# The Mechanism for the Degradation of Cystamine by Ionizing Radiation

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## INTRODUCTION

In a previous paper we have shown that irradiation of aqueous solutions of the radioprotective agents cysteamine and cystamine produced certain oxidation products. Cysteamine at a pH of 5 was oxidized to cystamine, the corresponding disulfide, with an ionic yield of 4. This yield compared well with values found by Barron for other sulfhydryl compounds, and the mechanism of oxidation is probably the same.

Irradiation of cystamine solutions yielded the oxidation products 2-aminoethane sulfinic acid and taurine with $G$ values of 0.8 and 0.4, respectively, after 240,000 r. The destruction by ionizing radiation of another disulfide, cystine, has been demonstrated by other workers (*1*, *2*), but the mechanism was not determined.

The purpose of the present paper is to demonstrate the mechanism by which the disulfide cystamine is oxidized by ionizing radiation to 2-aminoethane sulfinic acid and taurine. We found that the oxidizing agents were hydrogen peroxide and the highly reactive radicals formed when water is irradiated. The relative proportions of the two products formed by each of the oxidizing agents were determined. The analytical methods used were the same as those developed in our previous paper in which we used $S^{35}$-labeled cystamine and paper chromatography.

## METHOD

Cystamine dihydrochloride-$S^{35}$ was synthesized with a specific activity of 0.11 to 0.15 mc/mg (*3*). The solutions which were irradiated were $1.4 \times 10^{-3}$ $M$ cystamine in $M/15$ sodium phosphate buffer at pH 7.5. Solutions in which radicals were made chemically were prepared as described below. The water for all solutions was purified by boiling it with potassium permanganate and by distilling it several times from hard glass equipment.

In experiments in which the solutions were deoxygenated before irradiation the buffer was first boiled and then equilibrated with oxygen-free nitrogen. It was then

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placed in special vessels to which the cystamine was added. These solutions were alternately evacuated (1 mm Hg) and flushed with oxygen-free nitrogen under constant shaking until their volume was reduced by one-half. The nitrogen was freed of oxygen by passing it through a pyrogallol solution and then over copper filings heated to 400° C. Controls were run in vessels of the same type and with the same procedure except that they were flushed with air instead of nitrogen.

Solutions were irradiated by an 0.5-curie $Co^{60}$ source or by an X-ray machine with the factors 175 kv, 10 ma, and an 0.5 mm Cu filter. The dose rate of the X-ray machine and the cobalt source was measured by means of a Victoreen ionization chamber. In the latter case the ionization chamber was coated by 4 mm of Lucite. The dose rate with the cobalt source was 5500 r/hr and the rate with the X-ray machine was 1240 r/min.

The chemical production of hydroxyl radicals essentially followed the method used by Collinson *et al.* (*4*). A $1.4 \times 10^{-3}$ *M* solution of cystamine in distilled water was made $10^{-3}$ *M* in ferric alum. It was deoxygenated and then illuminated by a mercury arc ultraviolet lamp for 45 minutes. In the control solutions ferric alum was omitted. Hydroxyl radicals were also produced by Fenton's reagent. A $1.4 \times 10^{-3}$ *M* cystamine solution in distilled water containing $10^{-4}$ *M* ferrous sulfate was made $3.3 \times 10^{-4}$ *M* in hydrogen peroxide and allowed to stand at room temperature for 45 minutes. This reaction also produces some $HO_2$ radicals:

$$OH + H_2O_2 \rightarrow HO_2 + H_2O$$

The spontaneous action on cystamine of $H_2O_2$ at concentrations above $1 \times 10^{-3}$ *M* made it necessary to use the stated concentration of hydrogen peroxide. Controls were run with ferrous sulfate alone and with hydrogen peroxide alone.

