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Reconstitution Mechanisms in the Radiolysis of Aqueous Biochemical Systems: Inhibitive Effects of Thiols

THE hydrated electron, e^-_{aq}, and the OH radical are the principal products of the radiation induced decomposition of liquid water; with gamma rays the 100 eV yields[1] of these reactive intermediates are closely approximated by the values $G e^-_{aq} \simeq G_{OH} \simeq 2{\cdot}6$. While certain classes of organic compounds are known to react quantitatively with both e^-_{aq} and OH in oxygen-free solution, the observed yield for net destruction of such solutes may be quite low compared with 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 gamma radiolysis of oxygen-free solutions of the pyrimidine bases, both e^-_{aq} and OH add preferentially to the 5,6 double bond[2,3]

$$e^-_{aq} + B + H_2O \rightarrow \dot{B}H + OH^- \quad (1)$$

$$OH + B \rightarrow \dot{B}OH \quad (2)$$

where the rate constants k_2, k_3 fall in the range 10^9 to 10^{10} M^{-1} s^{-1} (refs. 4 and 5). Yet the observed yield for base destruction in millimolar solutions is uniformly low with $G(-B) < 0{\cdot}9$. The evidence is that a back-reaction[3]

$$\dot{B}H + \dot{B}OH \rightarrow 2B + H_2O \quad (3)$$

leads to a reconstitution of the base.

We have found that certain labile compounds such as ascorbic acid and the thiol, cysteine, are effective at low concentrations in blocking reaction (3) by virtue of the H atom transfer reaction

$$\dot{B}H + R'H \rightarrow BH_2 + R' \quad (4)$$

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 1*A*. 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 1*A* 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.

Table 1. EFFECT OF CYSTEINE AND ASCORBIC ACID ($R'H$) ON THE GAMMA RAY INDUCED REDUCTION OF PYRIMIDINE (C=C) AND PEPTIDE (C=O) LINKAGES IN OXYGEN-FREE SOLUTIONS*

(*A*) Cytosine→dihydrouracil (BH_2) + ammonia†

Solution	$R'H$‡	$G(BH_2)$
0·05 M cytosine, *p*H 7	None	< 0·9
0·05 M cytosine, *p*H 7	Cysteine, $2{\cdot}5 \times 10^{-3}$ M	2·8
0·05 M cytosine, *p*H 7	Ascorbic acid, $1{\cdot}5 \times 10^{-3}$ M	2·9

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

Solution	$R'H$	$G(R$CHO)
1 M N-ethylacetamide, *p*H 7	None	< 0·1
1 M N-ethylacetamide, *p*H 7	Cysteine, 4×10^{-4} M	2·8

* At dosages below $\sim 2{\cdot}5 \times 10^{18}$ eV/g.
† Dihydrocytosine is unstable and hydrolyses spontaneously to yield dihydrouracil and ammonia.
‡ The indicated concentrations of $R'H$ give the maximum enhancement in the yield of reduced products.

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

$$e^-_{aq} + R\text{CONH}R + H_2O \rightarrow R\dot{\text{C}}(\text{OH})\text{NH}R + OH^- \quad (5)$$

$$OH + R\text{CONH}R \rightarrow \dot{P} + H_2O \quad (6)$$

where k_6, k_7 fall in the range 10^7 to 10^8 M^{-1} s^{-1} (refs. 7 and 8). Combination of $R\dot{\text{C}}(\text{OH})\text{NH}R$ with like species or with the radical $\dot{P}$ would lead to formation of ketonic products. The combined yield of such products is low, however, with $G(>\text{CO}) \simeq 0{\cdot}2$; the evidence is that the reconstitution reaction

$$R\dot{\text{C}}(\text{OH})\text{NH}R + \dot{P} \rightarrow 2R\text{CONH}R \quad (7)$$

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

In accord with this, we find that cysteine effectively blocks the reconstitution reaction (7) through the step

$$R(\dot{\text{C}}\text{OH})\text{NH}R + R'H \rightarrow R\text{CHO} + \text{NH}_2R + R'$$

and, as shown in Table 1*B*, the reductive deamination of the peptide bond in the presence of cysteine at low concentrations is essentially quantitative with $G(CH_3CHO) \simeq 2{\cdot}5$.

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 3 and 7.

These results seem 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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