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Photo-promoted Hypochlorite Oxidation of α-Amino Acids. Kinetics and Irradiation Effect for the Strecker Degradation[1)]

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Hypochlorite oxidation of simple α-amino acids (the Strecker degradation) in aqueous solutions has been studied in the dark and under UV-irradiation. UV-spectra, iodometry, and amino acid determination (2,4-dinitrofluorobenzene method) suggest that the intermediate *N*-chloro amino acid is formed quickly at an initial stage, then its slow oxidative decomposition takes place to give aldehyde, carbon dioxide, and ammonia. The mechanism is also supported by the fact that the oxidation follows the first-order rate expression; $-d[RCH(NH_2)CO_2H]/dt = k_1[RCH(NHCl)CO_2H]$. UV-irradiation was found to promote remarkably the degradation of *N*-chloro amino acid. When equimolar amounts of amino acid and hypochlorite were used, the products such as unreacted amino acid, ammonia, and aldehyde were analogous to those of the dark reaction. The use of a large excess of hypochlorite under irradiation enables complete oxidative degradation of amino acid to carbon dioxide, water, and nitrogen. Its application to waste water treatment is discussed.

Simple α-amino acids often present in municipal waste water may be one of the major organic pollutants causing serious eutrophication in seas and lakes. Treatment of α-amino acids with sodium hypochlorite in the dark is known to cause the Strecker degradation leading to carbon dioxide, ammonia, and aldehydes possessing one less carbon atom.[2)] Langheld postulated a mechanism involving *N*-chloro amino acid (Eq. 1),[3)] but only limited information is available as to the kinetics and mechanism.[4,5)]

$$\mathrm{RCHCO_2H} \xrightarrow[\mathrm{NH_2}]{\mathrm{NaOCl}} \mathrm{RCHCO_2Na} \xrightarrow[\mathrm{NHCl}]{-\mathrm{HCl}} \mathrm{RCCO_2Na} \xrightarrow[\mathrm{NH}]{} \xrightarrow{-\mathrm{CO_2}}$$

$$\mathrm{RCH{=}NH} \xrightarrow{\mathrm{H_2O}} \mathrm{RCHO} + \mathrm{NH_3} \qquad (1)$$

Sodium hypochlorite exhibits under UV-irradiation a high oxidation power, decomposing effectively organic compounds and ammonia.[6)] It has been reported that irradiated hypochlorite effectively oxidizes some sulfonic acids,[7)] ethers,[8)] and aliphatic acids,[9)] which are fairly stable against hypochlorite in the dark. Hypochlorite oxidation of ammonia to nitrogen is greatly accelerated by irradiation.[10)] This photo-oxidation method might be important for waste water treatment, in which pollutants should be eliminated completely.

The present paper describes the kinetic behavior of the Strecker degradation in order to clarify the rate-determining step and also the effect of irradiation. We have found that on irradiation in the presence of large excess of NaOCl amino acids are decomposed rapidly into carbon dioxide and nitrogen.

Results and Discussion

Dark Reaction and Its Kinetics. The dark reactions of 5—10 mM (1 mM = 10^{-3} mol dm^{-3}) glycine, alanine, and valine with NaOCl were examined in various buffer solutions at room temperature by monitoring the concentration of amino acid and oxidant. Analysis was based on (A) iodometry which gives concentration of hypochlorite and even *N*-chloro amino acids, and (B) determination of amino acids by 2,4-dinitrofluorobenzene method after converting *N*-chloro amino acids into amino acids. When hypochlorite was used in a 5—10-fold amount to amino acid at various pH, degradation of amino acid at pH 3—11 was too fast to determine the accurate rate of oxidation.

Table 1. First-order rate constants (k_1)[a)] for the dark reaction of α-amino acids with NaOCl at 20 °C

Amino acid	pH	$k_1 \times 10^5/s^{-1}$
Glycine	7.0	0.74
Alanine	6.0	7.5
	7.0	8.0 (9.8)
	9.0	8.5 (9.0)
Valine	7.0	10.3 (12.0)

a) Determined by iodometry (method A). Figures in parentheses are based on consumed amino acid (method B).

