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Radiolysis of Cystine in Aqueous Solution by Gamma Irradiation

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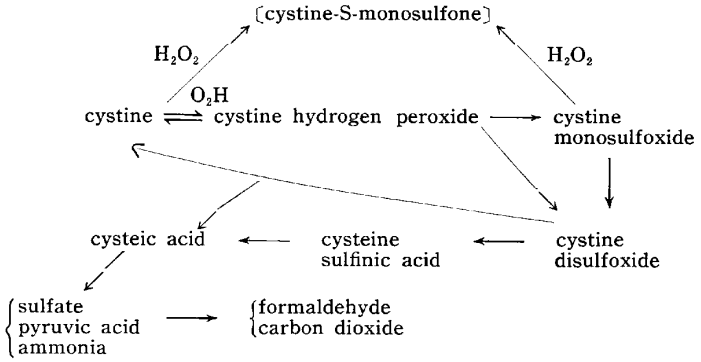
ABSTRACT

From several experiments on oxidative radiolysis of cystine in air-containing aqueous solution a scheme of radiolytic mechanism was proposed as follows:



Chromatographic behaviors of these intermediate products and the radiolytic process were described

INTRODUCTION

Several results on radiolysis of amino acids by ionizing radiation in aqueous solution have been reported recently by the authors[1-4]. From the results a mechanism of oxidative radiolysis of amino acid in air-containing solution has been proposed[1]. As already well known, sulfhydryl compounds such as cysteine[5],

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cysteamine[6] and glutathione[7,8] are more radiosensitive owing to characteristics of the sulfhydryl group, and sulfur-containing compounds such as cystine[9,10], and methionine[11] are also relatively more radiation-labile because of the property of the sulfur[8,9]. The present paper deals with radiolysis of cystine in air-containing aqueous solution by relatively small doses of γ-irradiation. A mechanism of the radiolysis of cystine and its derivatives is discussed.

EXPERIMENTAL

Materials DL-Cystine used in these experiments was extra pure chromatographically and a product of Tanabe Pharmacological Co., Ltd., Osaka. 2,4-Dinitrophenylhydrazine, methylcellosolve, citric acid, sodium hydroxide and hydrochloric acid were obtained from Nakarai Chemicals Co., Ltd., Kyoto. Water used in these experiments was prepared through a ion-exchange resin column and distilled twice by an all glass distillaton apparatus in order to avoid catalytic decomposition of cystine by a small amount of heavy metals in water. For all glass tubes, ampoules, and vessels arrangements were also made in order to avoid the decomposition by impurity of heavy metals.

METHODS

Irradiation of ^{60}Co γ-rays Ten mM solutions of cystine in 0.1 *N* sodium hydroxide were prepared using redistilled water by an all glass distillator in order to avoid some metallo-catalytic decomposition of cystine[12] and put into tubes (12 mm. in dia. and 100 mm. in length). The tubes were sealed and irradiated at room temperature by exposure to γ-irradiation with a dose rate of 1.2×10^5 roentgens per hour in a Two Kilo-curie ^{60}Co Gamma Ray Irradiation Facility[13]. For irradiation of relatively small doses of γ-rays, a 100 curie ^{60}Co Gamma Ray Irradiation Facility for Biochemical Research[14] was used. After the irradiation the tubes were put into a dry ice bath for further measurements.

Determination of carbon dioxide Amounts of gaseous carbon dioxide were measured manometrically by using a Warburg respiratory manometer.

Determination of acidic keto acids Keto acids among carbonyl compounds which were produced in alkaline solutions by the irradiation, were converted to 2, 4-dinitrophenylhydrazones by 0.1 *N* 2, 4-dinitrophenylhydrazine hydrochloride reagent.

The hydrazone mixtures were extracted with ethyl acetate and reextracted with 0.1 *N* sodium carbonate and sodium bicarbonate solution. The alkaline solutions thus obtained were made acidic by addition of hydrochloric acid, and extracted again with ethyl acetate. Qualitative analyses of the hydrazones of keto acids were carried out with a paper chromatography using a developing solvent of ethanol: n-butanol: 0.1 *N* sodium bicarbonate (10: 10: 3 v/v) and No. 50 Toyo filter papers. Quantitative determination of the keto acid hydrazones was performed spectrophotometrically at the wave-length 380 mμ in sodium carbonate and bicarbonate solution

by using Hitachi EPU-2 spectrophotometer.

Determination of volatile organic acids and aldehydes A gaschromatograph, Yanagimoto Model GCF-100, was used for measurements of volatile radiolytic products from the cystine solutions. For the chromatographic estimation, a 2.0 m column in which C-22 coated with polyethyleneglycol 1500 was filled, was operated at 130°C with a carrier nitrogen gas at a running rate of 30 m*l* per min. The results were recorded automatically.

