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Free Radical Reactions in Food

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The formation and decay of free radicals in food components lead to chemical changes that are discernible in the food. These reactions (some of which also take place in physiologically active systems) are influenced by many physical and chemical factors. The rate at which they are formed, the environment in which they migrate and react, and the presence of constituents having an affinity for these reactive intermediates, all affect the competitive pathways for reaction and the subsequent formation of stable products. Consequently, it is of considerable importance to the food scientist and technologist to understand how one might anticipate—and possibly control—free-radical-mediated reactions relevant to food quality that take place during processing and subsequent storage.

Part of this understanding involves appreciating that, since food is a heterogeneous system, the free radicals likely to be encountered will be derived from the major food components or from their reactive constituents and will be physically constrained in separate phases. The structural protein, lipid, and carbohydrate components along with the aqueous medium containing soluble vitamins, globular proteins, and simple sugars are considered in this context to make up four separate phases. Reactions of radicals in each phase will tend to involve only constituents of that phase; cross reactions between radicals derived from different major components might occur only at interfacial boundaries or in emulsified systems. This dependence of free radical chemistry on food structure and composition makes it possible to extrapolate results from model systems and simple foods to the more complex foods.

Another part of this understanding involves appreciating that, irrespective of whether such radicals are formed during processing by exposure to heat or ionizing radiation or during storage through the involvement of peroxides and oxygen, there is going to be a commonality in the chemistry in all cases. The types of radicals will be similar, but the predominance of some over others will depend on specific processing or storage conditions. The pathways for reactions will also be similar, but preference of one sequence over another will depend on the concentration of radicals and reactive constituents and on the physical environment. The course of free radical reactions in different foods—processed with heat or radiation to destroy microorganisms or stored at reduced temperatures or in the frozen state to retard microbial growth—is governed by a common but branched mechanism of radical-radical and radical-substrate interactions.

It is the intention in this presentation to illustrate, using selective examples, the reactions of free radicals that determine the chemistry of many fresh, processed, and stored foods. To put these reactions in perspective, some of the basic concepts relating to free radical structure, formation, and reactivity will be described briefly. Reactions involving ascorbic acid radicals, myoglobin radicals, and palmitate radicals, as representative of radicals derived from a vitamin, a metalloprotein, and a saturated lipid, will then be described.

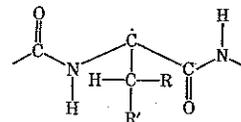
Basic Free Radical Concepts

Designation and Characterization

All atomic or molecular entities, with the exception of transition metal ions, having an odd number of electrons are designated as “radicals” or “free radicals.” Such entities can be neutral or can have a negative or positive charge. The unpaired electron can be localized in the orbital system of a specific atom, often referred to as a carbon-centered, nitrogen-centered, etc., radical, or can be distributed among overlapping orbitals of several atoms in the molecule, such as in the case of π -radicals. Some radicals are considered “stable,” but all are reactive and most are highly reactive and short-lived.

Typical examples are given in Table 1. Nitric oxide (in the absence of oxygen) is stable as a monomeric gas, but condenses with spins paired as a dimeric entity in the solid phase. The di(*t*-butyl)nitroxide radical is a stable entity whose analogs can be covalently bonded to large molecules and used as a “spin-label.” Shown also is the historically important “trityl” or triphenylmethyl radical, which Gomberg (1) proved existed in equilibrium with its dimeric form, hexaphenylethane. The other radicals all are short-lived in fluid media and include the simple, carbon-centered methyl radical, the anionic biphenylide radical, with the electron density spread over both rings, and the cationic tetramethylphenylenediamine radical.

Of particular importance in food is the peptide radical in proteins. Its general structure has been shown (2) to be



in which a hydrogen from an aliphatic amino acid has been removed from the carbon atom in the peptide chain. The R and R' groups can be the same or different or can be just simply hydrogen atoms. This radical is sometimes referred to as the “abstraction” or “backbone” protein radical.

Table 1. Examples of Typical Free Radicals

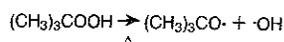
NO^\bullet	Nitric oxide
$(\text{CH}_3)_3\text{C}-\dot{\text{N}}\text{O}$	Di(<i>t</i> -butyl)nitroxide
$\cdot\text{CH}_3$	Methyl
$(\text{C}_6\text{H}_5)_3\text{C}^\bullet$	Triphenylmethyl
	Biphenylide anion
$[(\text{CH}_3)_2\text{N}-\text{C}_6\text{H}_4-\text{N}(\text{CH}_3)_2]^\bullet$	Tetramethylphenylenediamine cation

Modes of Formation

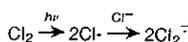
Several physical or chemical means can be used to generate free radicals. Among these are thermolysis, photolysis, radiolysis, metal ion catalysis, and enzyme catalysis. Illustrative reactions are shown in Table 2. In thermolysis, a weak bond can be broken giving rise to a pair of free radicals; the decomposition of *t*-butyl hydroperoxide is illustrative of a homolytic scission into two oxygen radicals: an alkoxy radical and the hydroxyl radical. Photolysis can also lead to either homolytic or heterolytic bond scission, depending on the excited states involved and the modes of molecular decompo-