When equimolar (1:1 mol) amounts of hypochlorite and amino acid were used, oxidation became slow enough to be followed by spectrophotometry, iodometry, and amino acid determination. The UV spectroscopy of the equimolar solution indicated the instantaneous and quantitative formation of *N*-monochloro amino acid at pH 5—11. Each *N*-chloro amino acid was assigned by its absorption maximum at *ca.* 250 nm and its observed molar absorption coefficient (see Experimental). In acidic solutions at pH 2—4, *N,N*-dichloro amino acids were detected.

For the reaction of one mole of amino acid with one mole of hypochlorite, the change in amino acid concentration (c) was measured from time to time by methods (A) and (B). The observed values were found to fit the first-order rate expression ($\ln c_0/c = k_1 t$), though an apparent deviation from the first-order was observed at conversion higher than 70%. The first-order rate constants (k_1) analogous in methods (A) and (B) are given in Table 1.

The observed first-order rate suggests that the Strecker degradation with hypochlorite involves the decomposition of *N*-chloro amino acids at a rate-determining step. Thus the rate is first-order with respect to *N*-chloro amino acid:

$-d[RCH(NH_2)CO_2H]/dt = k_1[RCH(NHCl)CO_2H]$.

TABLE 2. PHOTOLYSIS AND DARK REACTION OF AMINO ACID WITH HYPOCHLORITE[a]

Light	Substrate	Amino acid		Ammonia		Aldehyde	
		Unreacted (mM)	Consumed (%)	Produced (mM)	Yield[b] (%)	Produced (mM)	Yield[b] (%)
>290 nm	Glycine	1.50	70	3.57	102	1.35	39
	Alanine	1.07	78	3.53	90	c)	
	Valine	0.85	83	4.28	97	c)	
None	Glycine	1.90	62	2.30	74	0.50	17
	Alanine	0.96	81	4.11	102	c)	
	Valine	0.75	85	4.61	108	c)	

a) Initial concentration; amino acid 5.0 mM; NaOCl 5.02 mM. Photolysis at 22 °C for 2.0 h, dark reaction at 25±2°C for 52 h. b) Yield based on consumed amino acid. c) Unsuccessful in quantitative determination, but positive to 2,4-dinitrophenylhydrazine reagent.

This is in accord with the UV spectroscopic measurements indicative of rapid and quantitative *N*-chlorination of amino acid. Though monochlorination of amino acid with chloramine T giving nitrile is slow,[5] the *N*-chlorination with hypochlorite leading to Strecker degradation is very rapid as is seen in ammonia and alkylamines.[11]

Chloramine NH_2Cl is in equilibrium with ammonia and hypochlorite.[12] Thus, the following mechanism is proposed:

$$\underset{\underset{NH_2}{|}}{RCHCO_2H} + HOCl \underset{\text{fast}}{\overset{\text{very fast}}{\rightleftharpoons}} \underset{\underset{NHCl}{|}}{RCHCO_2H} + H_2O \quad (2a)$$

$$\underset{\underset{NHCl}{|}}{RCHCO_2H} \xrightarrow{\text{slow}} RCHO + NH_3 + CO_2. \quad (2b)$$

Fast *N*-chlorination takes place with the equilibration of Eq. 2a (the equilibrium is extremely shifted to the right), followed by slow decomposition of *N*-chloro amino acid (Eq. 2b), indicating that the reverse process in pre-equilibrium (Eq. 2a) should be faster than decomposition (Eq. 2b). Otherwise, the second-order rate instead of first-order would have been observed.

The oxidation rate at pH 7 increases in the order glycine<alanine<valine (Table 1). The order might indicate that the electron-releasing group (CH_3) accelerates the reaction by stabilizing the developing positive charge during the course of oxidation. The effect of pH on the rate is not significant.

Photo-oxidation. Equimolar solutions (5—10 mM) of hypochlorite and amino acid were irradiated with light of wavelength>290 nm at pH 2—11. By means of iodometry and UV spectroscopy, *N*-chlorinated amino acids were found to decompose quite readily at any pH. To attain 95% consumption of *N*-chloro amino acid (oxidant), only 15 min was sufficient with an internal irradiation (see Experimental), whereas a period of over two days was necessary with the dark reaction. In spite of the presence of *N,N*-dichloro amino acid at pH 2—4, the decomposition rate was the same as that at pH 5—11; the effect of pH on decomposition rate of *N*-chlorinated amino acids was negligible. This may be due to the rapid interconversion between *N,N*-dichloro amino acid and *N*-chloro amino acid.

$$\underset{\underset{NCl_2}{|}}{RCHCO_2H} + \underset{\underset{NH_2}{|}}{RCHCO_2H} \longrightarrow 2\underset{\underset{NHCl}{|}}{RCHCO_2H} \quad (3)$$

Aldehydes, ammonia, carbon dioxide, and nitrogen were identified as products of the equimolar reaction together with unchanged amino acids. These products are the same as those of the corresponding dark reaction. A parallel experiment between irradiated and dark reactions was conducted with an equimolar solution at pH 7.0 in order to examine their product distribution. The results are summarized in Table 2.

