

of the stereochemical course of the hydrolysis reaction catalyzed by the spleen exonuclease would be extremely difficult with phosphorothioate methodology; thus, the results we report in this communication demonstrate an unanticipated advantage in using oxygen chiral phosphate esters to determine the stereochemical course of an enzyme-catalyzed reaction, i.e., the ratio of potential reaction products need not be the same when phosphorothioates are used. This problem can occur only with enzymic reactions which involve the formation of an intermediate which can partition to different acceptors.

In addition, the ability of the enzyme to catalyze an efficient transphosphorylation reaction with phosphorothioate substrates may provide a useful synthetic approach to the synthesis of oligonucleotides which have internucleotide phosphorothioate linkages: with low concentrations of nucleoside 3'-[(4-nitrophenyl)phosphorothioates] and high concentrations of nucleoside or nucleotide acceptors, good yields of a wide variety of oligonucleotide analogues can be anticipated.

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### Comparison of the Capacities of the Peroxyhydroxyl and the Superoxide Radicals To Initiate Chain Oxidation of Linoleic Acid

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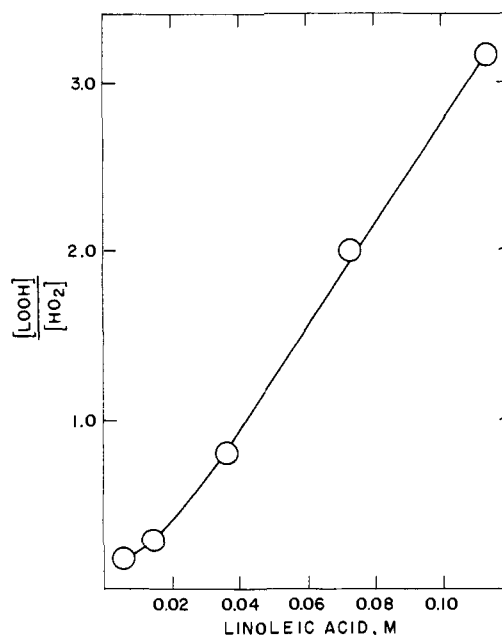
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The discovery that tissues of aerobic organisms contain an enzyme whose specific role appears to be the removal of superoxide radicals<sup>1</sup> has led to the suggestion that  $\text{O}_2^-$  is a potent agent of biological damage.<sup>2</sup> Many studies support this view. Conditions which lead to the generation of this radical have been associated with some significant pathological disorders<sup>3,4</sup> that can often be prevented by the addition or enhancement of naturally occurring levels of superoxide dismutase.<sup>2</sup> It has been shown that few biochemicals react directly with  $\text{O}_2^-$ ;<sup>5,6</sup> those which do react undergo electron-transfer reactions that occur under normal metabolic conditions and are therefore unlikely to cause cell injury.

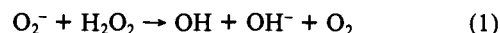
It was then proposed<sup>6</sup> that a derivative of  $\text{O}_2^-$ , an "active oxygen" species such as OH or  $^1\text{O}_2$  (singlet molecular oxygen),

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**Figure 1.** Effect of linoleic acid concentration upon the yield of linoleic hydroperoxide (LOOH) per  $\text{HO}_2$  added. 11.5 mL of a photolyzed ethanol solution (70% ethanol, 1.25 mM  $\text{O}_2$ , 0.01 M KOH) containing 88.15  $\mu\text{M}$   $\text{O}_2^-$  were added dropwise to 15 mL of a rapidly stirred 70% ethanol solution containing 0.09 M  $\text{H}_2\text{SO}_4$  and varying amounts of linoleic acid. The yields monitored at 233 nm (absorption maximum of LOOH) were computed on the basis of the final volume.

could be the agent of damage. The notion that OH is formed in the so-called Haber-Weiss<sup>7</sup> reaction



had to be abandoned when its rate constant  $k_1$  was shown to lie between 0.5 and 0.1  $\text{M}^{-1} \text{s}^{-1}$ .<sup>8</sup> On the other hand, the very short lifetime in water (about 2  $\mu\text{s}$ ) and high specificity of its reactions make  $^1\text{O}_2$  an unlikely candidate for the role of an important biological intermediate. Also, there is evidence accumulating which negates the formation of singlet molecular oxygen from  $\text{O}_2^-$ .<sup>9</sup> Little can be said at present about an alternative scheme which involves the participation of transition-metal complexes in reactions of  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$ . It has long been known that OH can be generated in Fenton-type processes,<sup>10</sup> but the chemical complexity of tissues has so far not allowed the unequivocal demonstration of their occurrence in vivo.