Table 2. Illustrative Free Radical Generating Reactions

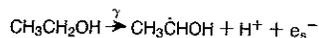
Thermolysis



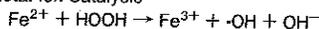
Photolysis



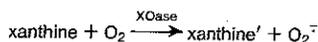
Radiolysis



Metal Ion Catalysis



Enzyme Catalysis



sition. A simple example is shown in which a chlorine atom photolytically formed in solution reacts rapidly with chloride ion to form the dichloride radical ion. In radiolysis, the formation of radicals is complex (3), involving excitation and ionization and leading ultimately to excited states, free radicals, and ions. In the case of ethanol, the main radical produced is the "abstraction" radical, characterized by the loss of a hydrogen alpha to the hydroxyl group. Chemical modes of formation can also be complex, but they formally involve a one-electron transfer. In the case of the metal ion reaction between ferrous ion and hydrogen peroxide, the Fenton reaction, an electron is transferred from the former to the latter leading to ferric ion and dissociation of the peroxide linkage into hydroxide ion and hydroxyl radical. Various enzyme-catalyzed reactions presumably proceed similarly so that, in the case of the xanthine-xanthine oxidase system, molecular oxygen accepts an electron and becomes the superoxide radical ion.

Modes of Detection

Because the presence of an unpaired electron imparts special magnetic, electronic, and redox properties to the radicals, several techniques can be used to detect them either when they are immobilized in solid matrices or when they are rapidly reacting in fluid media.

It is the interaction of the magnetic moment of the electron with both an external magnetic field and the magnetic moments of nuclei in the radical that forms the basis for electron spin resonance (ESR) spectrometry, a technique capable of providing detailed information on the identity and structure of free radicals (4). ESR is used mostly for radicals trapped either in powdered or crystalline matrices or in amorphous glasses at low temperatures; it has also been used for radicals rapidly reacting in media of low viscosity but amenable to detection either by observing them after a steady state concentration is attained or by monitoring their change in concentration with time. An example of an ESR spectrum is shown in Figure 1 for the abstraction radical of isobutyric acid in an aqueous glass (5). As predicted for a radical with the electron on the carbon interacting with six equivalent hydrogen nuclei, there are seven symmetrically spaced lines in the spectrum.

In a very general way, it is the possibility of additional and often more probable transitions among electronic states that forms the basis for a radical having intense optical absorption bands red-shifted relative to the parent molecule. Such spectra are not always distinctive and usually cannot be assigned to a specific radical species on a purely theoretical basis. However, optical absorption spectrometry, particularly in the ultraviolet and visible regions, has been used very effectively to discern free radicals in aqueous and organic systems that are transparent as crystals, glasses, and liquids over a wide

temperature range. The spectrum in Figure 2 attributable to the hydrogen adduct radical of phenylalanine in an aqueous glass (2) is illustrative of optical data obtainable on radicals formed and stabilized in a rigid matrix. Because of the excellent time resolution that is possible, optical techniques also have been used to characterize very fast radical reactions. An example of kinetic data obtained by rapid monitoring of the change in optical density at a specific wavelength following the instantaneous formation of a radical in a fluid system using a pulse of ionizing radiation will be given below.

It is the propensity for many radicals to be reduced or oxidized that forms the basis for their detection and characterization by electrochemical techniques (6). Rapid conductometric measurements provide information on the mobility of charged radicals, and rapid polarographic measurements can

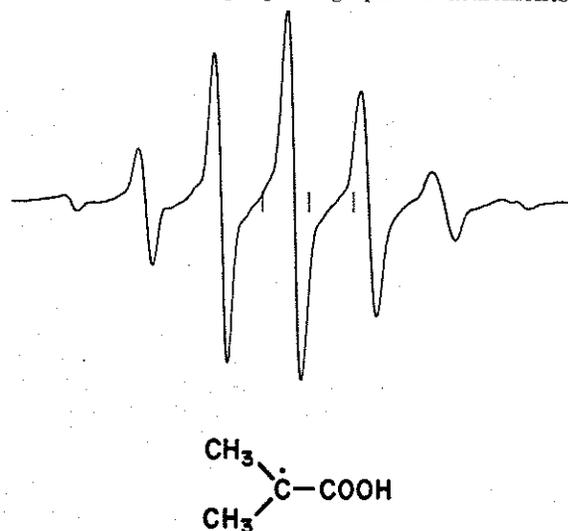


Figure 1. ESR spectrum of the abstraction radical of isobutyric acid in a 12 M LiCl-D₂O glass at 160° K. This radical, (CH₃)₂ĊCOOH, is a consequence of a series of reactions that starts with attachment at 77° K of photolytically generated electrons and continues upon subsequent annealing at higher temperatures (5). Spectrum was provided by M. D. Sevilla.