After the reaction or irradiation, the solutions were analyzed by a descending chromatographic technique with paper strips pretreated with 1 *M* KCl and *M*/15 phosphate buffer at pH 7.5. The solvent consisted of equal parts of isopropanol and ethanol with water added to a final concentration of 15%. To locate and quantitate the compounds resolved, the radioactivity along the strips was measured with a GM counter.

## RESULTS AND DISCUSSION

When dry, pure cystamine crystals were irradiated with 1,000,000 r and then dissolved in buffer, analysis of the solution revealed no irradiation products. The same result was obtained when the buffer was irradiated with 130,000 r and cystamine added subsequently. These experiments demonstrated that water was necessary in the radiation destruction of cystamine and that the agents produced in water were short-lived. Such findings are suggestive of a radical mechanism.

When radicals were formed by chemical means in cystamine solutions 2-aminoethane sulfinic acid and taurine were isolated. The amounts of these products formed cannot be compared with the amounts formed by irradiation, since we do

not know the number of radicals produced by the chemical reaction. However, the relative amounts of the two compounds formed indicate the efficiency of their production by the oxidative agents. This is represented by the ratio of taurine to 2-aminoethane sulfinic acid, and this value can be compared with those obtained in the irradiation experiments.

The role played by the radicals and hydrogen peroxide in the formation of the radiation products can also be determined by treating the cystamine solutions in such a way that on irradiation one or two of the oxidizing agents formed will predominate in concentration. Thus, deoxygenation of the solution prior to irradiation prevents formation of $H_2O_2$ and $HO_2$, allowing OH to predominate. The presence of catalase during exposure to X-rays destroys the $H_2O_2$ and leaves the OH and $HO_2$ radicals. The addition of potassium iodide to the solutions before irradiation results in a reduction of OH radicals by the following reaction (*5*):

$$I^- + OH \rightarrow OH^- + I$$

This accentuates the effect of $H_2O_2$ and the $HO_2$ radicals. In discussing the results of these manipulations we will treat together all experiments designed to define the role of each radical.

## The OH Radical (Table I)

The hydroxyl radical, produced chemically in cystamine solutions, forms one-third to one-half as much taurine as sulfinic acid. This is confirmed by the deoxygenation experiments where OH radical predominates and where the ratio

TABLE I

The Effect of the Hydroxyl Radical in the Production of 2-Aminoethane Sulfinic Acid and Taurine in Cystamine Solutions

| *Type of experiment* | *Experiment* | *$RSO_3H$ (cpm/1000 cpm on paper)* | *$RSO_2H$ (cpm/1000 cpm on paper)* | *$RSO_3H/RSO_2H$* |
|---|---|---|---|---|
| Production of OH; $Fe^{+++}$ + ultraviolet light | I | 17 (0)[a] | 56 (0) | 0.30 |
| | II | 12 (0) | 30 (0) | 0.40 |
| | III | 20 (0) | 44 (0) | 0.45 |
| Predominance of OH | | | | |
| Solution deoxygenated, then given 240,000 r | I | 23 (47) | 49 (80) | 0.47 |
| | II | 22 (43) | 41 (82) | 0.54 |
| | III | 27 (51) | 40 (51) | 0.67 |
| Solution aerated, then given 240,000 r | | 31 | 63 | 0.50 |
| Unaffected OH; solution aerated, then given 80,000 r | | 24 | 24 | 1.00 |
| Depression of OH; $10^{-3}$ *M* KI added and solution then given 240,000 r | I | 30 (32) | 28 (57) | 1.1 |
| | II | 31 (32) | 26 (54) | 1.2 |
| | III | 36 (43) | 20 (82) | 1.3 |

[a] Numbers in parentheses are control values.

between the taurine and the sulfinic acid is 0.47 to 0.67. It is also confirmed in experiments in which solutions equilibrated with air prior to irradiation were given a dose of 240,000 r. Here the ratio of 0.50 is explained by the fact that all the oxygen is exhausted from the solution after 60,000 to 80,000 r. For the remainder of the irradiation we have essentially a deoxygenation experiment with predominance of the OH radical. The control values (in parenthesis) for the deoxygenation experiments are higher than the controls in the other experiments. This is probably due to the extra handling in the deoxygenation procedure.