Analysis of ninhydrin positive products An automatic amino acid analyzer, Hitachi Model KLA-2, was used for determination of ninhydrin positive compounds produced from cystine in aqueous solution by the irradiation. For chromatographic separation of cysteic acid and cysteine sulfinic acid, a Dowex-1×8, 200 to 400 mesh, column (0.9 cm in dia. and 35 cm in length) was operated at 50°C and an eluting rate of 30 m*l* per hour with an eluting buffer of 0.01 *N* hydrochloric acid and 0.025 *N* sodium chloride. Acidic and neutral compounds were separated on an Amberlite CG 120×8,325 to 400 mesh column, (0.9 cm in dia. and 150 cm in length) by running 0.2 *N* citrate buffers, pH 3.28 and 4.25, at a rate of 30 m*l* per hour. Ammonia and basic compounds were analyzed by the procedure as follows: after γ-irradiation of the sealed test tubes containing the amino acid materials, 1 *N* hydrochloric acid was added air-tightly into the test tubes by a syringe through a rubber stopper until pH 1 to 2. The acidic solution containing the radiation products was analyzed chromatographically using an Amberlite CG 120, 400 mesh, column (0.9 cm in dia. and 15 cm in length) and a 0.35 *N* citrate buffer, pH 5.28, under the conditions described by Spackman *et al*[15].

Analysis of amines Amines in the radiation products were analyzed by a new method of procedure for analysis of basic amino acids with the following modification[16]: three kinds of buffers, 0.35 *N* citrate, pH 5.28, 0.025 M borate, pH 8.02, and 0.20 M salycilate pH 11.08, were employed successively for the amine analysis at 50°C and a running rate of 30 m*l* per hour. Specimens of cysteine sulfinic acid and cystine sulfoxide were prepared by oxidation of cystine with perbenzoic acid.

RESULTS AND DISCUSSION

1. *Radiolytic oxidation of cystine to cysteine sulfinic acid and to cysteic acid.* Cystine was decomposed oxidatively in the air-containing solutions irradiated with relatively large doses from 10^4 to 10^7 roentgens. Among the radiation products after exposure to several kilo roentgens, cysteine sulfinic acid, $H_2NCH(CH_2SO_2H)COOH$, appeared, and cysteic acid, $H_2NCH(CH_2SO_3H)COOH$, was found to be produced only by exposure to larger doses of irradiation. Further progress of the oxidative radiolysis made it possible to liberate the sulfuryl compound from the sulfuryl intermediates producing carbonyl compounds such as pyruvic acid and end products, carbon dioxide and ammonia. The amounts of these radiation products were increased linearly with increasing radiation doses.

A chromatogrphic separation of cysteine sulfinic acid and cysteic acid was shown

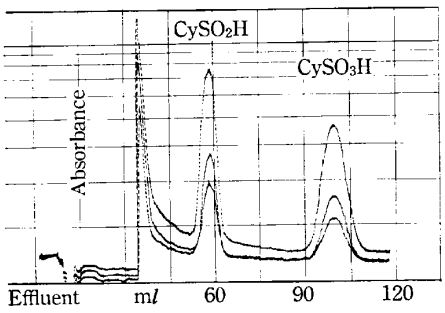

Fig. 1 A chromatographic separation of cysteine sulfinic acid and cysteic acid from the radiolytic products of aqueous cystine on the Dowex-1 column, 0.9×35 cm. The 20 mM cystine solution were exposed to 2×10^6 R of ^{60}Co γ rays. The 0.01 *N* HCl, 0.025 *N* NaCl buffer was used for the elution.

in Fig. 1. On a Dowex-1 column, 0.9 cm in dia. and 35 cm in length, retention volumes were found to be 60 m*l* for cysteine sulfinic acid and 100 m*l* for cysteic acid as shown in Fig. 1.

The formation of the amino sulfuric compounds with the increase of γ-ray doses was presented in Fig. 2. Production of pyruvic acid, ammonia and carbon dioxide by γ-irradiation was also shown in Figs. 2 and 3.

Because any other keto acids could not be found on paper chromatograms the carbonyl product from irradiated cystine was found to be only a pyruvic acid of which 2,4-dinitrophenylhydrazone was separated into two spots of cis- and trans-isomers on the paper chromatogram.