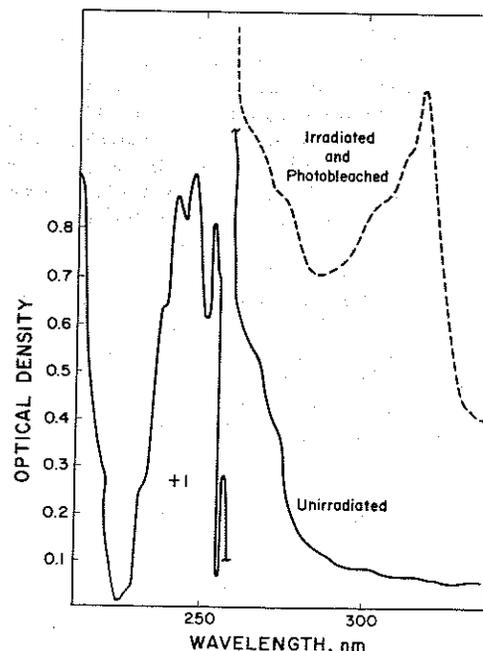


Figure 2. Optical spectrum of hydrogen adduct of phenylalanine in an ethanediol-water glass at 77°K. Glass was formed from an equimolar mixture of ethanediol and water containing 4×10^{-2} M phenylalanine, irradiated to approximately 0.4 Mrad and exposed at 77°K to visible light to bleach the trapped electrons. Solid curve corresponds to phenylalanine in the unirradiated glass; dotted curve is displaced vertically for clarity and corresponds to the $^+\text{NH}_3\text{CH}(\text{CH}_2(\text{C}_6\text{H}_5))\text{CO}_2^-$ radical.

Table 3. Representative Free Radical Reactions

Abstraction	$\text{CH}_3\text{CH}_2\cdot + \text{RCH}_2\text{COOH} \rightarrow \text{CH}_3\text{CH}_3 + \text{R}\dot{\text{C}}\text{HCOOH}$
Addition	$\text{CH}_3\text{CH}_2\cdot + \text{---CH=CH---} \rightarrow \text{---}\dot{\text{C}}\text{H---CH---} \begin{matrix} \text{CH}_2\text{CH}_3 \\ \end{matrix}$
Dimerization/Disproportionation	$2\text{CH}_3\text{CH}_2\cdot \begin{cases} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3 \\ \rightarrow \text{CH}_2=\text{CH}_2 + \text{CH}_3\text{CH}_3 \end{cases}$
Redox (Electron Transfer)	$(\text{CH}_3)_2\dot{\text{C}}\text{OH} + \text{cyt(III)-c} \rightarrow \text{cyt(II)-c} + (\text{CH}_3)_2\text{C=O} + \text{H}^+$
Decomposition	$\text{RCH}_2\text{CO}_2\cdot \rightarrow \text{RCH}_2\cdot + \text{CO}_2$

characterize the redox potentials of neutral and charged radicals.

Lifetime and Reactivity

Free radicals, because of the unpaired electron, are inherently unstable relative to the formation of entities with an even number of electrons through the pairing or transferring of electrons. Provided that they can diffuse through the medium, they will react rapidly and have only a short, transitory existence. In rigid media such as solids or glasses, they are constrained and react only very slowly, if at all; in fluid media, they could undergo reactions involving no or low activation energies that take place at or near diffusion-controlled rates.

Several types of representative radical reactions are given in Table 3. The bimolecular reaction between two ethyl radicals involves no activation and leads, through dimerization, to butane and, through disproportionation, to ethylene and ethane. Disproportionation formally amounts to the transfer of a hydrogen atom from one radical to the other and consequent formation of a double bond in the transferring radical. The addition of an ethyl radical to a double bond in a stable molecule is also a low activation process and leads to another, larger radical species. Abstraction of a hydrogen by the ethyl radical from an organic acid would involve activation and is driven by the formation of a stronger C—H bond in ethane at the expense of a weaker bond in the acid alpha to the carboxyl group. The reduction of cytochrome(III)-c by the isopropanol radical is typical of an electron transfer reaction involving a metal ion; the rates of such reactions depend on relative redox potentials and steric factors (7). Decomposition of an acyloxy radical into an alkyl radical and carbon dioxide is typical of a unimolecular reaction in which a stable molecule and another radical are formed.

The specific pathway for reaction that predominates among many alternative pathways depends on several physical and chemical factors. As an illustration, the competition among three pathways for reaction by an acyl radical



can be described as follows

$$\frac{-d[\text{RCH}_2\dot{\text{C}}\text{O}]}{dt} = k_1[\text{RCH}_2\dot{\text{C}}\text{O}][\text{RCH}_2\text{COOH}] + k_2[\text{RCH}_2\dot{\text{C}}\text{O}] + k_3[\text{RCH}_2\dot{\text{C}}\text{O}]^2 \quad (4)$$

If the instantaneous concentration of the radical is very high and if diffusion is unimpeded, then reaction (3) would predominate and the diketone would be formed. If the steady-state concentration of the radical is very low, then the com-

petition would be between reactions (1) and (2). At low temperatures or in rigid matrices, the bimolecular abstraction reaction would be unfavorable relative to the unimolecular decomposition reaction leading to carbon monoxide formation; at higher temperatures and in a medium in which the organic acid concentration is overwhelming, reaction (1) would predominate leading to another radical and an aldehyde. Depending upon the relative activation energies for reactions (1) and (2), a crossover from one set of products to another could occur at a specific temperature. Clearly, a knowledge of absolute rate constants and activation energies gives one a predictive capability.

