Scavenging Action in γ-Irradiated Aqueous Cysteine Solutions[1]

Sir:

One explanation offered for the mechanism of radiation protection of biological systems by cysteine and other sulfhydryl compounds is the trapping of "free radicals" originating from radiolyzed water molecules in body tissues.[2] A more recent theory which has gained wide acceptance is the repair of free radicals located on sensitive biochemical molecules by transfer of the –SH proton.[3] To add to the complexity of the interpretation of the protective effect, a recent report[4] indicates that one optical isomer of a sulfhydryl compound is more protective than another. Eldjarn and Pihl[5] have pointed out that very little quantitative data were available on the effectiveness of the sulfhydryl and other protective agents as scavengers of reactive intermediates of water radiolysis. More recently reports of the relative rate constants for OH radical scavenging reactions have become available for both protective agents and for other important biological molecules.[6]

In a program recently initiated in this laboratory, the kinetics of the aqueous radiation chemistry of various chemical protective agents is being investigated in order to provide a mechanism for their *in vitro* radiolysis with cobalt-60 γ-rays and to establish relative rate constants for various scavenging reactions.

We report here some preliminary results of a study of the radiolysis of oxygen-free cysteine solutions which illustrate the need for more detailed kinetic investigations. Previous studies of this system[7] have not included rigorous attempts to correlate product yields or kinetics with the modern theory of aqueous radiation chemistry.[8]

In our studies it has been shown that at initial cysteine concentrations [$(RSH)_0$] lower than 0.001 M, secondary reactions are very important after 1000 rads. Radiolytic yields are therefore reported only for initial slope data and are given as G values (the number of molecules formed or destroyed per 100 e.v. of energy absorbed in the system). The yields for the disappearance of the cysteine thiol group, $G(-RSH)$, were determined polarographically[9] and were found to be quite sensitive to both pH and $(RSH)_0$. For example, at pH 1 $G(-RSH)$ varied from 4 to 11 in the concentration range 2×10^{-4} to 4×10^{-3} M $(RSH)_0$, while at pH 7 $G(-RSH)$ varied from 8 to 20 in the same concentration region.

No complete mechanism is postulated in this communication for the radiolysis of aqueous cysteine both because of the above-noted dependence of $G(-RSH)$ on initial cysteine concentration and because of the large values of $G(-RSH)$ It would appear that a relatively simple mechanism at lower $(RSH)_0$ may change to a more complex partial chain mechanism at higher $(RSH)_0$. The important point to note here is that the type of mechanism appears to be concentration dependent. This point has not been made before in previous studies.[7]

It was therefore of interest to investigate the nature of the initial reactions between the reactive intermediates produced by the radiolysis of water, namely, $H\cdot$, $\cdot OH$, and the hydrated electron, e_{aq}^-,[10] and dissolved cysteine. The method used in this investigation was that of competition kinetics where an additive is used to compete with the RSH for each of these intermediates separately in primary steps which produced products which would not interfere with subsequent primary interactions or with the detection of RSH products originating from these primary interactions.

Acetone and nitrate ion were chosen as representative electron scavengers with very high rate constants for the scavenging reaction, 6×10^9 and 1.1×10^{10} l. mole^{-1} sec.$^{-1}$, respectively.[11] These are represented as k_1 and k_2 in the following reactions.

$$e_{aq}^- + CH_3COCH_3 \xrightarrow{k_1} \text{products} \qquad (1)$$

$$e_{aq}^- + NO_3^- \xrightarrow{k_2} \text{products} \qquad (2)$$

At pH 7 the $G(H_2S)$ value of around 3 was thought to arise from two sources, a small part from reaction 3

$$H + RSH \longrightarrow H_2S + R\cdot \qquad (3)$$

due to a reaction of part of the "residual" hydrogen yield[12] and the majority from reaction 4.

$$e_{aq}^- + RSH \xrightarrow{k_4} R\cdot + HS^- \qquad (4)$$

In order to test this hypothesis, separate experiments were performed in which the added acetone concentration was varied in the presence of a fixed initial RSH concentration. $G(H_2S)$ was determined from the initial slopes of H_2S yield–dose plots for each concentration of acetone. Then on the basis of the competition between RSH and acetone as represented in eq. 1 and 4, the following relationship should hold.

$$G(H_2S) = G(e_{aq}^-)\left[\frac{k_4(e_{aq}^-)(RSH)}{k_4(e_{aq}^-)(RSH) + k_1(e_{aq}^-)(CH_3COCH_3)}\right]$$

This may be rearranged to give

$$\frac{1}{G(H_2S)} = \frac{1}{G(e_{aq}^-)}\left[1 + \frac{k_1(CH_3COCH_3)}{k_4(RSH)}\right]$$

and a plot of $1/G(H_2S)$ *vs.* $(CH_3COCH_3)/(RSH)$ should be linear with a slope of $[1/G(e_{aq}^-)][k_1/k_4]$. Experimental plots were found to be linear with acetone and, in separate experiments, with added nitrate (KNO_3).

