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Kinetic Studies on Decomposition of Glutathione. I. Decomposition in Solid State

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The decomposition of crystalline glutathione (GSH) by water vapour was studied kinetically. The decomposition time course of GSH polymorphs (α and β forms) showed the pattern like autocatalytic reaction in a solid state. The logarithmic plots of the decomposed ratio *vs.* the reaction time were almost linear for the β form, but broken to two lines for the α form. Decomposition of the α form was more rapid than the β form under the same condition of temperature and humidity. The effect of oxygen in air on the rate was almost negligible. The reaction rate in the early stage of the decomposition was concluded to be larger than the rate calculated from the reaction model in which GSH decomposed in the saturated solution on the crystal surface. The rates were apparently proportional to about 3 to 5 powers of humidity. γ-Glutamyl bond was broken and some parts of products underwent intramolecular condensation in the decomposition. A small amount of oxidized GSH was formed but the decreased ratio of reductivity of the sample was considerable and increased with increasing decomposition ratio of GSH. Apparent activation energy for decomposition of both forms was 32 to 33 kcal/mol.

Keywords——glutathione; decomposition in solid state; kinetics; polymorph; relative humidity; decomposition product; activation energy

Decomposition of solid pharmaceuticals is an important problem on pharmaceutical science and technique, while many matters concerning this problem are still obscure and the kinetics reported is frequently semiquantitative. In a pure solid, the time course of decomposed fraction is often sigmoidal, divided into induced, accelerated, and decayed periods. Occasionally, it is assumed that the decomposition reaction progresses in the thick liquid layer on crystal surface. In a number of cases, nevertheless, the actual physical and chemical processes are ambiguous. In order to clarify the process concerning the decomposition of pharmaceutical solids, quantitative kinetic studies are necessary on many kinds of organic solids. Such kinetic studies are few on peptides in solid state, hence such a study on reduced glutathione (GSH), which is a very important oligopeptide as a medicine and biochemical agent, is significant. Because the stability of water-soluble drug becomes occasionally a difficult problem in practice, the studies on the decomposition of crystalline GSH, which is unstable in aqueous solution, are also expected to bring useful informations. Crystals of GSH are known to have two kinds of polymorph, α and β forms.[2] Their structures are assumed to be "folded" with intramolecular hydrogen bonds and "extended" with intermolecular hydrogen bonds, respectively.[3a,b] The difference in decomposition between the α and β forms, therefore, is interesting to investigate. Yamasaki, *et al.*[2] reported that after one month of storage at 37° and 74% relative humidity (R.H.), the amount of undecomposed GSH remaining are 99.7 and 99.5% for the α and β forms, respectively. Miyoshi, *et al.*[3a] also reported that after 17 days of storage at 40° and 100% R.H., the respective percentages are 15 and 75. Details of the decomposition concerning polymorphs of GSH, however, are

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2) K. Yamasaki, Y. Kobayashi, K. Takanobu, and M. Hamada, *Bunseki Kagaku*, **18**, 874 (1969).
3) *a*) M. Miyoshi, K. Kotera, H. Seko, K. Masukawa, S. Imado, and K. Okumura, *Bull. Chem. Soc. Jpn.*, **42**, 1749 (1969); *b*) W.B. Wright, *Acta Cryst.*, **11**, 632 (1958).

still ambiguous. This paper presents kinetic studies on the decomposition of crystalline GSH in varied moist atmosphere.

Experimental

Materials——GSH α form. Crystalline GSH (Yamanouchi Pharmaceutical Co.) was recrystallized from mixture of EtOH and H_2O (1:1).[4]

GSH β form. A part of the crystalline GSH was dissolved in 2 parts of H_2O with warming and filtered, and the half amount of H_2O was evaporated from the solution by a rotary evaporator. The concentrated solution was allowed to stand at about 50° for 40 min and then at room temperature overnight. The crystallized β form was collected by filtration and washed with a small amount of H_2O, and the crystals obtained were used as nuclei. A part of crystalline GSH was newly dissolved in 2.5 parts of H_2O with warming and filtered. The nuclei of β form prepared as above were seeded in the filtrate, and this was allowed to stand at room temperature. The crystallized β form was collected by filtration, washed with EtOH containing H_2O, and dried over P_2O_5 under reduced pressure.[2,3a]

With crystals prepared as above, X-ray diffraction patterns were measured. The α form showed a very strong peak at $2\theta=22.3°$ ($d=3.98$ Å) and β form at $2\theta=21.3°$ ($d=4.17$ Å).[2]

Kinetic Procedures——The reaction apparatus devised by Hasegawa, *et al.*[5] was used. About 100 mg of GSH powder was weighed into a Pyrex glass vial (12×20 mm) which was set in a reaction vessel. The reaction vessel was placed in a constant temperature bath that was regulated within ±0.1° precision. The relative humidity in the reaction vessel was maintained by using saturated solutions of inorganic salts (NaCl, $NaNO_3$, NaBr or NH_4NO_3) containing the same salt crystals in large excess. The whole amount of sample in the vial was removed and analysed at intervals.

Determination of GSH——In order to determine GSH specifically, GSH-*o*-phthalaldehyde method[6] was used. The sample dried under reduced pressure was weighed accurately, dissolved in distilled water containing sodium edetate at the concentration of 10 μg/ml, and the sample solution of about 10 μg/ml concentration was prepared. The sample solution (3 ml) was pipetted into a 25 ml flask, 3 ml of 0.3 M phosphate buffer (pH 8.0) and 1 ml of 0.1% *o*-phthalaldehyde MeOH solution were added and the solution was instantly diluted to 25 ml with distilled water. After 15 min, the fluorescence intensity was determined at 420 nm, under the excitation at 365 nm.

