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The Hydrogen Yields in the Radiolysis of Cysteine and Biscysteinatocobaltate(III) in 0.8 N Sulfuric Acid

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Radiation-induced oxidation of cysteine in an acid medium was studied by measuring the hydrogen yields at an initiation stage of radiolysis. In the deaerated system $G(H_2)=4.0$ along with the $G(-CySH)=7.1$ were observed and the results can be interpreted in terms of the yields of primary radicals from the radiolysis of water in 0.8 N sulfuric acid medium. From stoichiometric view point it is predicted that the radiolysis takes place through cysteine radical as intermediate to form cystine. In the aerated system on the other hand, the radiolysis of cysteine is much complicated. For a possible explanation satisfying all the G values obtained, 10.5 in the disappearance of cysteine, 4.0 in the formation of hydrogen peroxide and 0.4 in the hydrogen, the air oxidation of cysteine caused by the radicals, HO_2 and OH which produce H_2O_2 and O_2 by the radical recombination, is proposed as mechanism. The radiolysis of cysteine is greatly reduced by complexation with metallic ion as biscysteinatocobaltate(III) complex. The absence of hydrogen abstraction from cysteine by H atom, the isolation of cysteine from a ligand site and the extraordinary high G values in the disappearance of optical absorption of the complex can be cited as characteristics in the radiolysis of the biscysteinatocobaltate(III) complex. The intramolecular redox of the complex into Co(II) ion and cysteine is a best fit mechanism as over all reaction in the radiolysis of the biscysteinatocobaltate(III) complex.

In the literature so far appeared, the radiolysis of cysteine produces cystine along with some H_2S and alanine with G values around 3,$^{1-4)}$ depending on the concentration of cysteine as solute. In the presence of dissolved oxygen from air the irradiated cysteine produces sulfate instead of H_2S.$^{1)}$ A recent paper reported the formation of a lot of sulfur oxyacids and other several compounds as stable products from cystine in the radiolysis of its aerated solution.$^{5)}$ The disagreement on the stoichiometric balance and therefore, on the resulted mechanism appears likely to be derived from the technique employed by different workers. In their studies secondary reaction intermediates are picked up to account for the radiolysis and make the interpretation rather complicated. One of our principal concerns is the establishment of stoichiometry with the primary reaction between the solute and the primary radicals from the radiolysis of solvent water. As an initiation of radiation-induced oxidation of cysteine an assumption was made of cysteine radicals CyS· as intermediate which was subsequently reformed to cystine as final stable product. In this investigation the cysteine oxidation to cystine at an earlier stage of radiolysis was confirmed by the hydrogen yields

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1) A. J. Swallow, *J. Chem. Soc.*, **1952**, 1334.
2) S. L. Whitcher, *Nucleonics*, **11**, No. 8, 30 (1953).
3) P. Markakis and A. L. Tappel, *J. Am. Chem. Soc.*, **82**, 1613 (1960).
4) J. Parker, *J. Chem. Soc.*, **1963**, 2320.
5) J. W. Puradie, *J. Am. Chem. Soc.*, **89**, 226 (1967).

from the cysteine in 0.8 N sulfuric acid under γ irradiation in the presence and absence of dissolved oxygen.

Experimental

Materials. Triply distilled water was used for preparing samples throughout the experiments. Cysteine solution was prepared by dissolving an appropriate quantity of reagent grade cysteine (Wako Pure Chem. Ind. Ltd.) in 0.8 N sulfuric acid for acid medium radiolysis. The complex salt was prepared from cobalt(II) chloride by the method in Schubert's paper.⁶⁾ The purification by recrystallization was repeated three times. The analysis provided the composition of the complex together with theoretical values in parentheses: Co, 16.5% (17.6%); H, 4.57% (3.0%); C, 20.98% (21.4%); N, 8.09% (8.35%) corresponding to the formula $CoH(CyS)_2 \cdot 2H_2O$⁷⁾ All other reagents used, acids and salts, are of reagent grade.

Irradiation. Sample solutions were saturated with air or flashed with nitrogen in telextube and were irradiated with γ-rays from 60-Co source of one kilo curie facilitated by the Institute of Physical and Chemical Research. Dose rates were measured by Fricke Dosimeter taking the $G_{Fe^{3+}}$ value equal to 15.6, and the molecular extinction coefficient for Fe^{3+} $\varepsilon=2150$ at 304 mμ and 20°C was employed.