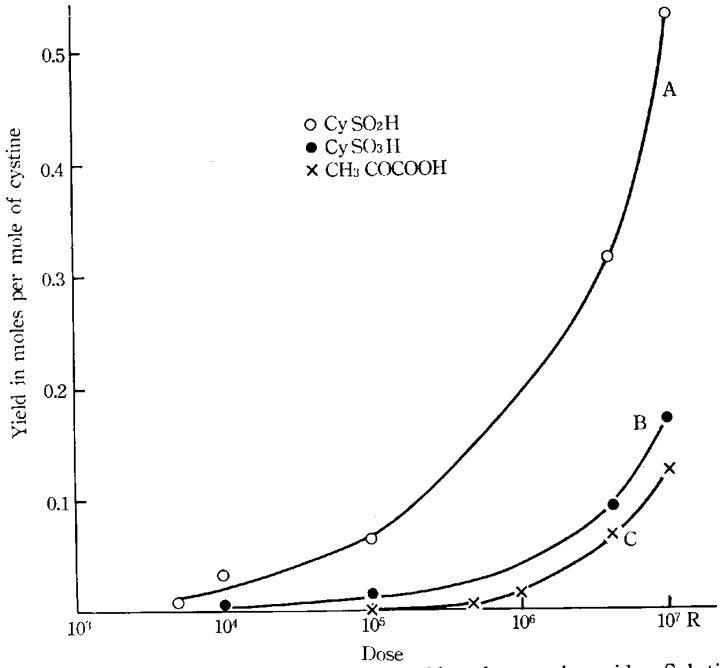

Fig. 2. Formation of cysteine sulfinic acid, cysteic acid and pyruvic acid. Solution of cystine, 10 mM was irradiated in 1.2×10^5 R/h dose rate at room temperature. Before analysis of the irradiation products, except pyruvic acid, this solution was adjusted to pH 2.2. Amount of pyruvic acid was measured by the Katsuki's method as described in text. A curve represents yields of cysteine sulfinic acid. B curve; cysteic acid and C curve; pyruvic acid.

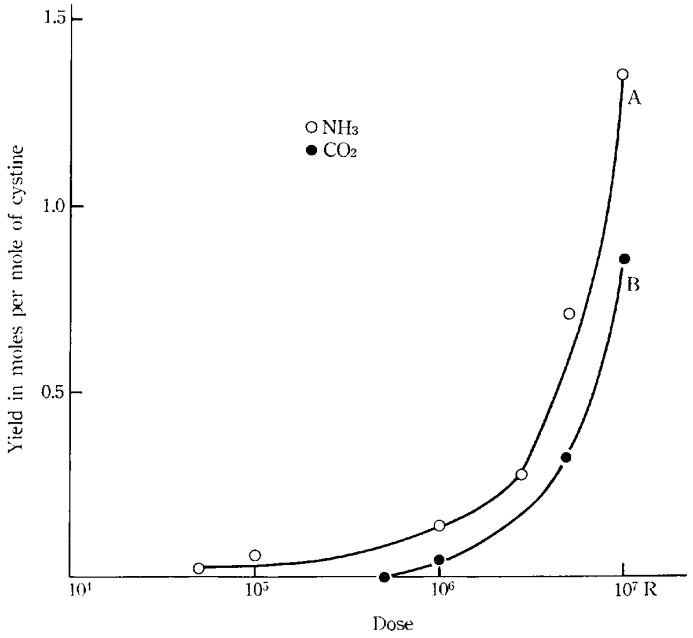

Fig. 3. Formation of ammonia and carbon dioxide. The solution of cystine, 10 mM, was irradiated in 1.2×10^5 R/h dose rate at room temperature. Ammonia was determined chromatographically using an automatic amino acid analyzer. Carbon dioxide was measured by the manometric method using Warburg's apparatus. A curve represents yield of ammonia, B curve; carbon dioxide.

Table 1. Rf-values of carbonyl compounds produced from cystine in aqueous solution by exposure to 1×10^7 R of γ-rays*

	trans form	cis form
Authentic 2,4-DNPH pyruvate	0.70	0.81
2,4-DNPH of irradiated product from cystine	0.69	0.83

* Mean of 5 experiments

Rf-values of 2,4-dinitrophenylhydrazones of carbonyl compounds produced in γ-irradiated cystine solutions on the paper chromatograms were presented in Table 1. Carbon dioxide decarboxylated from pyruvic acid was estimated and presented in Fig. 3.

Ammonia among irradiation products was estimated on a short Amberlite CG-120, 400 mesh, column being eluted at the retention volume of 80 m*l*, when no basic products such as basic amino acids and amines were found on the column. The amounts of ammonia determined at various doses of γ-irradiation were given in Fig. 3.

G-values of these irradiation products from cystine solutions were presented in Table 2.

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