(1) This research was supported in part by the U. S. Atomic Energy Commission.

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In the studies with added nitrate and acetone, both of these electron scavengers[11] reduced the H_2S yields to low values when present at high concentrations. With added acetone the limiting value of $G(H_2S)$ is about 0.3, roughly half of the "residual" H atom yield.[12] This yield might be expected from a partitioning of the "residual" hydrogen atoms between reactions 3 and 5.

$$H + RSH \longrightarrow H_2 + RS \qquad (5)$$

Added nitrate reduced the H_2S yield to nearly zero as would be expected if the nitrate acted as has been postulated[13] as both an efficient electron and hydrogen atom scavenger. From the linear plots obtained in each of these competition studies, ratios of rate constants were calculated and are reported here.

$$k_4(e_{aq}^- + RSH)/k_2(e_{aq}^- + NO_3^-) = 0.4$$

$$k_4(e_{aq}^- + RSH)/k_1(e_{aq}^- + CH_3COCH_3) = 0.9$$

From these ratios another ratio may be calculated, namely

$$k_2(e_{aq}^- + NO_3^-)/k_1(e_{aq}^- + CH_3COCH_3) = 2.2$$

This value may be favorably compared with 1.9 calculated from the data of Hart and co-workers[11] obtained with the pulsed-electron technique.

Similar competition kinetic studies with *p*-nitrosodimethylaniline, a compound whose chromophoric group has been shown[14] to be attacked specifically by OH radicals, show the rate constant for reaction 6 to be

$$OH + RSH \xrightarrow{k_6} \text{products} \qquad (6)$$

about 3×10^9 l. mole^{-1} sec.$^{-1}$, a nearly diffusion-controlled reaction rate constant. A preliminary study in which 2-propanol was used as an H atom competitor was complicated by the additional OH competition but showed that the total rate constant for reactions 3 and 5 is at least on the same order of magnitude as that for

$$H + \text{2-propanol} \longrightarrow \text{products}$$

It is apparent from the above experiments that cysteine, one of the better protective agents, is a quite effective OH and hydrated-electron scavenger. Preliminary studies in our laboratory indicate similar behavior for several other two- and three-carbon atom protective aminothiols.[15] These studies are being expanded to include other thiols which have shown no biological protective action.

Convincing experimental evidence has been presented for mechanisms of protection other than "radical scavening."[2-4] However, in view of the results reported above which support diffusion-controlled or nearly diffusion-controlled scavenging of the two major reactive intermediates known to exist in neutral, irradiated aqueous solutions, we wish to suggest that it is only reasonable to include the possibility that many of these reactive intermediates are scavenged *before* they are able to attack sensitive biological molecules. By combining the results of our studies with those reported by Hart, *et al.*,[11] it is observed that oxygen has a rate constant for combination with hydrated electrons which is approximately four times as large as cysteine. These results may help to explain the enhanced biological protective effect of *anoxia* in the presence of cysteine.[2]

More detailed studies on cysteine and related sulfhydryl compounds are in progress and will be reported elsewhere.[16]

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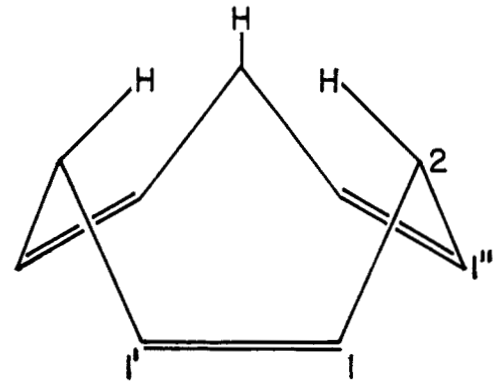
On the Question of Homoconjugation in *cis,cis,cis*-1,4,7-Cyclononatriene[1]

Sir:

cis,cis,cis-1,4,7-Cyclononatriene has recently been obtained independently in three different laboratories.[2-4] Proton magnetic resonance studies indicate that the substance possesses an interconverting crown-to-crown structure (I) in the liquid phase. On the basis of



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simple LCAO–MO calculations, Radlick and Winstein[2] have predicted a "nonzero" delocalization energy, resulting from "trishomo"[5] conjugation, for this triene. A more quantitative treatment by Untch[3] suggests that such delocalization energy is negligible. We wish to report experimental results which, within the limits of their uncertainties, exclude the possibility that homoallylic stabilization makes any significant contribution to the ground state of the cyclononatriene molecule.

The heat of hydrogenation of *cis,cis,cis*-1,4,7-cyclononatriene measured in acetic acid solution at 25° is -76.88 ± 0.05 kcal./mole. The value obtained for *cis*-cyclononene is -23.62 ± 0.07 kcal./mole.[6] The heat evolved in the hydrogenation of triolefin to monoolefin is therefore 53.3 kcal./mole, which represents an average value of -26.7 kcal./mole for the heat of

(1) This research was supported by grants from the National Science Foundation and the Robert A. Welch Foundation.

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