Title

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The hydrated electron, e^-_{aq}, and the OH radical are the principal products of the radiation-induced decomposition of liquid water; with γ-rays the 100 ev yields[1] of these reactive intermediates are closely approximated by the values: $G_{e^-_{aq}} \simeq G_{OH} \simeq 2.6$. While certain classes of organic compounds are known to react quantitatively with both e^-_{aq} and OH in oxygen-free solution, still, the observed yield for net destruction of such solutes may be quite low as compared to the G value for water decomposition. In interpreting the radiation chemistry of such systems the concept of a reconstitution or back-reaction must be invoked.

For example, in the γ-radiolysis of oxygen-free solutions of the pyrimidine bases, both e^-_{aq} and OH add preferentially to the 5,6 double bond[2,3]

$$e^-_{aq} + B + H_2O \rightarrow \dot{B}H + OH^- \qquad (2)$$

$$OH + B \rightarrow \dot{B}OH \qquad (3)$$

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[1]Czapski, G., Advan. Chem. Ser., 81 106 (1968).

[2]Weiss, J., Prog. Nucleic Acid Res. and Mol. Biol., 3 103 (1964).

[3]Kamal, A. and Garrison, W.M., Nature 206 1315 (1965).

where the rate constants k_2, k_3 fall in the range 10^9 to 10^{10} M^{-1} sec^{-1} (ref. 4,5). Yet, the observed yield for base destruction in millimolar solutions is uniformly low with $G(-B) < 0.9$. The evidence is that a back-reaction[3]

$$\dot{B}H + \dot{B}OH \rightarrow 2B + H_2O \tag{4}$$

leads to a reconstitution of the base.

We now find that certain labile compounds such as ascorbic acid and the thiol, cysteine, are effective at low concentrations in blocking reaction (4) by virtue of the H-atom transfer reaction

$$\dot{B}H + RH \rightarrow BH_2 + \dot{R} \tag{5}$$

where BH_2 represents the dihydro derivative of the pyrimidine nucleus. The effects of added cysteine on the yields for radiolytic reduction of cytosine in oxygen-free solution are shown in table 1A. Note that dihydrocytosine is unstable and spontaneously hydrolyses to yield ammonia and dihydrouracil[6] as the experimentally observed product. The product dihydrouracil was isolated and characterized both chromatographically and spectrophotometrically. The data of table 1A show that the yield for the radiolytic reduction of the 5,6 double bond of cytosine is essentially quantitative in the presence of either cysteine or ascorbic acid at low concentrations.

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[4] Hart, E.J., Thomas, J.K., Gordon, S., Radiation Res., Suppl. 4 74 (1964).

[5] Scholes, P., Shaw, P., Willson, R. L., and Ebert, M., "Pulse Radiolysis" p. 151 Academic Press, New York, 1965.

[6] Green, M., and Cohen, S.C., J. Biol Chem., 228 601 (1957).

Similarly, in the radiolysis of primary amides and monosubstituted primary amides (peptides) in oxygen-free neutral solution, the reducing and oxidizing species e^-_{aq} and OH are removed through reactions of the type

$$e^-_{aq} + RCONHR + H_2O \rightarrow R\dot{C}(OH)NHR + OH^- \quad (6)$$

$$OH + RCONHR \rightarrow \dot{P} + H_2O \quad (7)$$

where k_6, k_7 fall in the range 10^7 to 10^8 M^{-1} sec^{-1} (ref. 7,8). Combination of $R\dot{C}(OH)NHR$ with like species or with the radical $\dot{P}$ would lead to formation of ketonic products. However, the combined yield of such products is low with $G(>CO) \simeq 0.2$; the evidence is that the reconstitution reaction

$$R\dot{C}(OH)NHR + \dot{P} \rightarrow 2RCONHR \quad (8)$$

represents the major stoichimetry for removal of organic radical in such systems.[8]

In accord with this, we find that cysteine effectively blocks the reconstitituion reaction 8 through the step

$$R\dot{C}(OH)NHR + R'H \rightarrow RCHO + NH_2R + R'$$

and, as shown in table 1B, the reductive deamination of the peptide bond in the presence of cysteine at low concentrations is essentially quantitative with $G(CH_3CHO) \simeq 2.5$.

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[7]Willix, R.L.S., Garrison, W.M., Radiation Res., 32 452 (1967).

[8]Rodgers, M.A.J., Garrison, W.M., J. Phys. Chem., 72 758 (1968).

We find then that (a) the presence of cysteine (or ascorbic acid) at low concentrations leads to a very marked enhancement in the radiolytic lability of the pyrimidine and peptide moieties in oxygen-free solution, and that (b) such enhancement arises as a consequence of the blocking by the second solute of the reconstitution reactions formulated in equations 4, 8.

These results would appear to have interesting applications in the study and identification of reductive processes involving reactions of e^-_{aq} with unsaturated organic functions both in vitro and in vivo.

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Table 1. Effect of cysteine and ascorbic acid (ŔH) on the γ-ray induced reduction of pyrimidine (C = C) and peptide (C = O) linkages in oxygen-free solutions*.

A. Cytosine → dihydrouracil (BH_2) + ammonia†

solution	ŔH ‡	$G(BH_2)$
.05 M Cytosine, pH7	none	< 0.9
.05 M Cytosine, pH7	cysteine, 2.5×10^{-3} M	2.8
.05 M Cytosine, pH7	ascorbic acid, 1.5×10^{-3} M	2.9

B. N-ethylacetamide → acetaldehyde (RCHO) + ethylamine

solution	ŔH	G(RCHO)
1M N-ethylacetamide, pH7	none	< 0.1
1M N-ethylacetamide, pH7	cysteine, 4×10^{-4} M	2.8

*At dosages below ~ 2.5×10^{18} ev/gm.

†Dihydrocytosine is unstable and hydrolyzes spontaneously to yield dihydrouracil and ammonia.

‡The indicated concentrations of ŔH give the maximum enhancement in the yield of reduced products.