The depression of the OH radical by making the cystamine solution $10^{-3}$ $M$ with potassium iodide before irradiation results in a large reduction of sulfinic acid formed but does not affect the taurine. Thus the hydroxyl radical plays a leading role in sulfinic acid production but is of less importance in taurine production.

The mechanism of the OH reaction is probably

$$RSSR + OH \rightarrow RSOH + RS$$

The RS radical may recombine to form cystamine or pick up an H to form cysteamine. Aliphatic sulfenic acids have not been isolated, and attempts to do so have resulted in dismutation reactions of the type (*6*)

$$RSOH \rightarrow RSO_2H + RSSR + (RSO_3H)$$

There is some indication that a sulfonic acid can also be formed by this dismutation (*7*). Cysteamine sulfenic acid might dismutate in a similar manner to produce the sulfinic acid and possibly also some of the taurine found in the irradiated solution.

## Hydrogen Peroxide (Table II)

The addition of cystamine to $10^{-3}$ $M$ hydrogen peroxide solutions results in the formation of taurine and sulfinic acid. There is two or three times as much taurine formed as sulfinic acid. This effect is confirmed in the catalase experiments where

TABLE II

The Effect of Hydrogen Peroxide in the Production of 2-Aminoethane Sulfinic Acid and Taurine in Cystamine Solutions

| *Type of experiment* | *Experiment* | *$RSO_3H$ (cpm/1000 cpm on paper)* | *$RSO_2H$ (cpm/1000 cpm on paper)* | *$RSO_3H/RSO_2H$* |
|---|---|---|---|---|
| Addition of $H_2O_2$ (no irradiation) | | | | |
| $1.2 \times 10^{-3}$ $M$ | I | 24 | 9 | 2.7 |
| $3.0 \times 10^{-3}$ $M$ | II | 106 | 45 | 2.4 |
| Destruction of $H_2O_2$; catalase (5μg/ml) added | I | 16 (32)[a] | 57 (57) | 0.28 |
| and solution then given 240,000 r | II | 14 (32) | 60 (54) | 0.23 |
| | III | 12 (28) | 50 (52) | 0.24 |

[a] Numbers in parentheses are control values.

5 μg of crystalline catalase per milliliter of cystamine solution is added prior to irradiation. This reduces the hydrogen peroxide formed, and the result is that taurine is markedly reduced but the sulfinic acid is unaffected. Thus $H_2O_2$ plays a major role in taurine formation but is less important in sulfinic acid production. The catalase effect is not due to the protein of the enzyme, since an equal weight of albumin fraction does not affect the yield of radiation products.

Hydrogen peroxide is known to attack disulfides to form thiolsulfinic esters and usually the corresponding sulfonic esters:

$$\mathrm{RSSR} \rightarrow \mathrm{RS(O)SR} \rightarrow \mathrm{RS(O)_2SR}$$

The hydrolysis of these compounds would ultimately form sulfinic acid which might be further oxidized to taurine.

## The Hydroperoxyl Radical (Table III)

The $HO_2$ radical, produced chemically in cystamine solutions, in combination with the OH radical, produces taurine and sulfinic acid in different proportions than the OH radical does alone. With Fenton's reagent there is a much greater proportion of the yield as taurine than is obtained in the ultraviolet-illuminated ferric alum-cystamine solutions. This suggests that $HO_2$ has some effect on taurine formation. The reduction in sulfinic acid on deoxygenation prior to irradiation suggests that $HO_2$ also helps to form sulfinic acid. Hydrogen peroxide is the only other possible factor in this decrease in sulfinic acid formation, and it appears to have little or no effect on production of sulfinic acid. Further evidence of an $HO_2$-cystamine reaction is seen in the work of Alexander (*8*), who has shown that cystamine protects against the radiation depolymerization of methacrylic acid, a reaction produced only by the $HO_2$ radical.

