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NITROBENZENE RADICAL IONS AS INDICATORS FOR REDUCING SPECIES
FORMED DURING RADIOLYSIS OF AMINO ACID SOLUTIONS (a)

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A method is presented for detecting the formation of radicals derived from solutes such as amino acids during gamma irradiation of aqueous solutions. These radicals are expected to form as a result of hydroxyl-radical or hydrogen-atom attack on amino acids and other compounds. In our irradiator-ESR flow system, ⁽¹⁾ (Figure 1), they disappear too rapidly for direct observation and so we have resorted to an indirect method of detection. In this method, the short-lived amino-acid radicals reduce nitrobenzene to form the long-lived nitrobenzene radical ion.

In irradiated, oxygen-free alkaline solutions, nitrobenzene is converted to its radical anion which has a characteristic 54-line ESR spectrum with a lifetime of several seconds. ⁽²⁾ (Figure 2) This ESR spectrum can be eliminated by saturating the solution with nitrous oxide which results in removal of the hydrated electron through rapid reaction to form hydroxyl radicals. (Figure 3) If other reducing species, in addition to the hydrated electron, are present and capable of reducing nitrobenzene, then we can expect an enhancement of the nitrobenzene-radicals signal amplitude. If the ESR signal also persists after saturating the solution with nitrous oxide, this indicates that the new reducing species has been produced by hydroxyl-radical attack on the solutes, i.e., amino acids, present with the nitrobenzene. Both methanol and formate ion have been shown to form such reducing species ⁽²⁾ and now some of the naturally occurring amino acids have also been tested in this manner.

The results obtained with serine, alanine, valine and methanol are presented in Figure 4. There we see that the hydroxyl-containing amino acid, serine, is in the same class as methanol and forms a radical capable of reducing nitrobenzene. Its structure is most likely $\text{HOCHCH}(\text{NH}_2)\text{COO}^-$ in

analogy with radical formed from other simple alcohols^(3, 4). These alcohol radicals are thought to reduce nitrobenzene to its radical ion at essentially the same rate as does the hydrated electron.⁽⁵⁾ Much higher concentrations of alanine, the non-hydroxylated analog of serine, and valine are required to provide the same enhancement of the nitrobenzene-radical signal. When nitrous oxide is added, we see evidence for a reducing radical produced by hydroxyl-radical attack on alanine but this is not seen with valine. Since the alanine concentration is 0.1M, this reducing species might be due to secondary products of radiolysis. The enhancement of the ESR signal produced by alanine and valine in the absence of nitrous oxide is probably due primarily to the removal of hydroxyl radical from competition with the reduction by the hydrated electron.

Other compounds present in living tissue may also be expected to form free radicals during radiolysis and these can attack easily reduced substances and thus extend radiation damage. We expect to extend our technique for detecting such radicals into the neutral pH range by using more stable nitroaromatic radicals such as nitrobenzoic radical-anion. There seems to be a wide variety of uses for nitroaromatic or other long lived, easily produced radicals as indirect probes of radiation-chemical damage in biochemical systems where the chemical constituents as well as conformational variation leave a great number of interactions to be explored.

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FLOW SYSTEM FOR IRRADIATION OF SOLUTIONS

Figure 1

Figure 2

ESR Spectrum of a $9 \times 10^{-3} M$ Solution of Nitrobenzene in Aqueous $0.1 M$ Sodium Hydroxide Following Gamma-irradiation.

Rough Draft

Nitrobenzene $1 \times 10^{-3} M$
+ Mannitol at $4.8 \times 10^{-3} M$ (initial)

G100, MSO, R3, P0.3, 506/25 mm

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Figure 3. Effect of Adding Nitrous Oxide
to Nitrobenzene Solution During
Flow Through Co^{60} -ESR System
(Rough Draft)

↑
pH 11.16

↑
 N_2O
Bubbling

↑
 N_2O
stopped

↑
10 ml of $9 \times 10^{-1} M$
Mannitol
To $\frac{5.4 + 9}{11.2} = 1.27 \times 10^{-2} M$ in Mannitol

Effect of Added Solutes on ESR Signal Amplitude From Nitrobenzene Radical Ion

Figure 4. (Rough Draft)