Estimation of Reductivity——A sample dried under reduced pressure was weighed accurately, dissolved in distilled water to make a sample solution of about 3 mg/ml concentration, 1 ml of this solution was pipetted into a flask and 10 ml of 0.1 N HCl was added. This solution was cooled to 0 to 7° in an ice-bath. To the solution, 1 ml of 5% KI solution and starch reagent were added, and the titration was made with 0.01 N KIO_3 solution in an ice-bath.[7]

Estimation of Water Content——The weighed sample (80 to 100 mg) was dried over P_2O_5 under reduced pressure until a constant weight was obtained, and the weight loss was estimated as water content of the sample.

Paper Partition Chromatography (PPC) of Ninhydrin-positive Substances——The weighed sample (10 mg) was dissolved in 2 ml of 4% aqueous solution of N-ethylmaleimide and 10 μl of this solution was spotted on Toyo Roshi No. 50 paper. The developing solvents used were mixture of *n*-BuOH, AcOH, and H_2O (2:1:1) and a mixture of phenol and H_2O (454:114). The developed chromatogram was air-dried and colored by ninhydrin.[8]

Estimation of Cysteinylglycine——PPC of the sample was performed quantitatively with a developing solvent system of *n*-BuOH–AcOH–H_2O as described above. The standard solutions of cysteinylglycine were developed at the same time. The color intensity of the sample and the standard was compared and the amount in the sample was estimated.

Determination of Specific Surface Area——The specific surface area was determined by the B.E.T. method with MONOSORB (Yuasa-Quantachrome Surface Area Analyzer).

Results

Time Course of Decomposition and Water Content

The time courses of percentage of remaining GSH and water content in α (lot 1) and β (lot 1) forms after storage at 80° and 76.4% R.H. are shown in Fig. 1 as an example of kinetic

4) V. du Vigneaud and G.L. Miller, *Biochem. Prep.*, 2, 91 (1952).
5) J. Hasegawa, M. Hanano, and S. Awazu, *Chem. Pharm. Bull.* (Tokyo), 23, 86 (1975).
6) V.H. Cohn and J. Lyle, *Anal. Biochem.*, **14**, 434 (1966).
7) Y. Kuroiwa, *Nippon Nogeikagaku Kaishi*, **25**, 93 (1951).
8) S. Colowick, D. R. Schwarz, A. Lazarow, E. Stadtman, E. Racker, and H. Waelsch (ed.), "Glutathione, A Symposium," Academic Press, Inc., New York, 1954, p. 79.

data. In Fig. 2, the courses of α (lot 1, 2 and 3) and β (lot 1 and 2) forms at 80° and 76.4% R.H. are shown. The specific surface areas for lot 1, 2 and 3 of α form were 1.13, 2.16 and 2.21 m^2/g, respectively, and those for lot 1 and 2 of β form were 5.70 and 5.65 m^2/g, respectively. The decomposition rates of α and β forms increased markedly with increasing temperature and relative humidity throughout the experiment. In the early period, the decomposition of both forms was very slow and a kind of induction period was observed. After the induction period, it was accelerated exceedingly. In the case of α form, the rate always increased markedly, after the decomposition ratio reached to 10 to 20%. The reproducibility of decomposition pattern was indicated for various lots of α and β forms as shown in Fig. 2. Water content of the samples increased proportionally with increase of the decomposition.

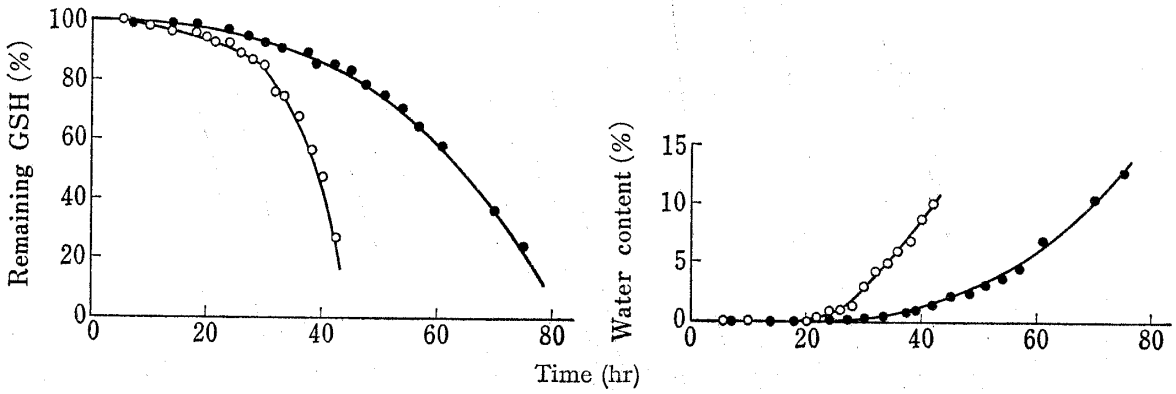

Fig. 1. Time Courses of Decomposition and Water Content of Glutathione Crystal at 80° and 76.4% R.H.

—○—: α form(lot 1), —●—: β form(lot 1).

