the ring closing reaction. This is in good agreement with the second pK_a of thiamine, 9.30, at 30°.

Discussion

That the change of the thiazolium salt to the pseudo-base may be the rate limiting step in the ring opening reaction is suggested by the findings that k_{obs} was a little smaller than k_1, and that the dependency of k_{obs} on the ionic strength was similar to that of k_1.

17) In the case of the ring closing reaction, the initial parts of the lines were used.
18) A.A. Frost and R.G. Peason, "Kinetics and Mechanism," 2nd ed., John Wiley and Sons, Inc., New York, 1962, p. 49.

From Eq. (1), the following results are obtained.[19]

$$(A) = (A)_0[C_1 \exp(r_1 t) + C_2 \exp(r_2 t) + k_2k_4/\beta]$$
$$(C) = (A)_0[C_3 \exp(r_1 t) + C_4 \exp(r_2 t) + k_1k_3/\beta]$$
$$(B) = (A)_0 - (A) - (C) \qquad \text{Eq. (4)}$$

where $(A)_0$ is the initial concentration of A. (A), (B), and (C) are the concentration of A, B, and C at time t, respectively, and

$$2\alpha = k_1 + k_2 + k_3 + k_4, \qquad \beta = k_1k_3 + k_2k_4 + k_1k_4,$$
$$r_1 = -\alpha + \sqrt{\alpha^2-\beta}, \qquad r_2 = -\alpha - \sqrt{\alpha^2-\beta},$$
$$C_1 = \frac{r_2(\beta - k_2k_4) + \beta k_1}{(r_2 - r_1)\beta}, \qquad C_2 = \frac{r_1(\beta - k_2k_4) + \beta k_1}{(r_1 - r_2)\beta},$$
$$C_3 = \frac{r_2k_1k_3}{(r_1 - r_2)\beta}, \qquad C_4 = \frac{r_1k_1k_3}{(r_2 - r_1)\beta}.$$

When k_2 and k_3 are considerably larger than k_1, and k_4 is nearly zero, then the following relation can be obtained.

$$|r_1| \ll |r_2| \qquad \text{(see appendix)}$$

In the present study the amount of base, (X), at the steady state is as follows.

$$(X) = (B) + 2(C)$$
$$= (A)_0[1 + (C_3 - C_1)\exp(r_1 t) + (C_4 - C_2)\exp(r_2 t) + (k_1k_3 - k_2k_4)/\beta]$$

Therefore

$$(X)_\infty - (X) = (A)_0[(C_1 - C_3)\exp(r_1 t) + (C_2 - C_4)\exp(r_2 t)] \qquad \text{Eq. (5)}$$

were $(X)_\infty$ is the total amount of base at equilibrium. After the reaction proceeds to some extent, Eq. (5) may be simplified to Eq. (6), since $|\gamma_1| \ll |\gamma_2|$.

$$\ln\{(X)_\infty - (X)\} = r_1 t + \ln(A)_0(C_1 - C_3) \qquad \text{Eq. (6)}$$

This can well explain the reason why the apparent velocity toward the equilibrium state was first order in the ring opening reaction.

Let

$$k_{obs} = -r_1$$

then

$$k_{obs}/k_1 = k_3/(k_2 + k_3) \qquad \text{(see appendix)}$$

From Eq. (2) and Eq. (3), we have

$$k_3/(k_2 + k_3) = k_{obs}'/k_1'$$

The values of k'_{obs}/k'_1, which were found to be about 0.5 as in Table I, indicate that k_2 is close to k_3 in magnitude. The same result can also be shown in the following manner. It is well known that thiamine has two pK_a's, one of which, 4.80 at 30°, is due to the dissociation of amino group of the pyrimidine moiety. The other higher pK_a, 9.30 at 30°, was formerly considered as the dissociation exponent of the thiol of B_1S^-, but now it is considered as the result of two step dissociation owing to the high reactivity of the pseudo-base.[6] Higuchi, *et al.*[16a] proposed the pK_{av} for this higher pK_a, defining as

$$K_{av}^2 = \frac{(C)_e}{(A)_e}(H^+)^2 \qquad \text{Eq. (7)}$$

19) M. Oiwa, "Han-no Sokudo Keisanho," Asakura Shoten, Tokyo, 1962, p. 136.

where subscript e represents the equilibrium state and (A), (C), and (H^+) are the concentration of A, C, and hydrogen ion, respectively. If the ring opening-closing reaction is summarized as

$$A + OH^- \underset{k_2}{\overset{k_1'}{\rightleftarrows}} B$$

$$B \underset{k_4'}{\overset{k_3}{\rightleftarrows}} C + H^+ \qquad \text{Eq. (8)}$$

then the following equation can be obtained.