Analysis. Decrease of cysteine and increase of cystine in a solution caused by irradiation was determined by the polarographic method described in the published work.⁸⁾

Spectrophotometric and polarographic measurements of biscysteinatocobaltate(III) is effected by the procedure as follows. A solution of 1×10^{-4} M biscysteinatocobaltate(III) was taken as reference and irradiated sample solutions were diluted to a concentration nearly same as above. Photoabsorption maxima of biscysteinatocobaltate(III) lie at the wavelengths of 276, 345 and 430 mμ. By differential spectrum method the decrease of absorption was measured against gamma doses at 276 mμ.

Analysis of Hydrogen and Hydrogen Peroxide. The amount of hydrogen evolved from irradiated liquid sample was determined by gas chromatographic method using a GC-1B type Shimadzu Gas Chromatograph to which a liquid-separating line and a precolumn consisting of Molecular Sieve was attached. A similar method has been reported in detail by one of present authors as well as Swinnerton *et al.*⁹,¹⁰⁾

Hydrogen peroxide in the irradiated solutions was colorimetrically determined by titan yellow method known as common usage.

Result

The Yields of Hydrogen from Cysteine. The radiolysis of cysteine proceeds in linear function of absorbed γ dose. The slope of the curve drawn by the plots of the amount of cysteine lost against the received γ dose provides a G value for a given cysteine concentration. The G values thus obtained are dependent on the cysteine concentration, hardly attainable to a maximum value independent of the cysteine concentration. Then, an inverse-plots method is required to find such a maximum G value which is independent of the cysteine concentration. The estimated G values are available for establishing stoichiometric balance relation. Concerning the disappearance yields of cysteine, the G values from our earlier data⁷⁾ are represented by the formula in Table 1 as a function of cysteine concentration.

TABLE 1. G VALUES OF CYSTEINE LOST
[CySH] designates the concentration
of cysteine in M

	O_2 present
$G(-CySH)$	$\dfrac{10.5}{1+(1/6.2\times10^{3}[CySH])}$
	O_2 absent
$G(-CySH)$	$\dfrac{7.5}{1+(1/2.5\times10^{3}[CySH])}$

Figure 1 shows the amount of hydrogen determined by gas chromatographic analysis towards the received γ dose in the radiolysis in the deaerated system. In the deaerated system the maximum $G(H_2)$ was determined at a cysteine concentration higher than 2×10^{-3} M from the curve shown in Fig. 2. In the aerated system, on the other hand, the hydrogen yields were found to be no more than 0.4 over all the range of cysteine concentration.

This means that there is no hydrogen derived from the hydrogen abstraction from cysteine by

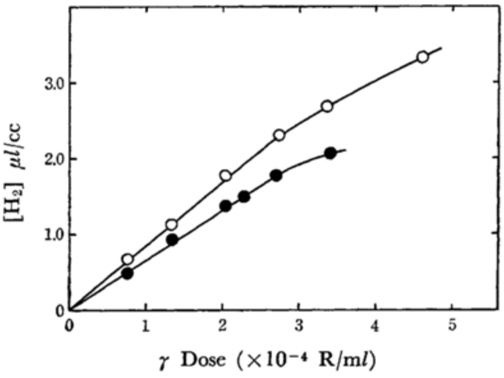

Fig. 1. Hydrogen evolution from deaerated cysteine solution.
Initial concentration of cysteine
○ 200×10^{-3} M, ● 2.00×10^{-4} M

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6) M. P. Schubert, *ibid.*, **51**, 3851 (1931).

7) N. Matsuura and K. Muroshima, *Sci. Papers of College of General Education, Univ. Tokyo*, **14**, 197 (1964).

8) N. Matsuura, K. Muroshima and M. Takizawa, *Japan Analyst*, **13**, 324 (1964).

9) J. W. Swinnerton, V. J. Linnenbom and C. H. Cheek, *Anal. Chem.*, **34**, 483 (1962).

10) J. W. Swinnerton, V. J. Linnenbom and C. H. Cheek, *ibid.*, **34**, 1509 (1962).

