**RADIOMIMETIC EFFECTS OF PEROXIDIZING LIPIDS ON NUCLEIC ACIDS AND THEIR
BASES**

BNL--33304

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Introduction

Lipid peroxidation has been identified in a wide variety of pathologies, and a possible role in carcinogenesis is the subject of great current research interest (1). Although oxidative cell death appears to depend mostly on membrane damage (and cell membrane changes are also important in cellular transformation and the promotion stages of carcinogenesis), we have proposed that lipid peroxidation may also provide a significant linkage between oxygen radical effects in cells and sublethal damage to DNA, perhaps by way of the nuclear membrane (2,3). Persistent mutational or clastogenic sequelae from co-oxidation of DNA and/or chromatin with lipids might then be important with regard to either the initiation or promotion of carcinogenesis. Nonetheless, cause-and-effect relationships directly linking lipid oxidation to DNA-dependent damage in tissues have yet to be established.

In model system studies, free radical intermediates of oxidizing lipids have been shown to be radiomimetic, that is, capable of inducing free radicals and subsequent radiation-like damage, in amino acids and proteins (4,5). It seems reasonable to expect, then, considering this reactivity, that oxidizing lipids should be radiomimetic towards nucleic acids and their base components as well as other important cellular macromolecules, and we present here data showing that this indeed is so.

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Results

Since free radicals are stabilized in dry matrices, we have begun our investigations using dry molecular mixtures similar to those used by Henriksen for radiation studies (6). Unoxidized or pre-oxidized methyl lineolate was mixed directly with DNA and purine and pyrimidine bases (1:5 w/w), then incubated at room temperature or at 37°C for varying periods of time, at which point samples were analyzed for free radical content by electron spin resonance spectroscopy.

Our findings may be summarized as follows (typical ESR signals are shown in Figure 1):

1. DNA facilely produced singlet envelope signals similar to the somewhat structureless signals induced by gamma and UV radiation (7-10). The rapidity of signal production and the intensity and stability of the signals were all substantially greater than observed for individual base components, probably reflecting radical transfer and stabilization along the DNA backbone.

2. Pyrimidines produced ESR signals readily, with the ease of formation of stable radicals for cytosine > uracil >> thymine. In each case spectra indicated more than one radical site, but resolution in powder spectra was generally insufficient for precise site identification. Thiol or methyl-mercapto substituents, when present, provided preferential sites for radical attack by oxidizing lipids, which appear as low-field ESR signal components.

3. Purines formed stable radicals with difficulty. Adenine and its nucleoside did not form detectable radicals at all. Guanine and guanosine required long incubation periods (about 20 days) before ESR signals were detected. The guanine signal was a clean singlet, possibly reflecting substantial delocalization of the electron. The guanosine signal formed somewhat less facilely, and showed at least five lines arising from more than one center; it is likely that the ribose moiety was also a site for radical attack (11).

4. That purines did not form stable radicals does not indicate they are not susceptible to attack by oxidizing lipids. In aqueous model systems, incubated for three days at 37°C, TLC of the reacted bases

showed formation of products (as yet unidentified) in the purines but no decomposition in the pyrimidines; concentrations were too dilute to allow ESR detection of any radicals produced. Two alternative explanations are plausible:

- a) oxidizing lipids indeed transfer free radicals to purines (i.e., abstract labile hydrogens), but the resulting purine radicals are unstable and further intramolecular rearrangement or decomposition occurs quite rapidly, or

Figure 1. Typical ESR spectra from solid samples of DNA, pyrimidines, and purines incubated with oxidizing methyl linoleate for periods of from 1 day (DNA) to 3 weeks (guanosine).

b) $LO\cdot/LOO\cdot$ radicals from oxidizing lipids add to purines, e.g., at C-8, rather than abstracting hydrogens. [This has been shown in ESR flow studies to be the preferred mechanism of $\cdot OH$ reaction with purines (11).]

5. The powder spectra detected and the patterns of reactivity exhibited for DNA and pyrimidine and purine bases are all remarkably similar to those reported for gamma and UV irradiation (12-15). Although much more definitive information must be obtained regarding the kinetics and reaction mechanisms involved and the specific products formed, it is clear that oxidizing lipids are capable of exerting radiomimetic effects on nucleic acids and bases.

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