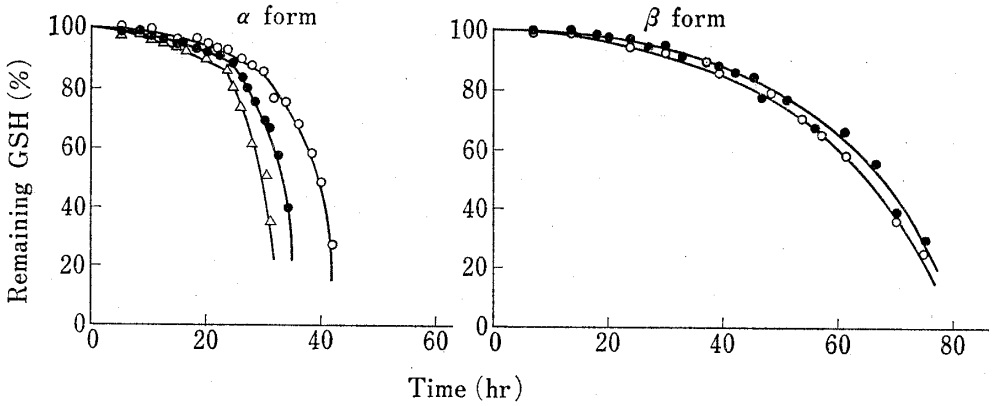

Fig. 2. Decomposition Time Courses of Glutathione Crystal at 80° and 76.4% R.H.

—○—: lot 1, —●—: lot 2, —△—: lot 3.

Empirical Formula

Decomposition time courses of GSH samples showed the pattern of an autocatalytic decomposition,[9] because the logarithmic plots of percentage decomposed *vs.* reaction time were as shown in Fig. 3. In the case of the β form, the plots were found to fit a straight line from the start to the end. In the case of α form, however, the straight line always broke when the decomposition ratio reached to 10 to 20%, so that two phases, early and late stages, were recognized. In the late stage, the decomposition were markedly accelerated. Eq. (1) expresses the time course of decomposition,

9) *a*) W.E. Garner (ed.), "Chemistry of the Solid State," Butter-worth Scientific Publication, London, 1955, p. 191; *b*) T. Kagiya, "Kagaku-hanno no Sokudoronteki Kenkyuho," Kagakudojin, Kyoto, 1970, p. 408.

$$\log \alpha = l \log t - a \quad (1)$$

where α is the percentage of decomposed GSH, t is the reaction time, l and a are parameters. Values of l and a were estimated by the least square method regarding the data shown in Fig. 3, and the resultant values are listed in Table I. The l values of both forms in the early stage varied from 2 to 4. At each temperature, unambiguous regularity of the l values could not be found. The l values of α form in the late stage varied from 4 to 10, and were about 2 to 3 times large than those in the early stage.

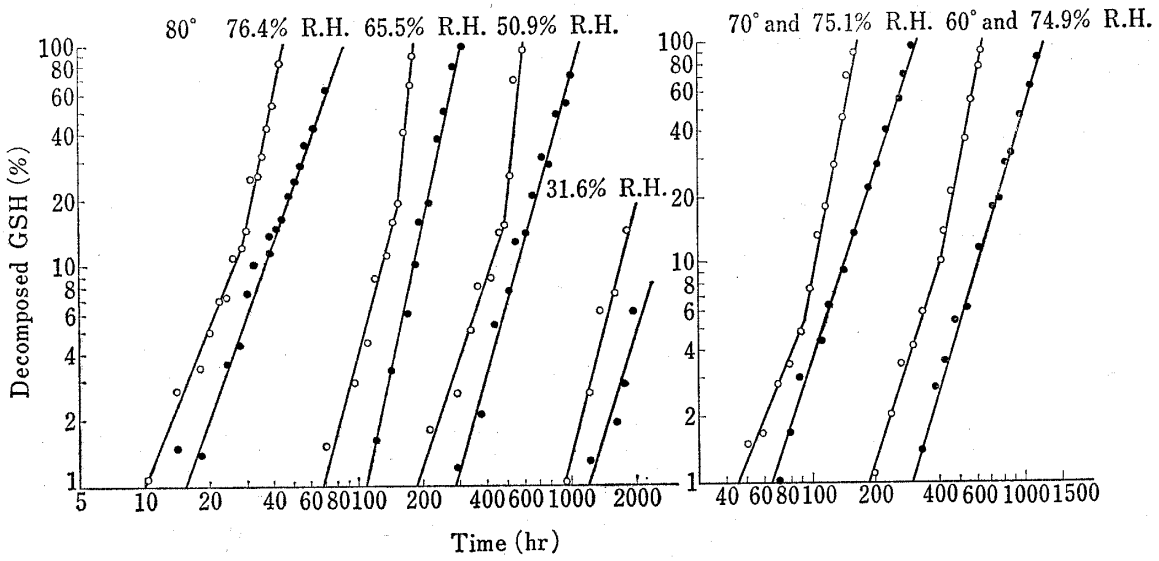

Fig. 3. Logarithmic Plots of Percentage of Decomposed GSH against Reaction Time
—○—: α form (lot 1), —●—; β form (lot 1).

Table I. Estimated Parameters, l and a, for Kinetic Equation[a] of Glutathione Decomposition in Solid State

Glutathione crystal form	Temperature (°C)	Relative humidity (%)	Lot No.	l	a
α (Early stage)	80	76.4	1	2.33	2.30
	80	76.4	2	2.05	1.78
	80	76.4	3	2.22	1.82
	80	65.5	1	3.46	6.27
	80	50.9	1	2.84	6.45
	80	31.6	1	3.75	11.10
	70	75.1	1	2.32	3.84
	60	74.9	1	2.94	6.68
α (Late stage)	80	76.4	1	4.41	5.32
	80	76.4	2	4.46	5.09
	80	76.4	3	4.21	4.55
	80	65.5	1	9.65	19.86
	80	50.9	1	10.12	26.09
	70	75.1	1	5.02	9.16
	60	74.9	1	4.93	11.89
β	80	76.4	1	2.59	3.01
	80	76.4	2	2.74	3.30
	80	65.5	1	4.33	8.75
	80	50.9	1	3.36	8.18
	80	31.6	1	3.00	9.22
	70	75.1	1	2.95	5.39
	60	74.9	1	3.14	7.76

a) $\log \alpha = l \log t - a$ [Eq. (1)].