Application to Foods

These basic concepts and considerations are applicable to any food in which free radicals react. Irrespective of how a specific radical is generated, its reactions will be determined by the nature of the medium, the temperature and/or viscosity, the concentrations of radicals and other reactive molecules, and the rate constant for each competing reaction.

Ascorbic Acid Radicals

Free radical intermediates have been implicated in a wide variety of physiological, analytical, and biochemical reactions involving ascorbic acid (8). In food, the catalytic decomposition of ascorbic acid, the interaction of ascorbate with nitrites in meats, and the antioxidant action of this vitamin most likely involve the same or similar radicals. Information has become available in recent years on the nature and reactivity of ascorbic acid radicals that puts their potential pathways for reaction into a more definitive framework.

The interrelationships of some presumed or proven ascorbic acid radicals with the parent oxidized or reduced forms of ascorbic acid are shown in Figure 3. To simplify the notation, ascorbic acid is written as AH₂, dehydroascorbic acid as A, and

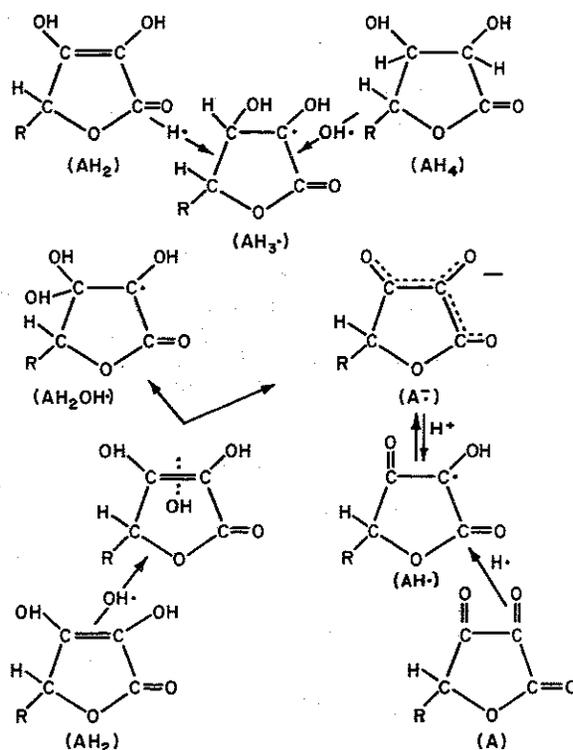


Figure 3. Interrelationships among ascorbic acid radicals, parent ascorbic acid and derivatives, and the reactive hydroxyl radical (OH·) and hydrogen atom (H·). Ascorbic acid is denoted as AH₂, dehydroascorbic acid as A, and gulonicacetone as AH₄. The presumed short-lived association of OH· with the double bond of AH₂ prior to formation of a sigma bond or acceptance of an electron is designated by the dotted lines.

gulonolactone as AH_4 ; the free radicals derived from them are written as variations of these designations depending upon the modifications made to the parent molecule. A reduction radical, AH_3^{\cdot} , could be derived by adding a hydrogen atom to AH_2 or by abstracting a hydrogen atom from AH_4 . An oxidation radical, $A^{\cdot-}$, could be derived by addition of a hydrogen to A or by loss of an electron from AH_2 , followed by dissociation, respectively, of one or two hydrogen ions. Other radicals are possible by other addition or abstraction reactions. The addition of the hydroxyl radical to the double bond gives AH_2OH^{\cdot} .

Reduction Radical

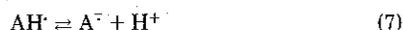
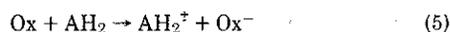
The radical derived by reducing AH_2 with H^{\cdot} , for which only limited data are available, probably has the structure shown in Figure 3 for AH_3^{\cdot} or a comparable structure with the hydrogen on the carbon that is alpha to the carbonyl group. That this reduction takes place by H^{\cdot} adding to the double bond is indicated by the high rate constant estimated for the reaction and by the decrease in molecular hydrogen formed when AH_2 competes effectively against isopropanol for the H^{\cdot} produced upon gamma radiolysis (9).