A transient species always formed in presence of  $\text{O}_2^-$  in water whose possible role in biology has been almost completely ignored is its conjugate acid, the peroxyhydroxyl radical ( $\text{HO}_2$ ). From systematic studies of the rates of reaction of  $\text{HO}_2/\text{O}_2^-$  with a number of biochemicals as a function of pH (Table I) a picture emerges that suggests that  $\text{HO}_2$  may be responsible for some of the biological effects presently attributed to  $\text{O}_2^-$ . Not only does  $\text{HO}_2$  react faster than  $\text{O}_2^-$  with the compounds listed, but its

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Table I. Comparison of Reactivities of HO<sub>2</sub> and O<sub>2</sub><sup>-</sup> with Compounds of Biological Interest

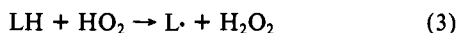
compound	$k_{\text{HO}_2+\text{X}}, \text{M}^{-1} \text{s}^{-1}$	$k_{\text{O}_2^{-}+\text{X}}, \text{M}^{-1} \text{s}^{-1}$	ref
alanine	<44.0	<0.06	5b
linoleic acid	<300.0	0.00	this work
cysteine	~600.0	<15.0	5b
NADH	$1.8 \times 10^5$	<<27.0	11, 12
ascorbic acid	$1.25 \times 10^6$	$5.75 \times 10^4$	13
lactate dehydrogenase-NADH complex	$1.20 \times 10^6$	$3.6 \times 10^4$	14
cytochrome <i>c</i>	$2.00 \times 10^6$	$5.0 \times 10^4$	15
glyceraldehyde-3-phosphate dehydrogenase-NADH complex	$2.00 \times 10^7$	0	16
horseradish peroxidase, compd I	$2.20 \times 10^8$	$1.6 \times 10^6$	17
O <sub>2</sub> <sup>-</sup>	$1.02 \times 10^8$	<0.35	18

simultaneous presence under any condition of pH is ensured by the equilibrium

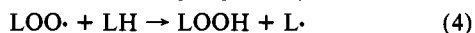


whose pK is 4.7.<sup>18</sup> Thus, in physiological conditions, nearly 1% of any O<sub>2</sub><sup>-</sup> formed is in the protonated form.

The purpose of the present investigation was the study of the reactivity of HO<sub>2</sub> with linoleic acid (LH), a C<sub>18</sub> unsaturated fatty acid with two double bonds. The water, ethanol, and all solutes used in this study were rigorously purified to ensure absence of metal and organic contaminants. The linoleic acid was freed of peroxide by passage through a silica gel column.<sup>19</sup> The HO<sub>2</sub> was formed from O<sub>2</sub><sup>-</sup> generated by photolysis of oxygenated alkaline (0.01 M KOH) ethanol solutions in an apparatus described previously.<sup>20</sup> Exposure to a vacuum-UV Xe-plasma lamp for a few seconds yielded 200–300 μM concentrations of O<sub>2</sub><sup>-</sup> which decayed only slowly with a bimolecular rate constant of 22 M<sup>-1</sup> s<sup>-1</sup>, very close to the value measured in alkaline water.<sup>18</sup> When the alcohol solutions were mixed rapidly with up to 0.2 M linoleic acid dissolved in 70% aqueous ethanol, there was no increase in absorbance at 233 nm, indicating that the acid was not oxidized. In addition, the spontaneous rate of decay of O<sub>2</sub><sup>-</sup> measured at 270 nm was unaffected by the presence of linoleic acid, showing that the radical and the acid did not react. When, on the other hand, the O<sub>2</sub><sup>-</sup> was quantitatively converted to HO<sub>2</sub> by inclusion of 0.09 M H<sub>2</sub>SO<sub>4</sub> in the linoleic acid solution, the characteristic spectrum of linoleic hydroperoxide (LOOH) with a maximum at 233 nm was observed. A plot of the yield of LOOH per quantity of HO<sub>2</sub> added is shown in Figure 1. The molar ratio (LOOH)/(HO<sub>2</sub>) shows that a chain reaction operated at high concentrations of the acid. A typical experimental result obtained in the stopped-flow spectrophotometer is shown in Figure 2. The first part of the curve, recorded at a fast rate, shows the decay of HO<sub>2</sub> radicals in presence of 0.064 M linoleic acid. In absence of linoleic acid the kinetics were strictly second order with a rate constant  $k_{\text{HO}_2+\text{HO}_2} = (8.7 \pm 1.4) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ , close to the value recorded in water at pH 1–2.<sup>18</sup> The estimated rate constant of the chain-initiating reaction



is  $k_3 \leq 300 \text{ M}^{-1} \text{ s}^{-1}$ . The kinetic result recorded at a slower scan rate (Figure 2B) shows the rate of formation of the stable end product linoleic hydroperoxide, with a calculated rate constant of  $k_4 = 36 \pm 7 \text{ M}^{-1} \text{ s}^{-1}$ . This step is probably the reaction



which has an estimated rate constant of 50 M<sup>-1</sup> s<sup>-1</sup> for ethyl linoleate.<sup>21</sup>

