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The Peroxyl Radical

by

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Abbreviations:
CPO-Chloroperoxidase, **CRDS**-Cavity Ring Down Spectroscopy, **DMPO**-5,5-DiMethyl-Pyrroline Oxide, $\mathbf{E_a}$-Energy of activation, **EPR**-Electron Paramagnetic Resonance, **FTIR**-Fourier Transform Infrared Spectroscopy, **MDA**-Microwave Dielectric Absorption, **PUFA**-Poly Unsaturated Fatty Acid, **UV**-Ultraviolet Spectroscopy

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Abstract:

The peroxyl radical is very important in many biological systems, including lipid peroxidation, DNA cleavage, and protein backbone modification. Peroxyl radicals can be created by several methods, such as chemically (KO_2), enzymatically, (Xanthine Oxidase) or physically (γ-irradiation). Detection methods range from the obvious, EPR, to the more complex, CRDS and microwave dielectric absorption. Finally, the rich reaction chemistry of the peroxyl radical will be discussed.

Introduction:

The conjugate acid of superoxide, $O_2^{\bullet -}$, is $HOO^{\bullet}$. This is the dioxyl radical, the simplest peroxyl radical. There are many more complex peroxyl radicals, including cholesterol derivatives,[1] fatty acids,[2] and many more. The chemistry of this type of molecule is varied due to the identity of the R group, the local environment, and concentrations of O_2 and other reactants. These molecules can be generated by a variety of means, and their spectroscopy is equally diverse.

Perhaps the most interesting feature of peroxyl radicals is the sundry reactions in which they participate. The complex set of reactions involved in lipid peroxidation [3] could be topic for a paper of this size, but will just be touched upon here. Peroxyl radicals are also involved in food spoilage [4], DNA cleavage [5], and protein backbone modification [6]. In addition, the identity of the R group of the peroxyl radical has a profound influence on the amount of self-reaction, which induces the formation of alkoxyl radicals and molecular oxygen [7]. Of course, this knowledge would be unobtainable without the proper spectroscopy, so a thorough discussion of the methods of detection is included.

Generation of Peroxyl Radicals

Several methods may be used to generate peroxyl radicals, ranging from chemical and physical to enzymatic techniques. Each system has its own nuances, which can be altered somewhat to allow the desired information to be obtained from the experiment. The general method of the chain reaction is shown below, as taken from Gardner [3]

$RH + X^{\bullet} \rightarrow R^{\bullet} + XH$ (R_i = Rate of initiation)

$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$ (R_{p1} = Rate of propagation)
$ROO^{\bullet} + R'H \rightarrow ROOH + R'^{\bullet}$ (R_{p2} = Rate of propagation)

$R^{\bullet} + ROO^{\bullet} \rightarrow$
$2\ ROO^{\bullet} \rightarrow$ {Non-radical products, i.e. Termination)
$2\ R^{\bullet} \rightarrow$

The most basic chemical method of initiation is to introduce a carbon centered radical, which then attacks a dissolved O_2 molecule, yielding a peroxyl radical. A slightly more complex method is the solvation of KO_2. Under a stream of N_2, 80/20 EtOH/H_2O will dissolve KO_2 to K^+ and O_2^-, which can then be oxidized by cytochrome *c*, or some other oxidant, to form hydrodioxyl radical, $HOO^{\bullet}$.[2] Another easy chemical method involves a metal with an affinity for hydrogen abstraction and an open site for binding. The basic reaction is diagrammed below:

$$X_4M^n(CH_3) + O_2 \rightarrow X_4M^{n-1}(CH_3)(O_2) \rightarrow X_4MH + CH_3O_2^{\bullet}$$

Eligible metals include Ce, Ti and others. It is important to realize that metals involved in these reactions may further interfere with reactions.

Physical methods of generating radicals usually depend on bombarding highly active molecules, such as *tert*-Bu-OOH, with radiation. The relatively weak O-H bond will break homolytically, leaving $ROO^{\bullet}$. For example, approximately 0.2 Mrad of γ-radiation at 77 K will induce a radical, if the reaction flask is something inert, such as quartz. In addition, flash photolysis is often used. A typical experiment would use UV light, at approximately 12 350 nm. Pulse radiolysis is also common. Typical parameters include a pulse width of 20 to 100 ns, which gives a maximum dose of 5 krad. All of

these methods add enough energy to the system to overcome the rather high E_a of radical formation. Sometimes, however, the energy added causes breakdown of other reactants and/or products.