Further evidence for AH_3^{\cdot} is available from spectral and kinetic data obtained from pulse radiolytic studies (10). The spectra determined from systems in which OH^{\cdot} reacts with AH_4 and H^{\cdot} reacts with AH_2 are similar, but not identical. The spectrum from the former shows a featureless, rising absorption from 400 nm down to the limit of observation at 280 nm; the spectrum from the latter shows a slight shoulder in the 350 nm region superimposed on a similar, rising absorption. Abstraction of hydrogen by OH^{\cdot} as indicated in Figure 3 would most likely take place at the carbons containing the hydroxyl groups (the 2 and 3 positions); addition of H^{\cdot} could be at the double bond to give the same AH_3^{\cdot} or at the carbonyl group to give another $>COH^{\cdot}$ radical (not shown). The kinetic data from experiments in which the changes in absorption at 290 nm and 366 nm were monitored as a function of time for both systems indicate a rapid decay on the same time scale.

These results suggest, but do not prove, that at least one AH_3^{\cdot} radical and possibly one other radical can be formed from AH_2 upon reaction with a strong reductant or hydrogen donor. Such radicals would be expected to decay rapidly by bimolecular reaction or by reaction with an oxidant.

Oxidation Radicals: Spectral Evidence

The radicals derived by oxidizing AH_2 , for which there is considerable evidence (11), have the structures as indicated in Figure 3 for $A^{\cdot-}$ and AH_2OH^{\cdot} . Oxidation of AH_2 in which simple electron transfer occurs leads to a cation radical that would rapidly deprotonate to AH^{\cdot} ; subsequent dissociation of the H^+ from the remaining hydroxyl group gives $A^{\cdot-}$. The following reactions, in which the oxidant is denoted by Ox, summarizes this sequence

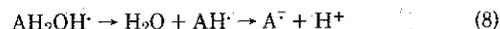


Addition of H^{\cdot} to any of the carbonyl groups in dehydroascorbic acid followed by dissociation of H^+ would also lead to $A^{\cdot-}$. Reaction of OH^{\cdot} with AH_2 , however, involves both electron transfer and addition: electron transfer leads to $A^{\cdot-}$, and addition leads to AH_2OH^{\cdot} . Whether or not the route to $A^{\cdot-}$ first involves addition is not certain. Data from both ESR and optical studies substantiate the nature of $A^{\cdot-}$ and AH_2OH^{\cdot} .

ESR Evidence. The identity and structure of the ascorbic acid anion radical, $A^{\cdot-}$, was conclusively established by Laroff, Fessenden, and Schuler (12), who obtained high resolution ESR spectra of this radical while it was maintained at a steady-state concentration during the continuous irradiation

of an N_2O -saturated solution of AH_2 at specific pH's. The key spectrum is shown in Figure 4 along with related information. It is characterized by a doublet of triplets, each line of which is further split into a doublet, as indicated in the simplified stick diagram which also shows the corresponding hyperfine splitting constants. Consistent with these results is the structure that is shown; carbon atoms 4, 5, and 6 are numbered for clarity. The main doublet is due to the C-4 hydrogen, $A_4 = 1.76$ G; the triplet with a weaker splitting of 0.19 G is due to the two equivalent hydrogens on C-6; and the additional, smaller splitting of 0.07 G is attributable to the hydrogen on C-5. The presence of only these resonances indicates that the unpaired electron density is spread over the three carbonyl groups. $A^{\cdot-}$ is therefore a trione radical. On the basis of the spectrum changing with decrease in pH, these authors further established that the equilibrium given by reaction (7) has a $pK = -0.4$.

Evidence for the structure of AH_2OH^{\cdot} was also obtained in this steady-state experiment as well as in a time-resolved study (13). The OH^{\cdot} addition to the double bond takes place at both the C-3 and C-2 positions. It appears, however, that the C-2 addition radical converts to $A^{\cdot-}$ by loss of H_2O followed by dissociation of H^+



Based on spectral changes with pH, it appears that AH_2OH^{\cdot} also undergoes a protolytic dissociation with $pK = 2$.

Optical Evidence. A broad optical absorption band with a maximum at 360 nm that is assigned to $A^{\cdot-}$ has been observed by several investigators. These investigations involved subjecting ascorbic acid solutions to a short pulse of irradiation and then monitoring the change in optical density at specific wavelengths with time. Two sample oscillograms are