Effect of Oxygen on Decomposition

When air in the reaction vessel is replaced with N_2 gas, decomposition of both forms after storage at 80° and 76.4% R.H. is shown in Fig. 4. The lines in this graph were calculated from the parameters under the corresponding aerobic conditions. The anaerobic decomposition data were plotted close to the lines, respectively.

Effect of Drying in Mid Course on Decomposition

In mid course of decomposition at 80° and 76.4% R.H., the sample was removed and dried over P_2O_5 under reduced pressure. The sample was reset in the reaction vessel under the same condition as before removal and the decomposition was allowed to proceed. Fig. 5 shows the plots of percentage decomposed *vs.* total time during the initial storage and after the reset. They were plotted close to the lines which are the calculated curves reproduced from Fig. 3 without drying in mid course of the decomposition.

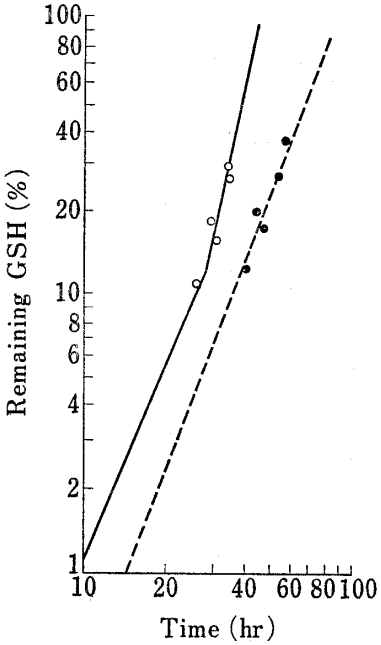

Fig. 4. Anaerobic Decomposition of Glutathione in Solid State at 80° and 76.4 % R.H.

○ and ●: anaerobic decomposition of α form (lot 1), and β form (lot 1), respectively.
—— and ------: calculated curves for aerobic decomposition of α form (lot 1) and β form (lot 1), respectively, which are reproduced from Fig. 3 at the correspondent condition.

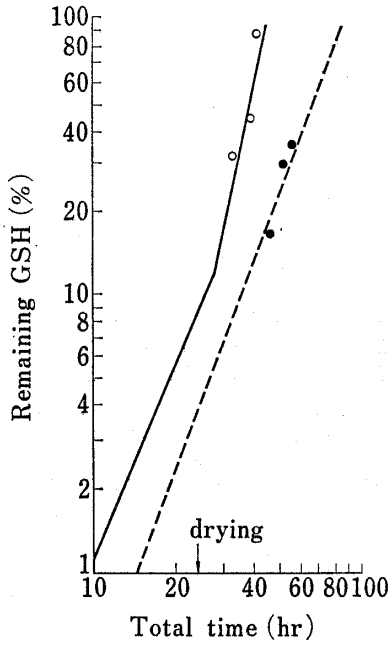

Fig. 5. Effect of Drying in Mid Courses on the Decomposition of Glutathione in Solid State at 80° and 76.4% R.H.

○ and ●: decomposition of α form (lot 1) and β form (lot 1) after drying and reset.
—— and ------: calculated curves for decomposition of α form (lot 1) and β form (lot 1), respectively, without drying in mid course which are reproduced from Fig. 3.

Effect of Salt Addition on Decomposition

Crystals of NaBr and $NaNO_3$ were ground in an agate mortar with a pestle and sieved in order to obtain a 200 mesh fraction. To 95 parts of the β form (lot 1), 5 parts of NaBr or $NaNO_3$ as described above were added respectively and mixed thoroughly. The remaining percentage of GSH in this sample after storage at 70° and 75.1% R.H. is shown in Fig. 6. Addition of either salt markedly accelerated the decomposition.

Cysteinylglycine Formed and Reductivity

Amount of cysteinylglycine formed and reductivity of samples after storage were estimated. In Fig. 7, the percentage decreased of the reductivity determined by iodometry was plotted against the decomposition ratio of GSH in the same sample measured by *o*-phthal-

dehyde method. In Fig. 8, the cysteinylglycine formed was plotted in the same manner. Reductivity of sample, due to its SH-group, decreased with increasing decomposition of GSH, but decrease of the former was less than the increase of decomposition to some extent. Formation ratio of cysteinylglycine was much less than the decomposition ratio of GSH.

Decomposition Products

(1) PPC of Ninhydrin-positive Substances. The PPC of the samples of α and β forms after storage at 80° and 76.4% R.H. for 34 and 48 hr are shown in Fig. 9 as an example. The

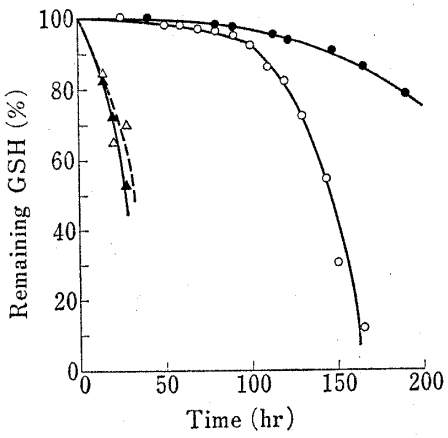

Fig. 6. Effect of Inorganic Salts on Decomposition of Glutathione β Form at 70° and 75.1% R.H.