Enzymes provide another route to overcome the E_a needed to generate a radical. Xanthine oxidase uses a complex electron transfer pathway to generate $HOO^{\bullet}$, which can then react with lipids or other biological molecules. Heme peroxidase enzymes are important in the breakdown of many biological materials, and they usually use a radical pathway. Chloroperoxidase catalyzes the breakdown of organic hydroperoxides. Enzymes easily generate radicals at ambient temperatures and mild conditions, but are subject to denaturation by heat or chemical means. The spectra shown below illustrate this fact.

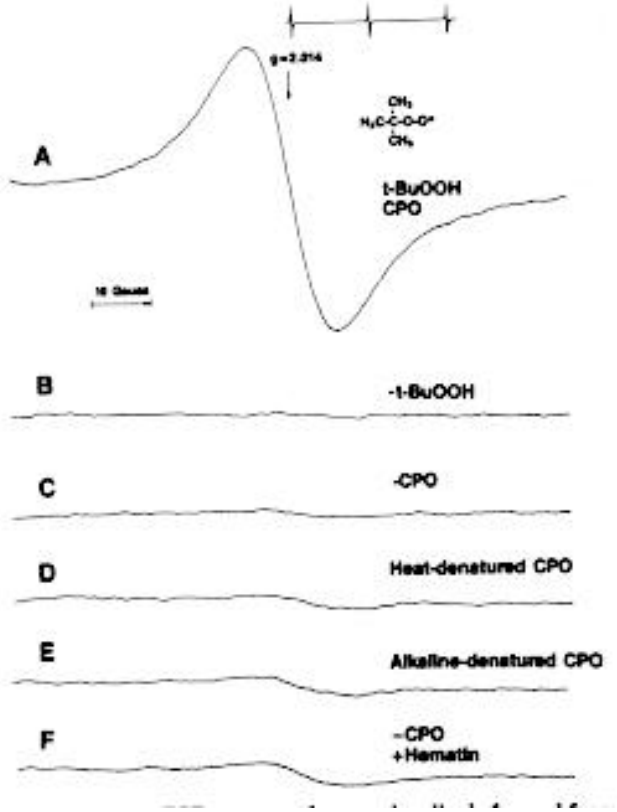


Figure 1. Direct EPR spectra of peroxyl radicals formed by incubation of *tert*-butyl hydroperoxide with CPO in 0.1M phosphate buffer at pH 6.4. no spectrum is observed when a vital component is missing, or the enzyme has been denatured. Adopted from [7].

Detection of Peroxyl Radicals

Nothing would be known about peroxyl radicals unless they could be detected in some fashion. The most common method is EPR, which relies on the absorption of radio waves by the free electron(s) while in a magnetic field. The characteristics of the peroxyl radical have also been determined by the use of UV spectroscopy, CRDS, FTIR, MDA, and electrochemical studies.

Some peroxyl radicals are stable enough to allow their investigation without the use of spin traps. Their spectra resemble Figure 1. Unfortunately, the relatively broad line observed in 1A doesn't allow for the elucidation of the radical environment. A spin trap will give much sharper, clearer lines, and give the researcher important keys to aid in the understanding of the radical. Paired with spectrum simulation software, the nature of the radical, whether peroxyl, alkoxyl or carbon-centered can be determined. An example of this was done by Mason, *et al*. [7] and is pictured in Figure 2.

Once radicals are discovered in a system, other means can be used to determine their properties. Peroxyl radicals absorb UV radiation and have a dipole moment. [8] Therefore, UV absorption spectroscopy could be done to determine quantitative amounts. Unfortunately, the transition involved has a half-width of nearly 40 nm, too broad and unstructured for definitive measurements. Peroxyl radicals also have a dipole moment, so IR spectroscopy could be done to determine stretching and bending modes. Unfortunately, traditional IR methods are too insensitive to be used in a biological situation. Recent developments, however, have yielded valuable information.