Our findings have added another compound to the list in Table I. The question whether oxidation of linoleic and probably other polyunsaturated fatty acids by HO<sub>2</sub> can lead to biological damage

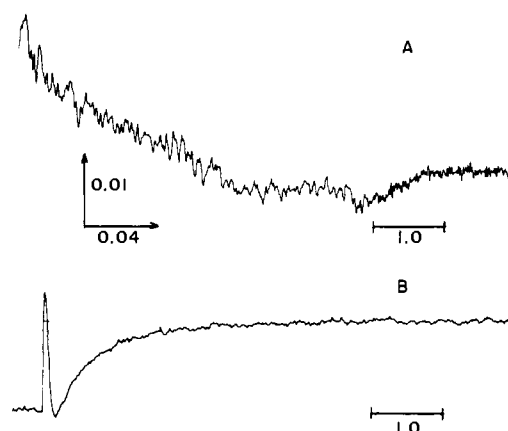


Figure 2. (A) Observed change in absorbance at 250 nm with time after rapid mixing of a O<sub>2</sub><sup>-</sup> solution (70% ethanol, 1.25 mM O<sub>2</sub>, 0.01 M KOH, photolyzed for 2 s) with an acidic (0.09 M H<sub>2</sub>SO<sub>4</sub>) 70% ethanol solution containing 0.032 M linoleic acid. Observe split time base (horizontal markers are equal to 0.04 and 1 s, respectively). The time constant for noise filtering was set at 0.1 ms. The vertical marker is equal to 0.01 absorbance units. The experiment was carried out in a modified Durrum D 110 stopped-flow spectrophotometer. (B) The experiment is similar to (A) except the photolysis time was 4 s and the time constant for noise filtering was set for 50 ms. The molar extinction coefficients for HO<sub>2</sub> and LOOH at 250 nm are 900 and 6440 M<sup>-1</sup> cm<sup>-1</sup>, respectively.

will require further work. However, it can be assumed that the most obvious sites for such damage are the plasma and intracellular membranes. Two circumstances are likely to contribute to this. First, the extent of conversion of O<sub>2</sub><sup>-</sup> to HO<sub>2</sub> by equilibrium (reaction 2) is likely to be increased in the immediate vicinity of a membrane due to the presence of an electrical double layer at the predominantly negatively charged surface. The pH of such surfaces is always lower than the bulk pH, with as much as a 3-pH unit difference in model systems.<sup>22</sup> Conversion of O<sub>2</sub><sup>-</sup> to HO<sub>2</sub> near a membrane, with the resultant loss of charge, would allow the radical to penetrate more easily into the lipid region, without experiencing the Coulombic forces at the surface. Once inside, the HO<sub>2</sub> could initiate oxidation which can lead to significant impairment of the membrane function.<sup>23</sup>

The second circumstance which can contribute to membrane damage lies in the tight molecular packing of the constituent lipids. The rate-controlling step in the oxidative chain (reaction 4) depends on the concentration of unoxidized unsaturated fatty acids. Earlier work has shown that molecular aggregation of linoleic acid in micelles leads to a great enhancement of its rates of oxidation.<sup>24</sup>

A short up-to-date review of the various mechanisms that have been proposed for the O<sub>2</sub><sup>-</sup> mediated oxidation of polyunsaturated fatty acids can be found in a recent report on the oxidation of

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arachidonic acid in micelles by the  $O_2^- - H_2O_2$  system.<sup>25</sup> Our preliminary studies of arachidonic acid indicate that it too reacts with the  $HO_2$  radical.

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### Production of Gas-Phase Bare Transition-Metal Clusters by Laser Photodissociation of Organometallic Cluster Compounds

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The characterization of the structure and reactivity of bare transition-metal clusters is currently an area of intense research.<sup>1</sup> Information concerning the electronic and vibrational spectra of these highly reactive species has been successfully obtained in low-temperature matrix isolation experiments.<sup>2</sup> However, the possibility of perturbing effects of lattice interactions on the spectra and structure of the sample and the difficulty of extracting information concerning a particular cluster size in a sample of mixed composition complicate the interpretation of matrix results. Bare metal clusters have been produced in the gas phase by aggregation of metal atoms vaporized by resistance<sup>3</sup> or laser<sup>4</sup> heating, but limited cluster size specificity may render these techniques inapplicable to the study of large clusters by non-mass-selective spectroscopic methods.