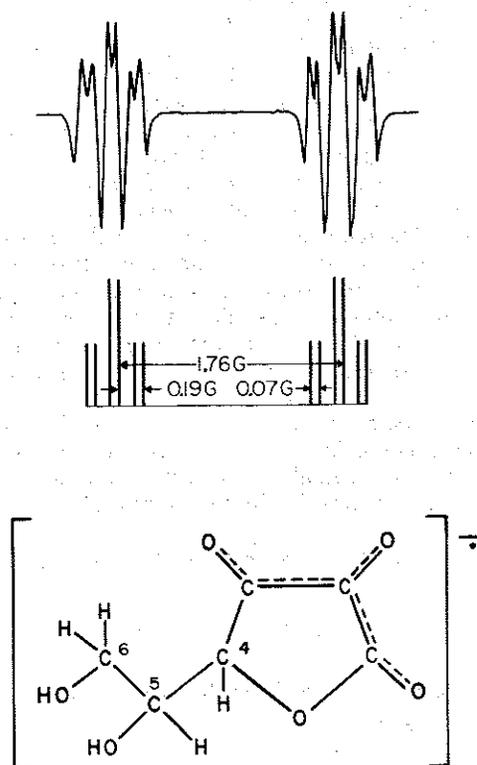


Figure 4. ESR spectrum of the ascorbic acid anion radical ($A^{\cdot-}$) in an irradiated, N_2O -saturated, 10^{-2} M solution of ascorbic acid at pH = 3. The radical is a consequence of hydroxyl radical reaction with ascorbic acid. The spectrum was recorded while the solution was continuously irradiated within the resonance cavity by a beam of electrons from a Van de Graaff accelerator. The stick diagram shows relevant hyperfine splitting constants. Adapted with permission from reference (12) (Copyright 1972 American Chemical Society).

shown in Figure 5 for monitoring at 366 nm and 289 nm. The upper frame for 366 nm shows traces at two different sweep rates; the lower frame for 289 nm shows an additional, still slower sweep rate of 200 msec/cm. Using similar oscillograms for different wavelengths and selecting a specific time after the pulse, one can construct a spectrum for the change in optical density at that time due to radicals present in the solution. Such a spectrum is shown by the dotted line in Figure 6 for the N₂O-saturated solution of AH₂ at *t* = 40 μsecs. It exhibits the peak at 360 nm attributable to A⁻ superimposed upon a featureless absorption that increases at wavelengths below 320 nm and is presumably attributable to AH₂OH⁻.

Several approaches have been taken to separate the absorption due to A⁻ from the total absorption. Some investigators have relied upon the spectrum constructed at longer times after the pulse, which allows the species responsible for the strong ultraviolet absorption to decay away. (Note in Figure 5 that, over the first 30–40 μsec, the absorption at 366 nm increases while the absorption at 289 nm decreases.) Some also have used Br₂⁻ or (SCN)₂⁻ as oxidants rather than OH⁻ to produce the A⁻ spectrum without incurring a contribution by the AH₂OH⁻ species. Schuler (14) has determined the spectrum presumably specific to A⁻ that shows distinctly the maximum at 360 nm and gives ε₃₆₀ = 3300 M⁻¹ cm⁻¹.

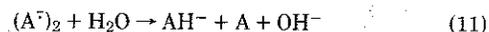
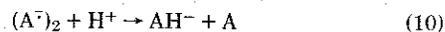
To demonstrate that the absorption attributable to A⁻ is reasonably a consequence of OH⁻ reacting with the double bond, a comparison was made with the spectrum obtained using reductic acid, which is similar to AH₂ except that C-4 has a hydrogen in place of the —CHOHCH₂OH substituent. The spectrum in this case shows a similar pattern with a peak at 360 nm and a rising absorption below 300 nm (15).

To demonstrate that the A⁻ absorption is also obtained by H⁻ reaction with A, a spectrum was obtained under conditions

arranged to eliminate OH⁻ and to selectively generate H⁻. This spectrum is shown by the solid line in Figure 6. While a peak at 360 nm is evident, it is only a small contribution to the overall absorption. Apparently, reactions involving other than addition to the carbonyl group could be taking place.

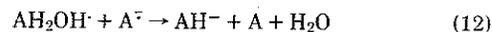
Oxidation Radicals: Decay Kinetics

The decay of A⁻ in the absence of other reactive entities is complex and not completely understood. It conforms to a second-order rate law and produces dehydroascorbic acid, but the observed rate constant, *k*_{obs}, is dependent upon pH and ionic strength. At pH = 1, *k*_{obs} ~ 1.2 × 10⁸ M⁻¹s⁻¹ and increases with increasing ionic strength; whereas at pH = 9, *k*_{obs} ~ 4 × 10⁴ M⁻¹s⁻¹ and is unaffected by ionic strength. One reaction scheme suggested to explain these observations (11) involves the formation of an intermediate complex that can decompose into final products



According to this scheme, high [H⁺] and high ionic strength would accelerate the decay, whereas low [H⁺] would limit the decay to an apparently slow hydrolysis reaction involving only one charged entity. The exact nature of the complex is not specified, and the basis for its stability is unclear.

The decay of A⁻ in the presence of other radicals would be expected to be rapid. Reaction of the other oxidation radical, AH₂OH⁻, with A⁻ according to reaction (12)