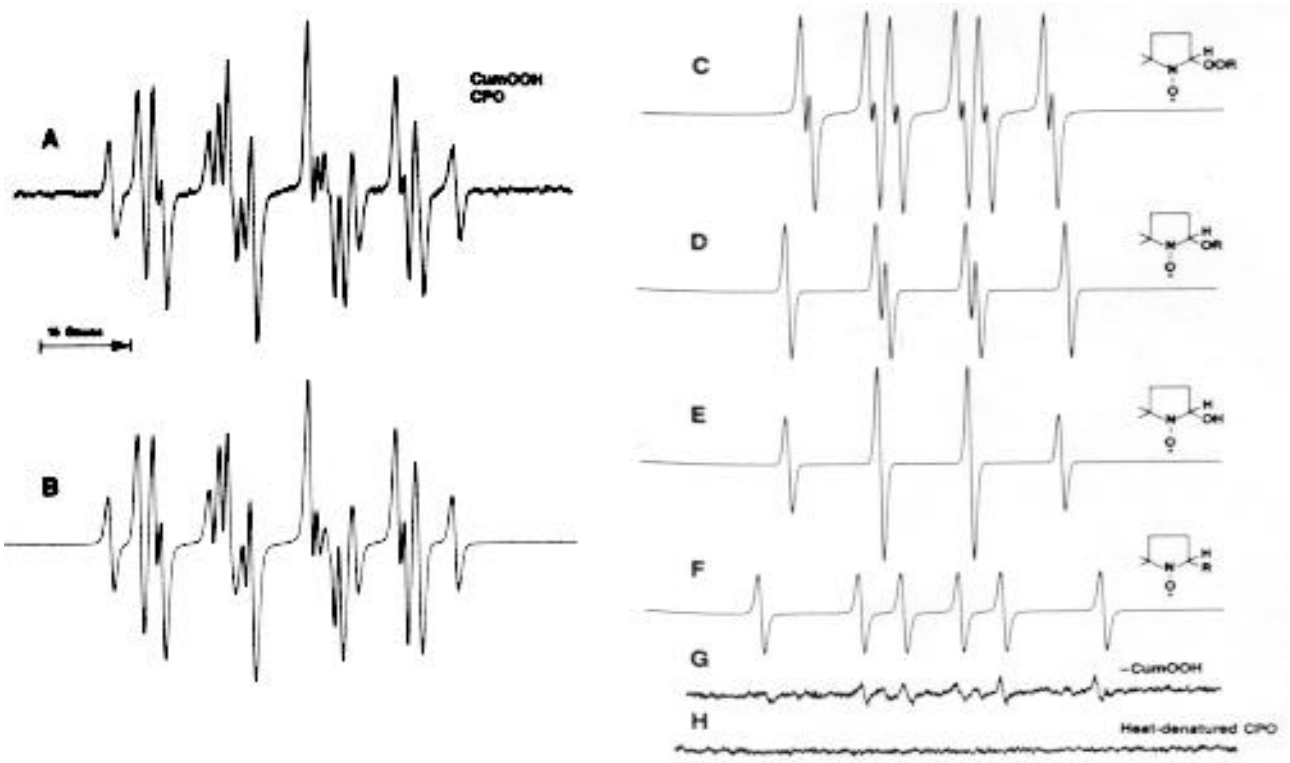


Figure 2. EPR spectra of DMPO radical adducts from incubations of *tert*-butyl hydroperoxide with CPO. 2A is the experimental EPR spectrum; 2B is a composite of 2C-E. F, G, and H are controls to prove the validity of the experiment. Adopted from [7].

Cavity ringdown spectroscopy is a powerful tool which recently has been put to work on the peroxyl radical system.[9] This IR transition is difficult to study, due to the small cross section, σ, which is approximately 2×10^{-21} cm^2. FTIR methods, such as CRDS, are much more sensitive, and can be measured on the small timescale of a radical's lifetime. Information about the rotational and vibrational states can be determined by CRDS. A typical spectrum would show the origin band of the peroxyl radical as well as