In the present communication, we propose laser photodissociation of organometallic cluster compounds as an alternative method for producing bare transition-metal clusters in the gas phase. The great variety of metal cluster sizes and geometries available in polynuclear organometallic compounds,<sup>5</sup> combined with the remarkable efficiency of multiphoton dissociation for producing neutral bare metal fragments from organometallic precursors,<sup>6</sup> suggests the potential of this technique for preparing well-defined bare metal clusters in sufficient concentrations for spectroscopic characterization. A further advantage of this preparative scheme is that it is applicable to highly refractory metals without the use of high temperature sources. The feasibility

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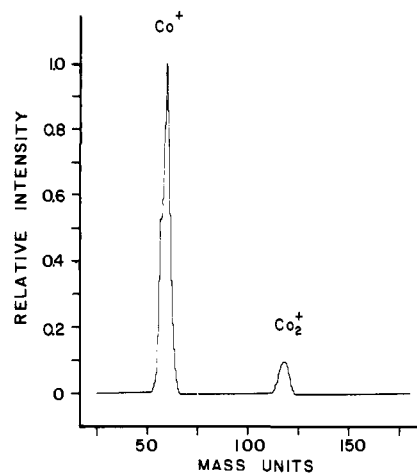
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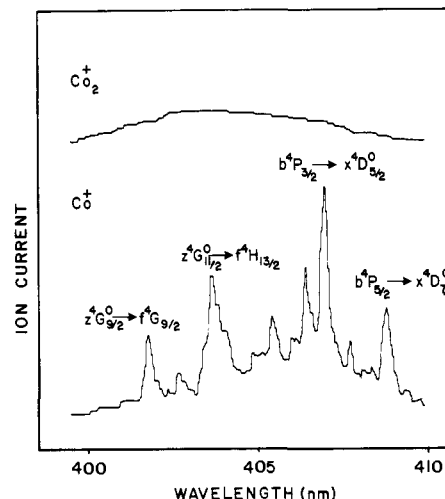
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**Figure 1.** Mass spectrum of photoproducts following multiphoton dissociation and ionization of  $Co_2(CO)_8$ . The laser was tuned to 406 nm and focused onto the molecular jet by a 7-cm focal length lens mounted inside the vacuum chamber. The apparatus is described in the text and ref 7. Scans up to the parent mass at 342 amu detected no additional ionic species. Peak positions of the spectral features shown were measured to  $\pm 1.0$  amu using several ions of known masses as calibration points. This precision is sufficient to positively assign the higher mass peak to  $Co_2^+$  (117.9 amu) rather than the closest likely ion,  $Co(CO)_2^+$  (115.0 amu).



**Figure 2.** Wavelength dependence of  $Co^+$  and  $Co_2^+$  signal intensities. The  $Co^+$  ion yield is enhanced when the laser wavelength is tuned to an allowed transition of  $Co(I)$ . The assignments indicated in the figure were made by using the atomic energy level data of ref 8b. The  $Co_2^+$  spectrum is expanded fivefold with respect to the  $Co^+$  spectrum. The spectra were taken with a resolution of 0.2 nm and were not corrected for the laser intensity, which maximizes at 407 nm.

of the proposed technique is illustrated below by the results of its application to  $Co_2(CO)_8$ .

In the present experiment, gas-phase  $Co_2(CO)_8$  was photodissociated, and the metal fragments were detected by mass-selective multiphoton ionization.  $Co_2(CO)_8$  was evaporated under its room temperature vapor pressure into a chamber with a background pressure of less than  $1 \times 10^{-5}$  torr, where it was irradiated with pulses of 10-ns duration from a Lambda Physics excimer-pumped tunable dye laser system. The sample number density was approximately  $10^{12}$  molecule  $mL^{-1}$  and the photon flux approximately  $10^8$   $W\ cm^{-2}$  in the region of intersection of the sample jet with the laser beam. Multiphoton absorption processes resulted in dissociation and ionization of the  $Co_2(CO)_8$  during a single laser pulse. The cations produced were collected, then mass selected by a quadrupole mass analyzer whose axis was perpendicular to both the molecular jet and the laser beam. The ion signal was detected by a channeltron electron multiplier and a boxcar averager.