presumably occurs concurrently with reaction (8). Reaction

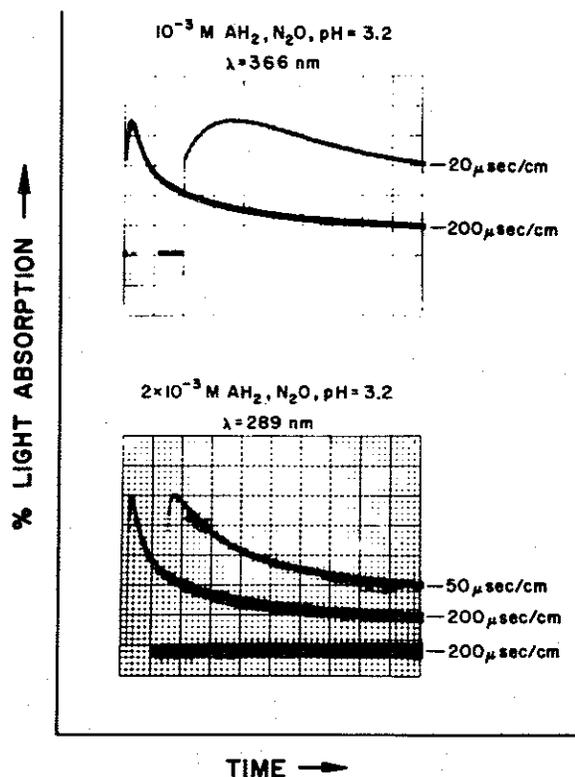


Figure 5. Oscilloscopic recordings of light absorption versus time corresponding to the formation and decay of ascorbic acid radicals following the pulse irradiation of an N₂O-saturated solution of ascorbic acid at pH = 3.2. The upper frame for λ = 366 nm shows traces at two sweep rates; the base line appears 2 cm from the bottom, and the pulse of irradiation representing zero time appears 2 cm from the left. The lower frame for λ = 289 nm shows traces at three sweep rates; the base line is coincident with the 200 μsec/cm trace triggered after the radicals have completely reacted.

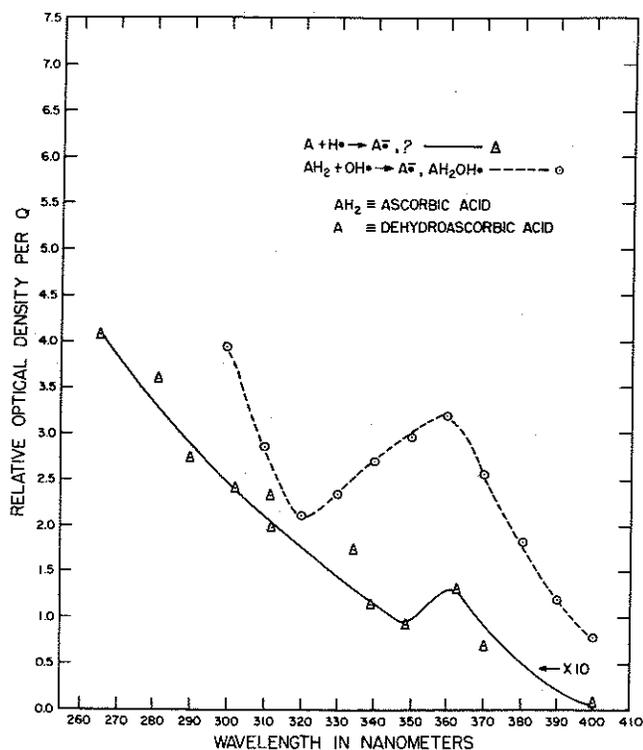
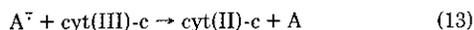


Figure 6. Optical absorption spectra for transient species in pulse irradiated solutions of ascorbic acid (AH₂) or dehydroascorbic acid (A). The spectra are derived from several oscilloscopic recordings of light absorption at different wavelengths versus time, by computing change in optical density normalized for dose absorbed (Q) at a specific time after the pulse irradiation. The dotted line corresponds to OH⁻-induced radicals in AH₂ solution at *t* = 40 μsec; the solid line (with optical densities multiplied by 10) corresponds to H⁻-induced radicals in A solutions.

of the hydroperoxy radical, HO_2^\cdot , with A^\cdot has been investigated (16), and its rate constant has been estimated as $2.7 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$, which is reasonable for electron transfer between radicals.

The rate constants for the reactions of A^\cdot with other entities, however, appear to be low. Reduction of ferricytochrome-c (17)



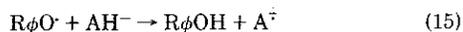
is characterized by $k_{13} = 6.6 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$, and reaction with O_2 presumably to form a complex (17)



has $k_{14} < 5 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$.

Implication for Food

Formation and decay reactions similar to those described above are expected to occur in food systems containing ascorbic acid or ascorbate. They are especially likely in systems also containing phenolic-type antioxidants. Direct measurements of the rate constants for the reaction of phenoxyl radicals, $\text{R}\phi\text{O}^\cdot$, with ascorbate (14) indicate that the formation of A^\cdot according to reaction (15)



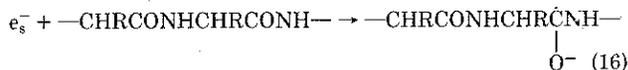
would be rapid. This regenerative reaction is relevant to α -tocopherol and ascorbate (18) acting synergistically in preventing lipid oxidation (see below).

