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September 6, 1968

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RECONSTITUTION ("REPAIR") MECHANISMS IN THE RADIOLYSIS OF AQUEOUS BIOCHEMICAL SYSTEMS: INHIBITIVE EFFECTS OF THIOLS

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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 $\gamma$-rays the 100 ev yields$^1$ of these reactive intermediates are closely approximated by the values: $G_{e_{aq}^-} \approx G_{OH} \approx 2.6$. 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 $\gamma$-radiolysis of oxygen-free solutions of the pyrimidine bases, both $e_{aq}^-$ and OH add preferentially to the 5,6 double bond$^2,3$

$$ e_{aq}^- + B + \text{H}_2\text{O} \rightarrow \text{BH} + \text{OH}^- $$

$$ \text{OH} + B \rightarrow \text{BOH} $$

---


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 $^3$

$$\hat{BH} + \hat{BOH} \rightarrow 2B + H_2O$$ (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

$$\hat{BH} + \hat{RH} \rightarrow BH_2 + H$$ (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.


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

$$e_{aq}^- + RCONHR + H_2O \rightarrow R\tilde{C}(OH)NHR + OH^-$$  \hspace{1cm} (6)

$$OH + RCONHR \rightarrow \dot{P} + H_2O$$  \hspace{1cm} (7)

where $k_6, k_7$ fall in the range $10^7$ to $10^8 \text{ M}^{-1} \text{ sec}^{-1}$ (ref. 7,8). Combination of $R\tilde{C}(OH)NHR$ 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 $G(>\text{CO}) \approx 0.2$; the evidence is that the reconstitution reaction

$$R\tilde{C}(OH)NHR + \dot{P} \rightarrow 2RCONHR$$  \hspace{1cm} (8)

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

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

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

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 $G(\text{CH}_2\text{CHO}) \approx 2.5$.

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 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.

This work was performed under the auspices of the United States Atomic Energy Commission.
Table 1. Effect of cysteine and ascorbic acid (RH) on the \( \gamma \)-ray induced reduction of pyrimidine \((C=C)\) and peptide \((C=O)\) linkages in oxygen-free solutions*.

<table>
<thead>
<tr>
<th>Solution</th>
<th>RH ‡</th>
<th>G(BH₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.05 M Cytosine, pH7</td>
<td>none</td>
<td>&lt; 0.9</td>
</tr>
<tr>
<td>.05 M Cytosine, pH7</td>
<td>cysteine, (2.5 \times 10^{-3}) M</td>
<td>2.8</td>
</tr>
<tr>
<td>.05 M Cytosine, pH7</td>
<td>ascorbic acid, (1.5 \times 10^{-3}) M</td>
<td>2.9</td>
</tr>
</tbody>
</table>

B. N-ethylacetamide \(\rightarrow\) acetaldehyde \((RCHO)\) + ethylamine

<table>
<thead>
<tr>
<th>Solution</th>
<th>RH ‡</th>
<th>G(RCHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M N-ethylacetamide, pH7</td>
<td>none</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>1M N-ethylacetamide, pH7</td>
<td>cysteine, (4 \times 10^{-4}) M</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*At dosages below ~\(2.5 \times 10^{18}\) ev/gm.

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

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