The consumption extent of amino acid is in the order glycine<alanine<valine, irrespective of irradiated and dark reactions. In irradiated and dark reactions (Table 2), each case of amino acid shows similar amounts of unreacted amino acid and produced ammonia. UV irradiation might essentially affect the rate of decomposition of *N*-chlorinated amino acid, accelerating the Strecker degradation. This is energetically possible since light energy supplied as high as 400 kJ/mol surpassed the N–Cl bond energy (about 190 kJ/mol) in *N*-chloro amino acid. Formation of formaldehyde resulted in a lower yield than expected from the stoichiometry (Eq. 1), though high yield was observed for ammonia (Table 2). Reactions of amino acid with one mole of hypochlorite were carried out in the presence of five moles of aldehyde. Increase in the amount of recovered amino acid was observed. Aldehydes produced should reduce *N*-chloro amino acid to the original amino acid.

Another kind of irradiation was carried out in order to attain complete oxidation of amino acid in water. According to the theoretical equation (*e.g.* Eq. 4), 5, 8, and 15 equivalent amounts (only slight excess) of hypochlorite were irradiated (>290 nm) with glycine, alanine, and valine, respectively.

$$2H_2NCH_2CO_2H + 9NaOCl \longrightarrow 4CO_2 + 5H_2O + N_2 + 9NaCl \quad (4)$$

Iodometry showed rapid comsumption of oxidants such as OCl^- (alkaline solution) or HOCl (in acidic solution). After completion of the reaction, no significant amount of starting amino acids nor their primary products (ammonia and aldehydes) was detected. Estimation of carbon dioxide produced gave 60, 43, and 23% yields for glycine, alanine, and valine, respectively, based on the complete oxidation stoichiometry. Attempts to detect formic acid as a pos-

sible oxidation product of glycine were unsuccesful. However, acetic acid for alanine, and 2-methylpropanoic acid for valine could be detected by analysis of their crude reaction mixtures. Carboxylic acids can be completely oxidized with a large excess of hypochlorite under irradiation,[9] the oxidation of ammonia to nitrogen being considerably accelerated under irradiation.[10] Photochemical oxidation of sulfonic acids,[7] ethers,[8] and carboxylic acids[9] has been attributed to active oxygen species derived from hypochlorite ion. The oxygen atom might take part in the complete oxidation of aldehyde and carboxylic acid. It is evident that the initial stage for amino acid oxidation involves the formation and subsequent decomposition of *N*-chloro amino acid.

Experimental

General. Aqueous sodium hypochlorite (1 M) was prepared by introducing gaseous chlorine to aqueous NaOH (2.1 M) at 0 °C, the concentration of which was determined by iodometry. All amino acids (guaranteed grade) were DL-compounds. A buffer solution of pH 7 for the reactions was prepared with 0.2 M KH_2PO_4 added to NaOH. Commercial reagents (guaranteed grade) were used for analysis. Visible and UV absorption spectra were measured on a Hitachi 124 spectrophotometer in 1 cm cells. The internal irradiation was carried out in a vessel equipped with a Halos 100 W high-pressure mercury lamp, and external irradiation with a merry-go-round apparatus equipped with a Halos 300 W high-pressure mercury lamp.

Analysis. The 2,4-dinitrofluorobenzene (DNFB) method[13] was selected for the determination of amino acid. To a sample adjusted at pH 10 (1+1 ml) was added 1 w/v % 2,4-dinitrofluorobenzene in 2-propanol (1 ml). After being kept at 40 °C for 15 min, 1 M HCl solution (2 ml) of 2-propanol (1:1) was added. The resulting coloration was determined spectrophotometrically at 460 nm. The presence of equal mol of ammonia was found to increase the estimation of glycine, alanine, and valine by 29, 22, or 17±2%, respectively. Hence, we corrected the apparent values of amino acid by substracting the contribution due to eventual amount of ammonia.