The chemical reactivity of the $HO_2$ radical is less than that of the OH radical (*9*),

TABLE III

The Yield of 2-Aminoethane Sulfinic Acid and Taurine Obtained by Addition of Fenton's Reagent to Cystamine Solutions

| *Type of experiment* | *Experiment* | *$RSO_3H$ (cpm/1000 cpm on paper)* | *$RSO_2H$ (cpm/1000 cpm on paper)* | *$RSO_3H/RSO_2H$* |
|---|---|---|---|---|
| Fenton's reagent (OH + $HO_2$) | I | 57 (0)[a] | 39 (0) | 1.5 |
| | II | 60 (0) | 46 (0) | 1.3 |
| | III | 50 (0) | 33 (0) | 1.5 |

[a] Numbers in parentheses are control values.

but it may still interrupt the S—S bond. Such a displacement reaction would result in formation of peroxysulfenic acid:

$$RSSR + HOO \rightarrow RSOOH + RS$$

Internal rearrangement could yield sulfinic acid. Such a rearrangement would be analogous to the suggested mechanism for the oxidation of sulfite by hydrogen peroxide (*10*). On the other hand $HO_2$ may act as a reducing agent (*11*):

$$RSSR + HO_2 \rightarrow RS^- + O_2 + H^+ \rightarrow O_2 + RSH$$

Figure 1 is a tentative diagram of reactions involved in the degradation of cystamine by ionizing radiation. The intermediate products are postulated from analogous reactions described in the literature; the reagents and the final products were determined by our own studies. The mechanism of the final oxidation of

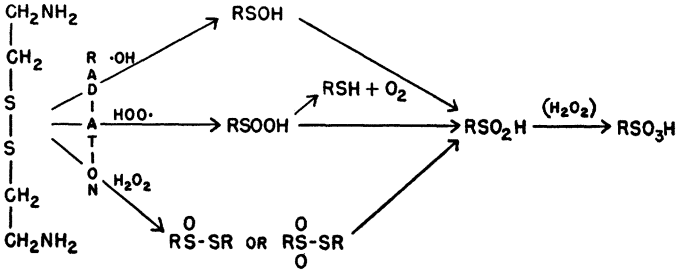


FIG. 1. The possible reactions leading to the formation of 2-aminoethane sulfinic acid and taurine in irradiated solutions of cystamine.

sulfinic acid to taurine is still obscure. It could occur by several means, one of which is certainly $H_2O_2$. However, since it occurs even in the absence of oxygen, $HO_2$ and $H_2O_2$ are not necessary and a dismutation reaction may also be responsible. The degradation of cystamine by radiation may occur over any of the pathways suggested, and the relative importance of each will depend on the conditions in the solution as they may affect the relative quantity of the radicals and hydrogen peroxide produced.

## SUMMARY

In order to test the hypothesis that the degradation of cystamine by ionizing radiation was produced by radicals and hydrogen peroxide formed from the water of the solution, pure, dry, $S^{35}$-labeled cystamine was irradiated by $Co^{60}$. Analysis of this material by paper chromatography did not reveal the 2-aminoethane sulfinic acid or taurine found after irradiation of cystamine solutions. The chemical production of the radicals OH and $HO_2$ in solutions containing cystamine did result in formation of 2-aminoethane sulfinic acid and taurine. The addition of hydrogen peroxide to cystamine solutions also yielded these two products. By treating aqueous

solutions of cystamine prior to irradiation it was possible to obtain a predominance or depression of one or two of the radicals and hydrogen peroxide. The results demonstrated the effect of each oxidizing agent in the production of 2-aminoethane sulfinic acid and taurine from cystamine. A tentative diagram of the pathways of cystamine degradation by ionizing radiation was presented.

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