$$\frac{k_1'k_3}{k_2k_4'} = \frac{(B)_e}{(A)_e(OH^-)} \times \frac{(C)_e(H^+)}{(B)_e} = \frac{(C)_e(H^+)^2}{(A)_eK_w}$$

Therefore

$$k_3/k_2 = (K_{a_v}{}^2k_4')/(K_wk_1') \qquad \text{Eq. (9)}$$

The value of k_3/k_2 at 30° is calculated to be about 1.0 by substituing the values of k'_1 and k'_4 into Eq. (9). Therefore it can be concluded that in the pH range studied here k_2 is nearly equal to k_3 but is considerably larger than k_1 and k_4 in magnitude.

The reason why thiamine showed the largest rate constant both in k'_1 and k'_4 among thiazolium salts studied here is not clear. It might be due to the electronic nature of the 2 position of the thiazolium ring through nitrogen atom or some neighboring group participation of the amino or alcohol group of thiamine, but in this respect further work is required.

In the preceding reports of this series,[20-22] the thiol exchange reactions between thiamine derivatives of disulfide type (IV) and some thiols have been studied. The thiol exchange reactions between disulfides and thiols are generally reversible, which can be written as Eq. (10) and Eq. (11),

$$RS\text{–}SR' + R''S^- \underset{k_b}{\overset{k_a}{\rightleftarrows}} R''S\text{–}SR' + RS^- \qquad \text{Eq. (10)}$$

$$R''S\text{–}SR' + R''S^- \underset{k_d}{\overset{k_c}{\rightleftarrows}} R''S\text{–}SR'' + R'S^- \qquad \text{Eq. (11)}$$

where k_a, k_b, k_c and k_d are the second order rate constants, respectively. It has been summarized that the larger are the values of pK_a of attacking thiols and also the smaller are the values of pK_a of leaving thiols, the greater are the rate constants.[22]

However, the thiol exchange reactions below neutral pH between cysteine (Cys^-) and such IV as thiamine propyl disulfide,[21a] thiamine disulfide (TDS),[21b] and thiamine tetrahydrofurfuryl disulfide[21c] were not reversible and thiamine was always released as a main product. The reactions were satisfactorily analyzed as two consecutive irreversible bimolecular reactions. The reverse reactions were slightly observed at higher pH region,[21a,23] while the reaction between diphenyl disulfide (PhSSPh) and Cys^- was reversible even below neutral pH.[22] The rate constants, k_a and k_c, of the reaction of TDS with Cys^- were only 10 times greater

20) H. Nogami, J. Hasegawa, and K. Noda, *Chem. Pharm. Bull.* (Tokyo), **17**, 219 (1969); H. Nogami, J. Hasegawa, S. Nakatsuka, and K. Noda, *ibid.*, **17**, 228 (1969); H. Nogami, J. Hasegawa, and K. Noda, *ibid.*, **17**, 234 (1969); H. Nogami, J. Hasegawa, and N. Ikari, *ibid.*, **15**, 693 (1967); H. Nogami, J. Hasegawa, N. Ikari, and K. Takeuchi, *ibid.*, **17**, 1541 (1969).

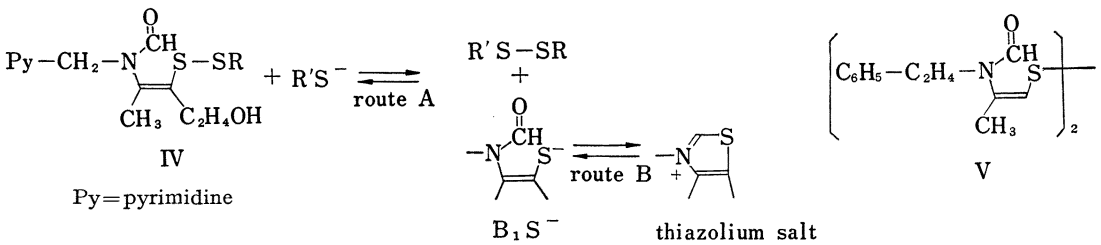
21) *a*) H. Nogami, J. Hasegawa, and N. Ikari, *Chem. Pharm. Bull.* (Tokyo), **15**, 685 (1967); *b*) H. Nogami, J. Hasegawa, T. Suzuki, and K. Hirata, *ibid.*, **16**, 1273 (1968); *c*) H. Nogami, J. Hasegawa, and K. Okazaki, *ibid.*, **16**, 1732 (1968).

22) H. Nogami, J. Hasegawa, N. Ikari, K. Takeuchi, and K. Ando, *Chem. Pharm. Bull.* (Tokyo), **18**, 1091 (1970).