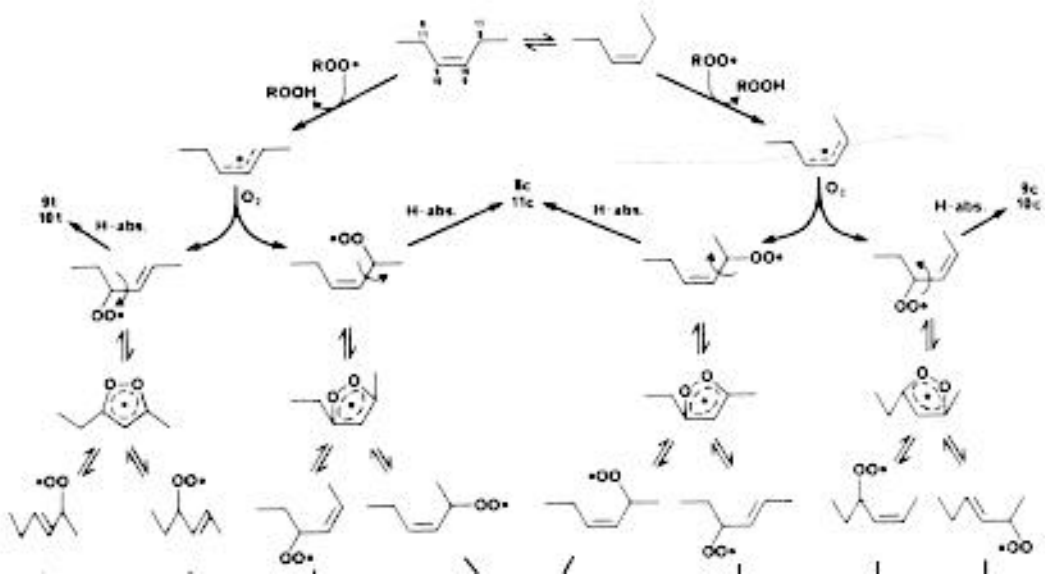
weaker absorptions at the low frequency range due to products from photolysis. This is valuable information, since it was not obtainable by conventional methods.

Reactions of Peroxyl Radicals

The reactions of peroxyl radicals are prevalent in all aspects of life, ranging from interactions with DNA to "knocking" in the internal combustion engines of automobiles. They are a high energy species, with a reduction potential ranging from 0.77 to 1.44 V, depending on the R group. [10] The reduction potential also varies among solvents, lowering in polar solvents and raising in nonpolar solvents.

A brief summary of all the reactions of peroxyl radicals would be enough to fill a small book, so only a few pertinent topics will be discussed. The reaction between PUFAs and peroxyl radicals yields several different products and interesting mechanisms, and therefore will be discussed here. The work of Gardner is pivotal in this field.[3] When a peroxyl radical attacks a PUFA, it bonds to the β-position of a double bond. From this position it can abstract an allylic hydrogen, cyclize, or cause a rearrangement in the fatty acid. Several options are shown below.

Figure 4. Several options are present for the autoxidation of oleic acid. Rearrangements to stable *trans* structures are thermodynamically preferred, but not always obtainable due to the steric constraints of the reaction. Adopted from [3].



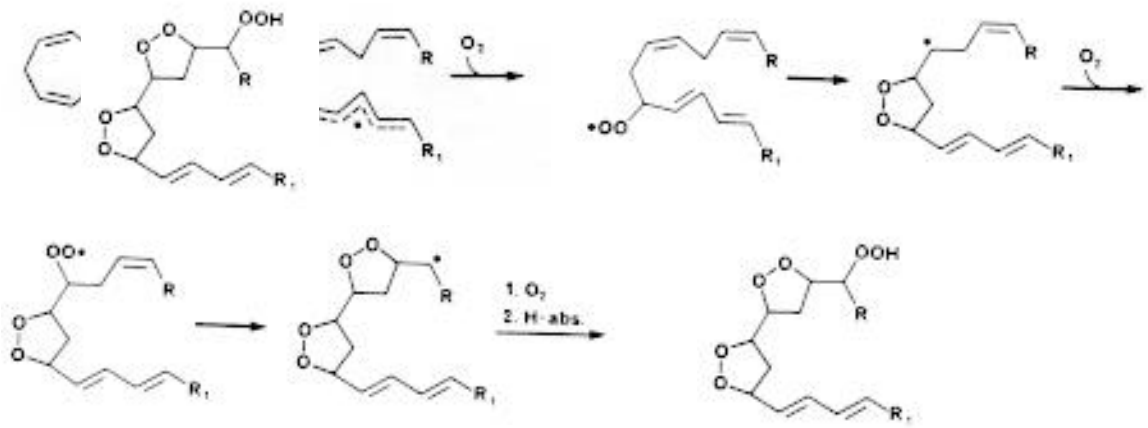


Figure 5. A chain reaction cyclization of *trans*-PUFA. Note the series of Initiation, Propagation, and Termination as described above. Adopted from [3].

The various peroxides are sorted out by HPLC. The mechanisms are very complex, and are elucidated in a paper by Porter and Wujek. [11]

Conclusion

The vast reactivity and properties of peroxyl radicals make them intriguing topics for study. Their presence in all aspects of life shows the importance they have, and makes their study worthy. New techniques are being developed to increase knowledge of these reactive species, as well as modifications of old methods. With new instruments being developed, the study of peroxyl radicals will continue to be fruitful for the near future.

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