The estimation of ammonia was based on the Nessler method[14] according to the procedure of JIS K 0101-1976. Chloramine, which is formed from ammonia and hypochlorite, disturbed the colorimetry at 450 nm. No interference was observed in the presence of amino acids. The presence of equimolar formaldehyde in an ammonia solution gave positive 28±3% error, acetaldehyde and 2-methylpropanal giving only *ca.* 5% error. No correction was made in the reactions of alanine and valine.

As regards aldehydes, 2,4-dinitrophenylhydrazine method was restricted only to qualitative analysis because of low sensitivity for quantitative analysis. Chromotropic acid reagent[15] was used for determination of formaldehyde, which was confirmed to be specific for formaldehyde. Colorimetry was carried out at 570 nm.

Dark Reaction. An equimolar amount of aqueous sodium hypochlorite was added to buffered solution of amino acid. Immediate iodometry showed a slight decrease in oxidant concentration, the concentration of amino acid based on DNFB method decreasing to 5—10% of the initial concentration. UV inspection of the solution showed a new absorption instead of HOCl (at 235 nm) and OCl^- (at 292 nm). After reduction with sodium thiosulfate solution, the DNFB method gave the expected value. This shows that since *N*-chloro amino acid is formed quickly, it is determined by iodometry but not by the DNFB method. The UV absorption data for *N*-chloro amino acid are as follows: absorption maximum (molar coefficient), *N*-chloroglycine 254 nm (342) (lit,[16] 256 nm (350)), *N*-chloroalanine 252 nm (349) (lit,[16] 253 nm (385)), and *N*-chlorovaline 254 nm (338). Each acidic (pH 2—4) solution of glycine, alanine, and valine containing an equimolar amount of hypochlorite showed an absorption at 302, 293, and 295 nm, respectively. These absorptions were assigned to the corresponding *N,N*-dichloro amino acid, based on lit.[16]

Kinetic runs were conducted with an equimolar solution of amino acid (7.0 mM) and sodium hypochlorite (7.0 mM) in buffer solutions at 20±1 °C. The change of concentration of *N*-chloro amino acid with time was followed by iodometry (method A). In order to know the true consumption of amino acid, determination by the DNFB method was applied after reduction of *N*-chlorinated amino acid with aqueous thiosulfate (method B). The results are given in Table 1.

Photo-oxidation. Internal irradiation was started immediately after addition of aqueous sodium hypochlorite to a buffer solution of glycine, alanine, and valine. Progress of each reaction was followed by iodometry together with UV spectroscopy. The irradiation was continued for 15—20 min. In order to detect aldehydes, the irradiated solution was treated with 2,4-dinitrophenylhydrazine dissolved in sulfuric acid and ethanol. 2,4-Dinitrophenylhydrazones of aldehydes (recrystallized from ethanol), obtained from glycine, alanine, and valine, respectively, showed following mps (mixed mps) and ^{1}H-NMR spectra: formaldehyde 168—169 °C (lit,[17] 166 °C), δ (d_6-DMSO) ppm 6.90 (d, J=12 Hz, N=C(H)–H) 7.71 (d, J=12 Hz, N=C(H)–H) 8.03 (d, J=10 Hz, arom. H^6) 8.44 (d, d, J=10 Hz, J'=2 Hz, H^5) 9.02 (d, J'=2 Hz, H^3) 11.7 (broad s, N–H); acetaldehyde 168—170 °C (lit,[18] 168 °C) δ ($CDCl_3$) 2.15 (d, J=6 Hz, CH_3) 7.63 (q, J=6 Hz, =CH); 2-methylpropanal 180—182 °C (lit,[19] 182 °C) δ ($CDCl_3$) 1.20 (d, J=6 Hz, CH_3) 2.68 (m, >CH). Carbon dioxide was trapped on acidification of the solution with $Ba(OH)_2$ solution as $BaCO_3$. Nitrogen was ascertained by GLC with a column packed with Molecular Sieve 5A.