---△---: GSH β form (lot 1)+NaBr (95: 5).
—▲—: GSH β form (lot 1)+$NaNO_3$(95: 5).
—○—: GSH α form (lot 1) without salts.
—●—: GSH β form (lot 1) without salts.

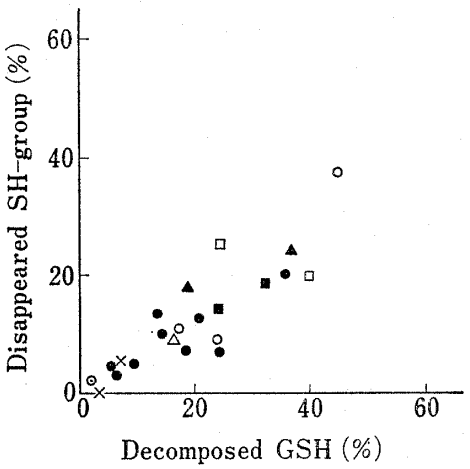

Fig. 7. Decrease of SH-group in Decomposition of Glutathione in Solid State at 80°

Relative humidity (%) in the case of α form (lot 1) are 31.6 (×), 50.9 (□), 65.5 (△) and 76.4 (○); in the case of β form (lot 1), 31.6 (⊙), 50.9 (■), 65.5 (▲) and 76.4 (●).

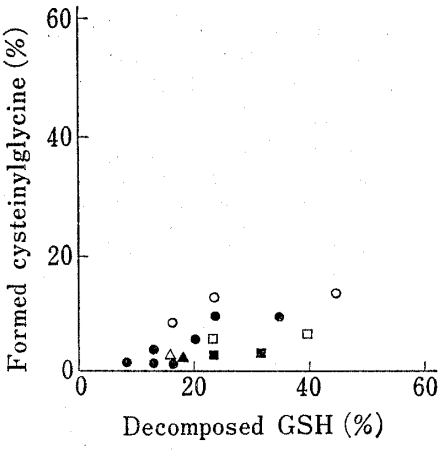

Fig. 8. Formation of Cysteinylglycine in Decomposition of Glutathione in Solid State at 80°

See Fig. 7 for keys.

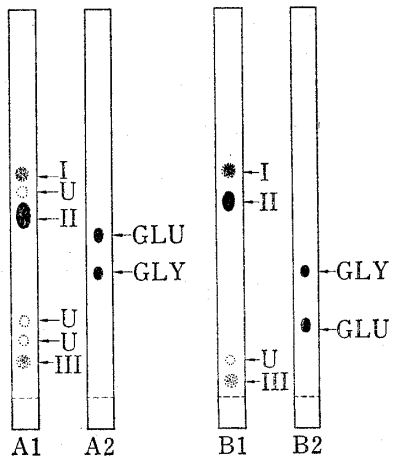

Fig. 9. Paper Partition Chromatogram of Partially Decomposed Glutathione in Solid State (Ninhydrin-positive substances)

Al and Bl are samples. A2 and B2 are mixtures of glycine and glutamic acid.
Developing solvent:
A1 and A2, *n*-BuOH·AcOH·H_2O (2: 1: 1); B1 and B2, Phenol·H_2O (454: 114).
I: cysteinylglycine, II: glutathione, III: oxidized glutathione, GLU: glutamic acid, GLY: glycine and U: unknown.

chromatogram developed with a mixture of *n*-BuOH, AcOH and H_2O showed the spots of GSH, cysteinylglycine and oxidized GSH, besides three very pale pink spots of unknown substances. The spots of glutamic acid and glycine were never found in the chromatogram of any sample. Chromatogram developed with a mixture of phenol and H_2O showed spots of GSH, cysteinylglycine, oxidized GSH, and an unknown substance, but none of glutamic acid or glycine.

(2) Water-insoluble Product. With increasing decomposition of GSH, a water-insoluble substance was produced. A sample of β form (0.25 g) after storage at 80° and 50.6% R.H., was dissolved in 15 ml of distilled water with warming, filtrated and allowed to stand overnight in a refrigerator. The crystallized product was collected by filtration, washed with a small amount of water, dried under reduced pressure, and was identified with 3,3'-(dithiodimethylene)-bis(2,5-piperazinedione)[10] (I) that is an oxidized product of 3-(mercaptomethyl)-2,5-piperazinedione[10b,11] (II) which is formed by intramolecular condensation of cysteinylglycine. *Anal.* Calcd., for $C_{10}H_{14}N_4O_4S_2$: C, 37.73; H, 4.43; N, 17.59; S, 20.14. Found: C, 37.83; H, 4.38; N, 17.33; S, 21.10. This product was ninhydrin-negative. It was hydrolyzed with 1 N HCl at 105 to 110° in a sealed tube for 4 hr. The hydrolyzate was neutralized with NaOH, and the PPC was developed with a mixture of *n*-BuOH, AcOH and H_2O, (2: 1: 1), or a mixture of *n*-PrOH and H_2O (4: 1), and colored with ninhydrin reagent. *Rf* values of two ninhydrin-positive spots for the sample solution agreed with those of cystine and glycine.

