Chemical Screening of Radiation Protecting Agents

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An aqueous solution of 2-propanol was chosen as chemical system for fundamental study of the chemical screening of organic compounds on the responsibility for ionizing radiation. The effect of additives on the yield of acetone was investigated by subjecting this system on gamma-radiolysis. The results show that this system is suitable for the estimation of radiation protecting character of the known radiation protecting agents and compounds which act as an OH scavenger. On the basis of these results, various nitrogen-containing additives having free amino group, guanidine group and hetero-cyclic amine were examined first of all in expectation of improvement for the toxicity of sulfur-containing compounds.

Key Words: 2-propanol, chemical screening, gamma-radiolysis, radiation protecting agents

1. Introduction

Numerous radioprotectors, especially sulfur-containing compounds, have been developed by extensive studies. None of these compounds, however, have yet been shown useful for practical application on radiation protection and clinical radiotherapy, because of their high toxicity. The mechanisms of radioprotection, i.e., radical scavenger hypothesis, mixed disulfide hypothesis, energy transfer hypothesis, hypoxia hypothesis and so on, have been proposed to explain the radiation-induced damaging of biological systems.

The protective action of sulfhydryl compounds in radiobiology is often explained by assuming a repair mechanism involving hydrogen atom transfer from the sulfhydryl group to a induced biological radical. Adams, et al. have demonstrated that the formation of simple organic free radicals, such as alcohol- and amino acid-radicals produced by hydrogen atom abstraction with OH radicals, is retarded in the presence of cysteamine. A similar reaction was also shown to occur in irradiated solutions of the polyethylene oxide. It is still necessary to examine the radioprotective effects in both chemical and biological systems.

We propose in this paper radiation protecting agents by examining the radical scavenger hypothesis. An acid aqueous solution of 2-propanol was chosen as a chemical system for fundamental work, and the effect of additives on the yield of acetone was investigated.

2. Experimental

2-Propanol (Wako Pure Chem. Extra Pure) and additives (Wako Pure Chem. and Tokyo Kasei Kogyo Co., Ltd. Guaranteed or Extra Pure) were used as received. Water was triply distilled by the ordinary procedures, and pH was adjusted by 0.1 M H2SO4 or 0.1 M NaOH.

Gamma-irradiation was carried out at an ambient temperature with 555 TBq (15 kCi) 60Co source at the Institute of Physical and Chemical Research. Dose rate, which was determined by using a Fricke dosimeter on the basis of $G(\text{Fe}^{3+}) = 15.6$, was 185 Gy (18.5 krad)·min⁻¹ throughout the study. Each sample of 5 ml was irradiated to a total dose of 40 kGy (4 Mrad)·m²⁻¹.
Analysis of acetone formed was made by gaschromatography using a 2-m polyethylene glycol-20 M column at 100°C. G-values of acetone formed were calculated after correction of adsorbed dose for electron densities of solution used.

3. Results and Discussion

3.1 Examination of chemical system

The reaction mechanism for the formation of acetone has been proposed by Cohen, et al. in the gamma-radiolysis of deaerated acid aqueous solution of 2-propanol (0.1 M): 

\[
\begin{align*}
\text{C}_3\text{H}_7\text{CHOH} + \text{H}(\text{or} \text{OH}) & \rightarrow \text{C}_3\text{H}_7\text{COH} + \text{H}_2(\text{or} \text{H}_2\text{O}) \quad (1) \\
\text{C}_3\text{H}_7\text{COH} + \text{H}_2\text{O}_2 & \rightarrow \text{C}_3\text{H}_7\text{C}=\text{O} + \text{H}_2\text{O} + \text{OH} \quad (2)
\end{align*}
\]

Thus, the yield of acetone, \( G(\text{acetone}) \), is thought to be decreased by adding the scavenger for H or OH. Since the hydrated electrons are converted to \( \text{H}_2\text{O}_2 \) in the aerated acid aqueous solution via reaction (3) \( \sim \) (5), the main reactive intermediate is OH radicals under the present experimental conditions.

\[
\begin{align*}
\epsilon_{\text{aq}} + \text{H}_2\text{O}^{-} & \rightarrow \text{H} + \text{H}_2\text{O} \quad (3) \\
\text{H} + \text{O}_2 & \rightarrow \text{HO}_2 \quad (4) \\
2 \text{HO}_2 & \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \quad (5)
\end{align*}
\]

Consequently, in the presence of additives, \( G(\text{acetone}) \) is affected by the competitive reactions between OH radicals and the additives for the hydrogen atom abstraction from 2-propanol. In the present study, known radiation protecting agents were used as additives.

3.1.1 Effects of coexisting air on \( G(\text{acetone}) \)

Aerated and deaerated acid aqueous solutions of 2-propanol (0.1 M, pH=3.0) were subjected to gamma-radiolysis at various dose rates to a constant dose of 40 kGy (4 Mrad). The value of \( G(\text{acetone}) \) for aerated solution decreases to some extent with an increase in dose rates as shown in Fig. 1, although that for deaerated solution is constant over the whole dose rate range examined. These results may be attributed to a specific dose rate effect on the radical reactions in which oxygen is involved. Although the mechanism is not clearly understood, a series of experiments were carried out at a constant dose rate of 185 Gy (18.5 krad) \( \text{min}^{-1} \) in practical means. The value of \( G(\text{acetone}) \) for aerated solution is nearly same as that for deaerated ones at this dose rate.