23) *a*) T. Matsukawa and S. Yurugi, *Science*, **118**, 109 (1953); *b*) *Idem*, *Yakugaku Zasshi*, **74**, 1373 (1954); *c*) I. Utsumi, K. Harada, and K. Kohno, *Bitamin*, **27**, 299 (1963); *d*) K. Kohno, *ibid.*, **31**, 470 (1965).

than those of the corresponding reaction of PhSSPh with Cys^- in 50% ethanolic buffered solution and the pK_a of the thiol of B_1S^- would be close to that of phenylmercaptan. Therefore the correlation of pK_a and rate constant above described was not enough to explain the disappearance of the reverse reaction in the case of IV.

A rationale may be given as follows. In the case of IV there are two possible reactions of B_1S^- which is produced by the exchange reaction with Cys^-. One path is the second order reverse reaction to produce the initial disulfide (route A), and the other direction is the first order ring closing reaction to thiazolium salt (route B), under the condition of a constant pH. The results of the present study indicate that the ring closing reaction is very fast below neutral pH. It is probable below neutral pH that the majority of B_1S^- produced from IV close to the thiazolium ring as suggested by Kohno[23d] and that the total amounts of B_1S^- are very small because the ring opening reaction is very slow. Consequently the reverse reaction to disulfide can hardly be observed. It will be allowable to use the value of k_b of the reaction of PhSSPh with Cys^- in order to estimate k_b of the reaction of TDS with Cys^- roughly, though the data were not obtained in the same reaction condition. Assuming that k_b of TDS is 2.0×10^2 liter $mole^{-1}$ sec^{-1} at 15°, which is about 10 times larger than k_b of PhSSPh, and that the activation energy is 10 kcal $mole^{-1}$ analogously to PhSSPh, then k_b of TDS at 30° will become 4.7×10^2 liter $mole^{-1}$ sec^{-1}. If the initial concentrations of thiamine-cysteine disulfide (B_1SSCys) and B_1S^- are both 10^{-4}M at pH 7 and the reaction proceeds solely through route A, then the initial velocity of the disappearance of B_1S^- will be 4.7×10^{-6} mole $liter^{-1}$ sec^{-1}. On the other hand the initial velocity of the ring closing reaction of B_1S^- in concentration of 10^{-4}M to the thiazolium salt at pH 7 and 30° is 1.5×10^{-5} mole $liter^{-1}sec^{-1}$. Most of the working concentrations of IV and Cys^- in the studies of this series were both 10^{-4}M or below. Therefore the concentration of B_1S^- would be much smaller than 10^{-4}M, so that the reaction velocity through route A would be very small as compared with that through route B.



IV

Py=pyrimidine

B_1S^- thiazolium salt

V

Chart 2

An additional evidence in support of the mechanism described above can be seen in the reaction of a derivative of II. The compound II can be considered as one of good analogous compounds of thiamine with respect to the thiazolium moiety, because k_{obs} of the ring closing reaction was slightly smaller than that of thiamine and the value of pK_{av}, which was obtained from k_{obs} similarly to that of thiamine, was about 9.5 at 30°. In parallel with present study, an exchange reaction between Cys^- and bis(N-phenylethylformamido)propenyl disulfide (V), which is a disulfide type of II, was investigated[24] and the reversible reaction between V and Cys^- was observed above pH 8.45, but not at pH 5.0. Therefore it can be concluded that the ring closing reaction of thiazolium moiety of IV hinders the reverse reaction in the thiol exchange reactions between IV and thiols.

24) H. Nogami, N. Ikari, K. Ando, and K. Takeuchi, *Yakugaku Zasshi*, **90**, 418 (1970).

Appendix

If k_2 and $k_3 \gg k_1$, and $k_4 \fallingdotseq 0$ in Eq. (4), the following relation can be obtained.[19]

$$\frac{\beta}{\alpha^2} \fallingdotseq \frac{4k_1k_3}{(k_2+k_3)^2} \ll 1$$

therefore

$$\sqrt{\alpha_2-\beta} = \alpha\sqrt{1-\frac{\beta}{\alpha^2}} \fallingdotseq \alpha\left(1-\frac{\beta}{2\alpha^2}\right)$$

$$r_1 = -\alpha + \sqrt{\alpha^2-\beta} \fallingdotseq -\frac{\beta}{2\alpha} \fallingdotseq -\frac{k_1k_3}{k_2+k_3}$$

$$r_2 = -\alpha - \sqrt{\alpha^2-\beta} \fallingdotseq -2\alpha + \frac{\beta}{2\alpha} \fallingdotseq -(k_2+k_3)$$

thus

$$|r_1| \ll |r_2|$$

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