Discussion

Kinetics of Decomposition

The kinetics of a solid decomposed autocatalytically, such as salicylic acid derivatives, often exhibits decelerated period. On the other hand, two forms of GSH do not show decelerated reaction period. The decomposition can be expressed by Eq. (1), as shown in Fig. 3. The percentage of decomposed β form was expressed by one equation with the constant parameters until decomposed to about 90%, whereas that of α form was expressed by two equations with the respective parameters, *i.e.* one for the early stage and the other for the late stage. The results shown in Fig. 2 indicate that decomposition rates are almost equal provided the specific surface area of lots is similar to one another and tend to increase with increasing area. The area in α form was found to be smaller than in β form in this study, but the decomposition in β form was nevertheless less than in α form. The decomposition reaction is considered to occur on the crystal surface and, therefore, the results shown in Fig. 4 indicate that the β form is more stable than the α form. The molecule of GSH contains an SH-group which is thought to be easily oxidized, but the effect of oxygen in air on the decomposition rate is almost negligible. When the sample was removed in mid course of decomposition, dried under reduced pressure for the exclusion of water and volatiles, and decomposed again under the same condition, the decomposition rate after the reset was almost similar to that of the experiment without drying at the same duration of the reaction. The amount of water or volatiles in the sample, therefore, is not considered to be the principal factor controlling the decomposition rate, but the state of the surface as well as temperature, relative humidity, and specific surface area.

At each reaction time, the actual decomposition rate, $(d\alpha/dt)_I$, is expressed by Eq. (2) derived from Eq. (1).

$$\left(\frac{d\alpha}{dt}\right)_I = \frac{l-1}{10^a} t^{l-1} \qquad (2)$$

10) *a*) J.C. Sheehan and D.H. Yang, *J. Am. Chem. Soc.*, **80**, 1158 (1958); *b*) L. Prizont, *Rev. Farm.* (Buenos Aires), **97**, 5 (1955) [*Chem. Abstr.*, **50**, 1207*h* (1956)].

11) E.M. Crook (ed.), "Glutathione, Biochemical Society Symposium No. 17," Cambridge University Press, 1959, p. 9.

When the reaction model is assumed that the whole amount of water contained in a sample forms a thick saturated solution layer of GSH on the surface and decomposition of GSH proceeds in this layer, the decomposition rate can be calculated by Eq. (3) and is termed as the assumed decomposition rate, $(d\alpha/dt)_{II}$ (%/hr).

$$\left(\frac{d\alpha}{dt}\right)_{II} = \frac{A/100 \times W \times S/100 \times K \times 100}{W} = \frac{A \times S \times K}{100} \tag{3}$$

Where A is the water content (%), W is the weight of dried sample, S is the solubility (g/100 g H_2O), and K is the rate constant as the first order in saturated solution (hr^{-1}). The solubilities and the rate constants in saturated solution were determined to calculate the assumed decomposition rate. The solubility was determined at 25, 35, 45 and 55° because of the rapid degradation at higher temperature. The results are shown in Table II. Fig. 10 illustrates the linear plot of the logarithm of solubility *vs.* reciprocal of temperature. The values of solubility at 60, 70, and 80° which are estimated by extrapolation are shown in Table II.

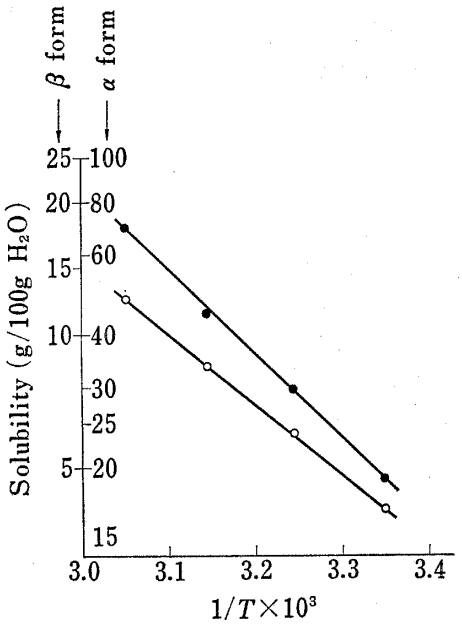

Fig. 10. Temperature Dependence of Solubility of Glutathione
—○—: α form, —●—: β form.

Table II. Solubility of Glutathione Crystals in Water

	Solubility (g/100 g H_2O)						
	Obtained				Calculated		
Temperature	25°	35°	45°	50°	60°	70°	80°
α Form	16.20	23.77	33.44	48.28	56.5	77.3	104.0
β Form	4.68	7.39	11.34	17.32	21.0	30.7	44.0

Table III. Decomposition Rate Constant of Glutathione in Saturated Aqueous Solution

	Decomposition rate constant (hr^{-1})		
Temperature	60°	70°	80°
α Form	7.64×10^{-2}	1.96×10^{-1}	4.80×10^{-1}
β Form	6.28×10^{-2}	1.60×10^{-1}	3.87×10^{-1}

The rate constants of GSH decomposition in aqueous solution have been reported to increase with increasing concentration.[12] The apparent rate constants as the first order in saturated solution at 60, 70, and 80° were determined as shown in Table III. The actual decomposition rate was calculated by Eq. (2) using the parameters listed in Table I, and the assumed decomposition rate was calculated by Eq. (3) using the data of water content, the solubility, and the rate constant in saturated solution. Both calculated values were plotted as a function of time in Fig. 11. The assumed rate of the β form is less than the actual rate under each condition. The difference is marked during the first half of reaction time. If decomposition occurs only in the aqueous solution layer adsorbed on the crystal surface, the assumed rate should be the maximum rate in this model because, in the actual decomposition, the amount of water and the concentration of GSH in the reacting solution must be less to some extent than the total water content of the sample and its solubility. Consequently,