3.1.2 Effect of known radiation protecting agents on \( G(\text{acetone}) \)

Known radiation protecting agents were used as additives to examine their effect on \( G(\text{acetone}) \). The initial concentrations of these agents were kept constant (0.003 M) throughout the experiments. The values of \( G(\text{acetone}) \) and the ratios of \( G(\text{acetone}) \) for the system with additives to that without the additives are given in Table 1. All the additives, except adenosine, give smaller than 1.0. A ratio of 0.74 was obtained with the extract from roots of Penax ginseng whose radioactive effect was noted by Yonezawa and Takeda, et al. These results indicate that the known radiation protecting agents tested were found to inhibit the formation of acetone, and that this system is regarded as being suitable

<table>
<thead>
<tr>
<th>Agent</th>
<th>( G(\text{acetone}) )</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Cysteine ethylester + HCl</td>
<td>0.80</td>
<td>0.45</td>
</tr>
<tr>
<td>Histamine + 2HCl</td>
<td>1.35</td>
<td>0.76</td>
</tr>
<tr>
<td>Adenosine</td>
<td>2.21</td>
<td>1.24</td>
</tr>
<tr>
<td>Adenine</td>
<td>1.10</td>
<td>0.62</td>
</tr>
<tr>
<td>Ethylene thiourea</td>
<td>1.46</td>
<td>0.82</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>1.78</td>
<td></td>
</tr>
</tbody>
</table>

* Ratios of \( G(\text{acetone}) \) for solution containing additive to that for solution containing no additive
Table 2 Effect of the additives whose rate constants with OH radicals are higher than that for 2-propanol with OH radicals

<table>
<thead>
<tr>
<th>Agent</th>
<th>Rate constant* (pH) (M⁻¹·s⁻¹)</th>
<th>Acetone (M×10⁵)</th>
<th>Ratio (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Ascorbic acid</td>
<td>1.2×10¹⁰ (1.0)</td>
<td>10.7</td>
<td>2.58</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>5.7×10⁹ (3.0)</td>
<td>5.98</td>
<td>1.43</td>
</tr>
<tr>
<td>1-Butanol</td>
<td>4.6×10⁹ (2.0–2.2)</td>
<td>6.58</td>
<td>1.58</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>5.9×10⁹ (2.0–2.2)</td>
<td>6.34</td>
<td>1.53</td>
</tr>
<tr>
<td>2-Methyl-4-phenyl-2-butanol</td>
<td>6.8×10⁹ (1.7–1.8)</td>
<td>6.10</td>
<td>1.47</td>
</tr>
<tr>
<td>3-Pentanol</td>
<td>2.4×10⁹ (1.7–1.8)</td>
<td>6.72</td>
<td>1.62</td>
</tr>
<tr>
<td>1-Phenyl-1-propanol</td>
<td>1.2×10¹⁰ (1.7–1.8)</td>
<td>5.26</td>
<td>1.27</td>
</tr>
<tr>
<td>2-Phenyl-2-propanol</td>
<td>5.3×10⁹ (1.7–1.8)</td>
<td>5.93</td>
<td>1.43</td>
</tr>
<tr>
<td>Suberic acid</td>
<td>4.0×10⁹ (2.0–2.2)</td>
<td>6.49</td>
<td>1.56</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>1.2×10⁹ (2.8)</td>
<td>7.76</td>
<td>1.87</td>
</tr>
</tbody>
</table>

* Rate constants for the reaction of OH radical with agents

for the estimation of radiation protecting character. Since G(acetone) is decreased by scavenging OH radicals in this reaction system, we made a point of testing to the additives whose rate constants with OH radicals are higher than that for 2-propanol with OH radicals. The results are shown in Table 2 for the some selected additives⁸. The values of G(acetone) for all compounds tested, except for 1-ascorbic acid, are lower than those of the run containing 2-propanol.

3.2 Effect of nitrogen-containing additives

Nitrogen-containing compounds were also examined by the present method. Since no protecting tendency was observed for primary, secondary, tertiary and quaternary aliphatic amines, the compounds which have free amino group, quanidine group and heterocyclic amines were examined. Results are shown in Figs. 2, 3 and 4. The ratio of G(acetone) for guanidine bicarbonate is much less than 1.0, although those for glycine-methylester and -ethylester, and β-phenethylamine are rather close to 1.0. These compounds, therefore, may be worth radiation protecting agents. As seen in Fig. 4, aromatic amines and relatively simple heterocyclic amines, such as 1,2,4-triazole, pyrrole, imidazole and aniline give low ratios. The effect of substituents on the radiation protecting character for aniline and picoline derivatives indicates the order of -CH₃ > -OH > -NH₂. The size of heterocyclic ring seems to affect on the ratios in a series of hexamethyleneimine, piperidine and pyrrolidine. Rinaldi, et al.⁹ have been reported the consistent results with the present data for the radiation protective character of imidazole. Carbonyl groups supposedly act as a sensitizer from the...
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Fig. 3 Variation of ratio of G(acetone) for hydrazines, amidines and amides.

results of Nos. 4, 12, 13 and 14 in Fig. 2, and Nos. 13, 14 and 15 in Fig. 4. Biological tests may be started by accumulating these informations required for the molecular design of radiation protecting agents.

References

8) Farhataziz and A.B. Ross: The National Standard Reference Data System-NBS 59
9) R. Rinaldi, Y. Bernard and M. Guilhermet: Comp. Rend., 261, 570 (1965)
要　旨

放射線防護剤の化学的選別法

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放射線防護剤を化学的な系を用いて選別するために，2-プロパノール酸性水溶液の放射線照射で
できるアセトンの収率に対する添加物効果を検討した。添加物として，既知放射線防護剤および
OH ラジカル捕捉剤を用いた実験では，アセトン収率が抑制されることが分かり，この系が放射線
防護剤の評価に十分利用できることを提案し，さらに，従来毒性が強いといわれたイオウ化合
物に代わる化合物を開発する目的で，まず N-化合物を検討した。