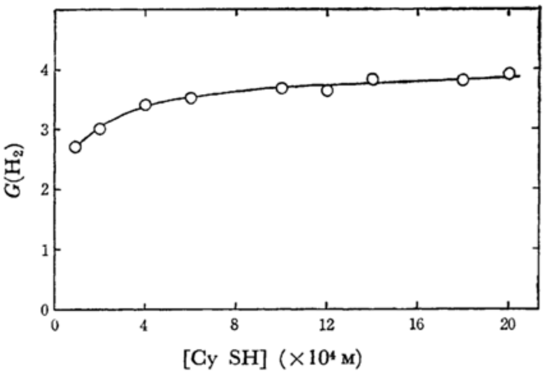

Fig. 2. $G(H_2)$ as function of cysteine concentration in deaerated system.
γ dose: 1.3×10^4 R/m*l*
Dose rate: 4.0×10^4 R/m*l*/hr

H atom and all of produced hydrogen is composed of unscavengeable H atom, whose yield is 0.4 in *G* value.

The Hydrogen Peroxide Yields in Aerated System. The determination of hydrogen peroxide yields is of much importance for the radiolysis of cysteine in the aerated system. The $G(H_2O_2)$ were 4.0 for the aerated system and 0.0 for the deaerated system. The dependency of the *G* values on cysteine concentration is illustrated in Fig. 3.

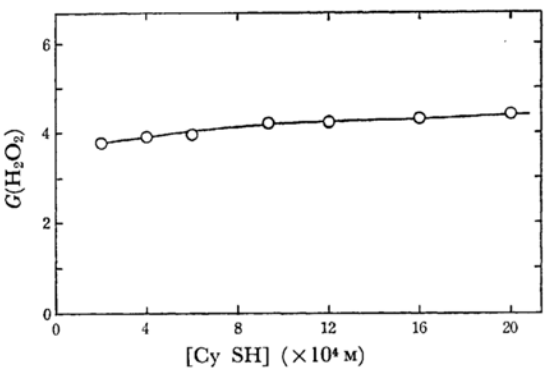

Fig. 3. $G(H_2O_2)$ as function of cysteine concentration (aerated system).
γ dose: 1.3×10^4 R/m*l*

The Hydrogen Yields from Biscysteinatocobaltate(III). The $G(H_2)$ are plotted in Fig. 4 against the concentration of the complex at a given γ dose of 2×10^4 R/m*l* or 1.2×10^{18} eV/m*l*. The observed *G* values did not exceed 0.4, corresponding to the primary molecular hydrogen yield. This fact suggests the chelated cysteine is no longer so sensitive to γ radiation as free cysteine is, even in deaerated system. In the radiolysis of this complex the *G* values with respect to the disappearance have been given by the formula in Table 2 established from our earlier data.7)

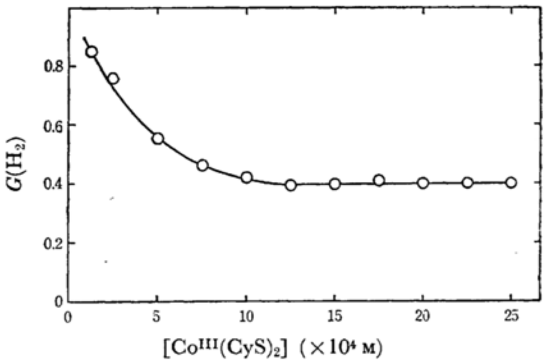

Fig. 4. Variation of $G(H_2)$ with the concentration of $Co^{III}(CyS)_2$ in deaerated system.