12) T. Matsuki and Y. Sumi, *Nippon Nogeikagaku Kaishi*, **47**, 185 (1973).

it seems to be impossible that decomposition of the β form is presumed to occur in the saturated solution layer on the surface. It should be considered to be the so-called gas-solid reaction. In the early stage, the α form presumably undergoes decomposition similarly as the β form, but in the late stage the actual rate approaches to the assumed rate. It would be assumed that the α form commences to undergo decomposition in the liquid layer partially after the early stage. As shown in Fig. 6, decomposition of the β form added with NaBr or $NaNO_3$ was accelerated at 70° and 75.1% R.H. Since these salts fuse with deliquescence, an aqueous solution layer should be formed on the crystal surface. In the newly formed solution layer, GSH will be dissolved and decomposed. Because of the sufficiently rapid reaction in solution, the sample of β form added with these salts is considered to be decomposed very rapidly. This experimental result on β form also supports the possibility that the α form is decomposed in the liquid layer at the late stage.

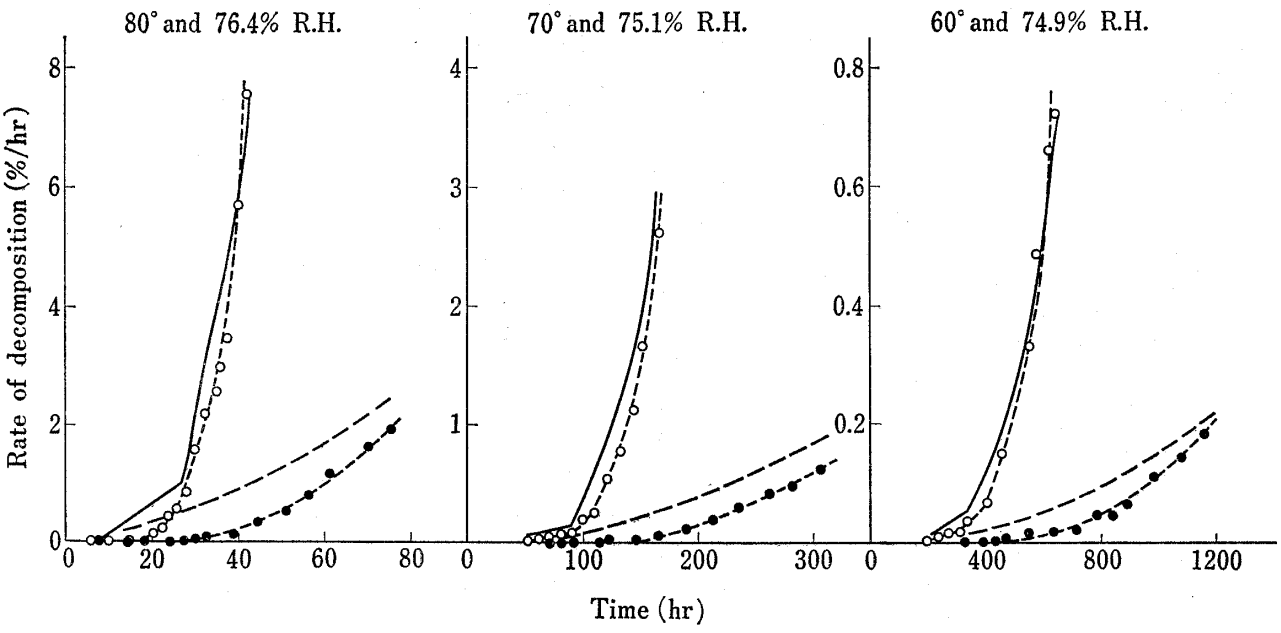

Fig. 11. Comparison between the Actual Decomposition Rate and the Assumed Rate by the Liquid Layer Reaction Model

---○--- and —●—: rate estimated by the liquid layer reaction in α form (lot 1) and β form (lot 1), respectively.
— and ---: actual rate calculated by the kinetic equation in α form (lot 1) and β form (lot 1), respectively.

Effect of Relative Humidity

Decomposition of both forms of GSH increased markedly with increasing the relative humidity as shown in Fig. 3. The half life, t_{50}, and the time for 90% remaining, t_{90}, were calculated from Eq. (1) using the parameters listed in Table I. The reciprocals of the time can be taken as a relative index of the decomposition rate. Their reciprocals at 80° were plotted against the relative humidity in Fig. 12. The half life of the α form is omitted in this plot because of the possibly different mechanism of decomposition in the late stage. In the cases of *p*-aminosalicylic acid (PAS),[13] acetyl-5-nitrosalicylic acid (ANSA),[5] and aspirin,[14] the reciprocals calculated from the reported data at 80° were plotted in a similar manner, respectively. Fig. 12 shows that the decomposition of both forms is markedly affected by the relative humidity, compared to other compounds.

The apparent reaction order, n, was defined by Eq. (4) or (5).