A solution of amino acid and sodium hypochlorite (50 mM, 5-fold equivalent to glycine, 8-fold to alanine and 15-fold to valine) was irradiated internally at pH 4, 7, and 10 until over 99% hypochlorite was consumed (for 15—20 min), 100% amino acid was consumed with negligible formation of ammonia and aldehydes. By titration using $Ba(OH)_2$, the yield of carbon dioxide was 60, 43, and 23% (average values) for glycine, alanine, and valine, respectively. Several kinds of detection of formic acid (reduction with magnesium in acidic solution followed by coloration of resulting formaldehyde with chromotropic acid; IR spectroscopy of the dried-up material) were attempted, but were unsuccessful. Acetic acid and 2-methylpropanoic acid were detected successfully by GLC method (with a column packed Chromosorb WAW).

Comparison of Products between Photo Reaction and Dark Reactions. To a 10.0 mM amino acid solution prepared in a buffer solution of 7.0 was added in one portion 50 ml of a buffered 10.3 mM NaOCl (existing as HOCl). Two samples of the solution (each 25 ml) were subjected to external irradiation at 22 °C until iodometry showed zero titer (for 2.0 h). The other two quarters were kept in the dark at 25±2 °C for 52 h. In the case of glycine, the oxidant

remained 6% after 52 h. All determinations were performed within 1 d. The results are given in Table 2.

Decomposition of N-*Chloro Amino Acid in the Presence of Aldehydes.* Solutions containing 5.0 mM amino acid, 5.0 mM hypochlorite, and 12.5 mM aldehyde were kept standing in the dark for 2 d. Addition of aldehyde increased the recovery yield of starting amino acid from 19% to 36% in the case of alanine and from 15% to 34% in the case of valine.

References

1) Contribution No. 279.
2) A. Schönberg and R. Moubacher, *Chem. Revs.*, **50**, 261 (1952).
3) K. Langheld, *Ber. Dtsch. Chem. Ges.*, **42**, 2360 (1909).
4) H. L. Slates, D. Taub, C. H. Kuo, and N. L. Wendler, *J. Org. Chem.*, **24**, 1424 (1964); E. E. van Tamelen, V. B. Haarstad, and R. L. Orvis, *Tetrahedron*, **24**, 687 (1968); N. Konigsberg, G. Stevenson, and J. M. Luck, *J. Biol. Chem.*, **235**, 1341 (1960); Y. Matsushima, *Bull. Chem. Soc. Jpn.*, **24**, 17 (1951).
5) A. Kumar, A. K. Bose, and S. P. Mushran, *Monatsch. Chem.*, **106**, 13 (1975).
6) Reviews; Y. Ogata and M. Kimura, *Yuki Gosei Kagaku*, **37**, 581 (1979); S. K. Chakrabartty "Oxidation in Organic Chemistry," ed by W. S. Trahanovsky, Academic Press, New York (1978), Chap. V.
7) M. Nakamura and Y. Ogata, *Bull. Chem. Soc. Jpn.*, **50**, 2396 (1977).
8) Y. Ogata, K. Takagi, and T. Suzuki, *J. Chem. Soc. Perkin Trans. 2*, **1978**, 562.
9) Y. Ogata, T. Suzuki, and K. Takagi, *J. Chem. Soc. Perkin Trans. 2*, **1979**, 1715.
10) M. Kimura and Y. Ogata, *Bull. Chem. Soc. Jpn.*, **53**, 3198 (1980).
11) I. Weil and J. C. Morris, *J. Am. Chem. Soc.*, **71**, 1664 (1949); C. R. Edmond and F. G. Soper, *J. Chem. Soc.*, **1949**, 2942.
12) R. E. Corbett, W. S. Metcalf, and F. G. Soper, *J. Chem. Soc.*, **1953**, 1927.
13) D. T. Dubin, *J. Biol. Chem.*, **235**, 783 (1960); F. C. McIntire, L. M. Clements, and M. Sproull, *Anal. Chem.*, **25**, 1757 (1953).
14) W. Massmann, *Z. Anal. Chem.*, **193**, 332 (1963).
15) E. Eegriwe, *Z. Anal. Chem.*, **110**, 22 (1937); C. E. Bricker and W. A. Vail, *Anal. Chem.*, **22**, 720 (1950).
16) W. S. Metcalf, *J. Chem. Soc.*, **1942**, 148.
17) N. R. Campbell, *Analyst*, **61**, 391 (1936).
18) B. E. Gordon, F. Wopat, Jr., H. D. Burnham, and L. C. Jones, Jr., *Anal. Chem.*, **23**, 1754 (1951).
19) O. L. Brady and G. V. Elsmie, *Analyst*, **51**, 78 (1926).