TABLE 2. *G* VALUES OF BISCYSTEINATOCOBALTATE(III) LOST

$[Co^{III}(CyS)_2]$ designates the concentration of $Co^{III}(CyS)_2$ in M

	O_2 present
$G(-Co^{III}(CyS)_2)$	$\dfrac{12}{1+(1/5.5\times10^3[Co^{III}(CyS)_2])}$
	O_2 absent
$G(-Co^{III}(CyS)_2)$	$\dfrac{13}{1+(1/8.5\times10^3[Co^{III}(CyS)_2])}$

The Yields of Cysteine Liberation. In the radiolysis of biscysteinatocobaltate(III) a break point was observed as shown in Fig. 5A, indicating that there had been a secondary reaction accompanied by the retardation of radiolysis at a critical dose of 2×10^4 R/m*l*. The cause of secondary reaction, occuring in slower rate, could be ascribed to a primary radiolysis product as prohibitor toward the degradation of the complex. The presence of break point suggests that hydrogen abstraction from cysteine as ligand molecule was suppressed by the reduction of cobalt(III) complex to cobalt(II) which resulted in the liberation of

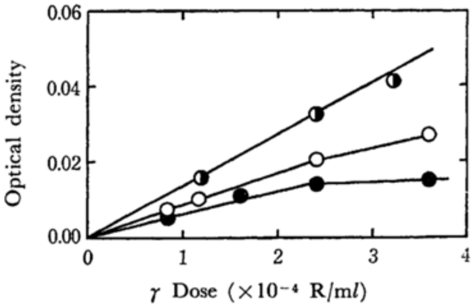

Fig. 5A. Disappearance of $Co^{III}(CyS)_2$ as function of received γ dose.
Initial concentration of $Co^{III}(CyS)_2$
● 1.00×10^{-4} M, ○ 6.00×10^{-4} M,
◑ 1.02×10^{-3} M

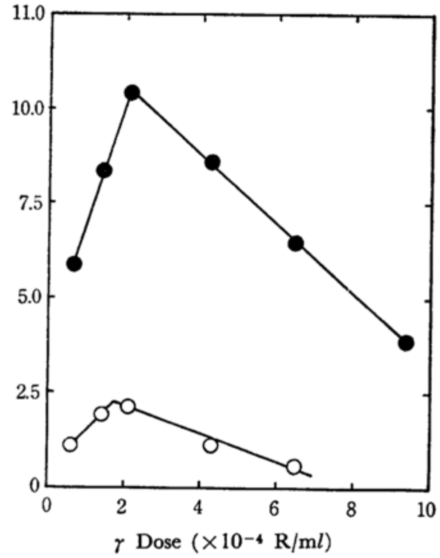

Fig. 5B. Liberation of cysteine from irradiated biscysteinatocobaltate(III) solution.
○ Aerated, ● Deaerated

cysteine from ligand position. The free cysteine thus liberated could be determined polarographically and by PCBM method[11] as function of received γ dose and a maximum yield was found at the corresponding critical dose cited above. The maximum yield of cysteine in the radiolysis of the complex, shown in Fig. 5B, can be accounted for by the loss of cysteine by oxidation after isolation from the complex in concurrence with the increase by isolation.

Discussion

Cysteine is regarded as one equivalent redox reagent when it is converted into cystine by the radiolysis in aqueous phase. If the final oxidation product of cysteine is assumed as cystine alone, so far as an earlier stage of radiolysis is concerned, the *G* values with regard to cysteine disappearance should be equal to the sum of primary radical yields, $g_H + g_{OH}$, attaining $3.6+2.9=6.5$ as a maximum *G* value in the deaerated system. The difference between the theoretical and experimental values, amounting to 1.0, is not small enough to be ignored as an experimental error. However, the observed $G(H_2)$ can account for the initiation of radiolysis by hydrogen abstraction to yield cysteine radical as intermediate product which forms stable cystine under our condition of the radiolysis at high scavenger concentration.

$$\mathrm{CySH} + \mathrm{H(or\ OH)} = \mathrm{CyS}\cdot + \mathrm{H_2(or\ H_2O)} \qquad (1)$$

$$\mathrm{CyS}\cdot + \mathrm{CyS}\cdot = \mathrm{CyS : SCy\ (cystine)} \qquad (2)$$

From Eqs. (1) and (2) the $G(H_2)$ is just equal to the sum $g_H + g_{H_2} = 3.6+0.4=4.0$ in good agreement with the value experimentally found. The radiolysis in deaerated system gave no hydrogen peroxide which might be responsible to the shortage of the *G* value 1.0 as stated above. Since hydrogen peroxide is inert against cysteine because of its slow reaction rate, the oxidation of cysteine by hydrogen peroxide must be induced by the radiolysis through a certain reactive intermediate, like a cysteine radical. Nevertheless, a presumed value of $g_H + g_{OH} + 2g_{H_2O_2} = 3.6+2.9+1.6=8.4$ based on the two equivalent oxidation of H_2O_2 is too large to explain the experimental *G* value of 7.5.