13) S.S. Kornblm and B.J. Sciarrone, *J. Pharm. Sci.*, **53**, 935 (1964).
14) L.J. Lesson and A.M. Mattocks, *J. Am. Pharm. Assoc.*, **47**, 329 (1958).

$$(P_i/P_s)^n = (1/t_{50})_i/(1/t_{50})_s \quad (4)$$

$$(P_i/P_s)^n = (1/t_{90})_i/(1/t_{90})_s \quad (5)$$

where P is the relative humidity and the suffixes s and i indicate the decomposition experiments at the lowest and higher humidity, respectively. The calculated n values are shown in Table IV. The values of both forms had a tendency to increase with increasing relative humidity, and were about 3 to 5. On the other hand, the n value for ANSA and PAS was approximately 1, and was 1 to 2 for aspirin. Because n is 1, the decomposition rate of ANSA and PAS is considered to be apparently proportional to humidity in atmosphere, and the rate of aspirin to be proportional to 1—2 powers of it. The n values of GSH support that its decomposition is a gas-solid reaction as mentioned above, and it is suggested that the GSH molecule takes more gaseous water molecules than the molecule of salicylic acid derivative.

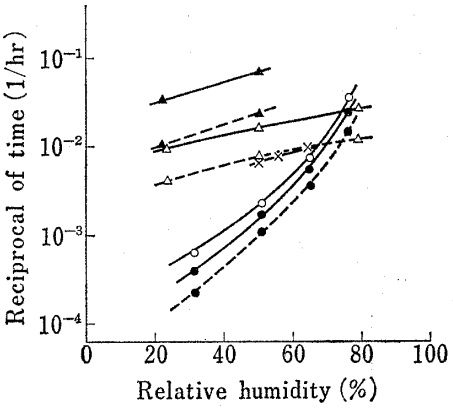

Fig. 12. Relationship of the Half Life and the Time for 90% Remaining *vs.* Relative Humidity at 80° for Decomposition of Glutathione and Other Compounds

---: half life, —: time for 90% remaining.
○: glutathione-α form (lot 1).
●: glutathione-β form (lot 1).
▲: *p*-aminosalicylic acid.[13]
△: acetyl-5-nitro-salicylic acid.[5]
×: aspirin.[14]

Effect of Temperature

The reciprocals of t_{50} and t_{90} at 60, 70, and 80° were calculated from the data at 75 to 76% R.H. shown in Fig. 3. Arrhenius plots of logarithms of $1/t_{50}$ and $1/t_{90}$ were found to be linear. The apparent activation energies calculated from the plots are shown in Table V. The values of both forms are 32 to 33 kcal/mol, while those of PAS[13] and ANSA[5] are 29.5 and 30 kcal/mol, respectively.

With regard to the decomposition rate constant (K) in saturated solution of GSH, Arrhenius plots of the logarithm of K were also linear. The apparent activation energies calculated from the plots were 21.6 and 21.4 kcal/mol in the solution for α and β forms, respectively. The energies in solid are greater by 11 to 12 kcal/mol than in solution.

Table IV. Apparent Reaction Orders, n Values, of Kinetic Equation in Decomposition of Various Crystals

Crystals	Reciprocal[a] of the time	Relative[b] humidity (%)	Relative humidity (%)[c] 50.9	56.0	65.5	76.4	79.5
GSH α form (lot 1)	$1/t_{90}$	31.6	2.9	n.d.	3.5	4.7	n.d.
GSH β form (lot 1)	$1/t_{90}$	31.6	3.2	n.d.	3.6	4.8	n.d.
	$1/t_{50}$	31.6	3.4	n.d.	3.9	4.6	n.d.
Acetyl-5-nitrosalicylic acid[5]	$1/t_{90}$	23.3	0.7	n.d.	n.d.	n.d.	1.0
	$1/t_{50}$	23.3	0.9	n.d.	n.d.	n.d.	1.1
Aspirin[14]	$1/t_{90}$	50.9	n.d.	2.0	1.4	n.d.	n.d.
p-Aminosalicylic acid[13]	$1/t_{90}$	22.8	1.0	n.d.	n.d.	n.d.	n.d.
	$1/t_{50}$	22.8	1.0	n.d.	n.d.	n.d.	n.d.

a) t_{50}, half life; t_{90}, time for 90% remaining.
b) The lowest humidity in decomposition experiment.
c) The higher humidity in decomposition experiment for the estimation of the n value.
d) n.d.: not determined.

Decomposition Products

The spots of cysteinylglycine and oxidized GSH were found on the paper chromatogram colored by ninhydrin, but glutamic acid and glycine were not detected. The absence of glycine

Table V. Apparent Activation Energy of Glutathione Decomposition in Solid State

	Time for estimation	Activation energy (kcal/mol)
α Form	Time for 90% remaining	33.4
β Form	Time for 90% remaining	32.3
	Half life	31.4

reveals that the peptide bond between glycine and cysteine are not broken. Because of the detection for cysteinylglycine, γ-glutamyl bond between glutamic acid and cysteine is considered to be broken. The absence of glutamic acid is because ninhydrin-negative pyroglutamic acid is produced. The very pale color of the spot corresponding to oxidized GSH indicates that a little autoxidation takes place in solid state. It is known that cysteinylglycine undergoes intramolecular condensation followed by dehydration, II is formed by this reaction,[11] and the oxidation of II produces I.[10] The both products are negative to ninhydrin. A water-insoluble and ninhydrin-negative substance was formed by the decomposition of both forms. Both the elemental analysis agreeing with the calculated values for I and the hydrolysis producing cystine and glycine reveal that the water-insoluble substance is I. The formation of cysteinylglycine, which is less than the decomposition of GSH, is considered to be due to further decomposition to I and II. With the progress of decomposition, the sample smelt of mercaptan-like odor, because H_2S and the other smelling compounds were produced from GSH. In spite of a little oxidation, the decrease in reductivity was large. It was partially due to the production of I and H_2S.

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