As for the aerated system the stoichiometric relation is much more complicated, probably because of the dissolved oxygen taking part in the radiolysis of cysteine by the formation of some radical intermediate like a $\mathrm{CySO_2}\cdot$ radical. In the aerated system the maximum *G* values were 10.5 with the $G(-\mathrm{CySH})$, 4.0 with the $G(H_2O_2)$ and 0.4 with $G(H_2)$. For possible explanation satisfying all these *G* values the schema (3) and (4), has to be introduced.

$$\begin{aligned}&2\mathrm{CySH} + \mathrm{HO_2(H+O_2)}\\&\quad = \mathrm{CyS : SCy} + \tfrac{1}{2}\mathrm{H_2O_2} + \mathrm{H_2O}\end{aligned} \qquad (3)$$

$$\begin{aligned}&2\mathrm{CySH} + 2\mathrm{OH} + \tfrac{1}{2}\mathrm{O_2}\\&\quad = \mathrm{CyS : SCy} + \mathrm{H_2O_2} + \mathrm{H_2O}\end{aligned} \qquad (4)$$

Under the assumption made for the schema (3) and (4) the stoichiometric balance between *G* values is quite self-consistent, because $G(-\mathrm{CySH}) = 2g_H + g_{OH} = 2\times3.6+2.9 = 10.1$, $G(H_2O_2) = (1/2)g_H + (1/2)\ g_{OH} + g_{H_2O_2} = 4.1$ and $G(H_2) = g_{H_2} = 0.4$. It is interesting to indicate that the reaction schema described in (3) and (4) are nothing but the recombination of radicals HO_2 and OH to H_2O_2 in the presence of cysteine as solute and the oxidation of cysteine by air oxygen induced by radiolysis.

The radiolysis of biscysteinatocobaltate(III) shows several characteristic features: the absence of hydrogen abstraction, the liberation of free cysteine from a ligand position and the high *G* value, 12—13, with regard to the disappearance of the complex. The latter yields are approximately twice as large as the sum of the primary radical yields $g_H + g_{OH} = 6.5$. These facts could be interpreted in terms of the inner sphere reduction of the complex from Co(III) to Co(II) due to radicals H and OH.[13]

$$\begin{aligned}2\mathrm{Co^{III}(CyS)_2}^- &\xrightarrow{\mathrm{H,\ OH}} 2\mathrm{Co^{2+}} + 2\mathrm{CySH}\\&\qquad + \mathrm{CyS{:}SCy} + \mathrm{H_2O}\end{aligned} \qquad (5)$$

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[11] P. B. Boyer, *J. Am. Chem. Soc.*, **76**, 4331 (1954).

[12] M. Haissinsky and M. Magat (edt.) Tables of selected Constants Radiolytic Yields, Pergamon (1963).

[13] N. Matsuura, M. Muroshima and M. Takizawa, *Nippon Kagaku Zasshi (J. Chem. Soc. Japan, Pure Chem. Sect.)*, **85**, 268 (1964).

The reaction (5) is based on the one molecule cysteine liberation per one molecule of the complex lost at an earlier stage of radiolysis with a dose below the critical value of 2×10^4 R/m*l*. It is indicated that the overall reaction (5) is nothing but an intramolecular redox of the complex caused by the radicals.

Generally speaking, so far as maximum *G* values are concerned or secondary reactions are excluded that might occur between free radicals and an intermediate product of radiolysis, a quantitative stoichiometric balance holds well. As initiation of radiolysis in the system of cysteine or metal-cysteine complex, the major product is likely the cystine produced by the recombination of cysteine radical CyS. Namiki *et al.*(14) have evidenced a stationary mixture of cysteine and cystine in equilibrium attained by the radiolysis of either cysteine or cystine used as starting solute. A number of products different from cystine reported by several workers could be ascribed to the results involving secondary reaction products in a large γ-dose region.

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14) M. Namiki, The 9th Symposium on Radiation Chemistry (Osaka, Japan, 1966).