Myoglobin Radicals

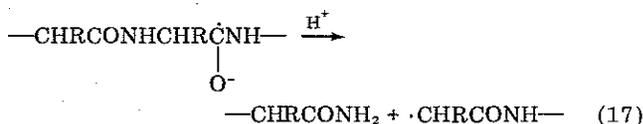
Free radicals derived from myoglobin have been implicated in irradiated meats, in enzymatic processes, in Fenton-type reactions, and in systems containing peroxidizing lipids. Because of the heme group and globular structure of myoglobin, several types of radicals are possible and several pathways for reaction, both intramolecular and intermolecular, are available. This complex reactivity arises because myoglobin can react as a multivalent positive ion, as a protein, or as an isolated, metal ion-protein system. Some of these radicals and the reactions they undergo are described to illustrate their potentially complex chemistry in foods.

Types of Radicals

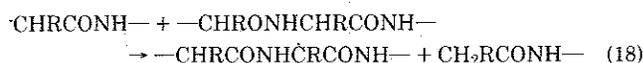
Several radicals involving only the protein portion of the myoglobin molecule have been observed by direct detection or inferred by product analyses (2, 19). Among these are the carbonyl anion radical, the alkyl radical, the peptide radical, the hydrogen and hydroxyl adduct radicals of cyclic amino acid moieties, and the peroxy radicals. The carbonyl anion radical in the peptide chain ($-\text{CHRCONHCHRCONH}-$) presumably forms, as in other non-metalloproteins, by electron addition



It can then decompose to an amide and an alkyl type radical



which in turn can react with the protein to give the peptide radical



Reaction of H^\cdot or OH^\cdot to abstract a hydrogen from the protein backbone would also lead to the peptide radical; however,

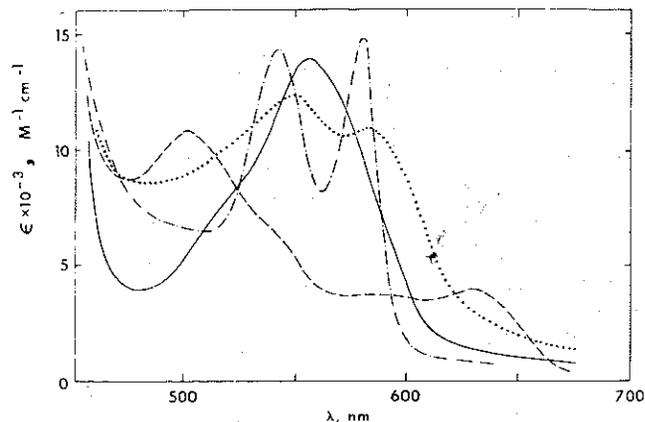
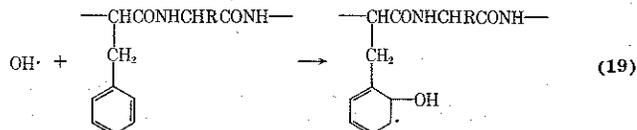


Figure 7. Visible region absorption spectra of myoglobin derivatives at pH 7.3. Ferrimyoglobin, ---; ferromyoglobin, —; ferrylmyoglobin, ···; oxymyoglobin, - · - ·. From reference (21).

these primary radicals are more likely to react by adding to phenylalanine, tyrosine, histidine, and tryptophan moieties to form isomeric adduct radicals



Reaction of O_2 with the longer-lived peptide or adduct radicals would lead to a peroxy radical at the same sites.

Some other radicals involving the heme group are possible, judging from experiments with models for iron and other metal ion porphyrin systems (20). These will not be considered here.

Experimental Evidence

Since the optical absorption spectra of the four major myoglobin derivatives—ferromyoglobin, ferrimyoglobin, oxymyoglobin, and ferrylmyoglobin—are very distinctive (Fig. 7), most of the evidence for the involvement of radicals comes from studies in which transient or permanent changes in the spectral properties of the system have been determined. Studies on the OH^\cdot reaction with ferrimyoglobin and oxymyoglobin are illustrative (21–25).

Rapid monitoring of optical density changes at specific wavelengths in the 290–750 nm region following the pulse irradiation of N_2O -saturated solutions of ferrimyoglobin (21) shows that more than one OH^\cdot -induced radical is formed and that complex reactions take place leading to other derivatives. There is, for example, a very rapid and then a less rapid rise in absorption at 430 nm; there is also an initial formation of an absorption at 320 nm that subsequently decays away. Spectra constructed for 3 μsec , 80 μsec , and 400 msec after the pulse are complex, but the latter shows a decrease in the 490 nm ferrimyoglobin band.

Slow scanning of the optical absorption in similar solutions at different times after gamma irradiation (21) shows that still other, slower changes in the solution composition among several myoglobin derivatives take place (Fig. 8). The initial change is attributed to the formation of globin-modified ferromyoglobin and ferrylmyoglobin (formed through the involvement of H_2O_2 co-generated upon irradiation); the subsequent change is attributed to the reaction of ferromyoglobin with a ferrylmyoglobin derivative to regenerate some ferrimyoglobin.

Optical studies of irradiated aqueous glasses containing ferrimyoglobin also indicate the formation of unstable intermediates absorbing in the ultraviolet region (26), and ESR

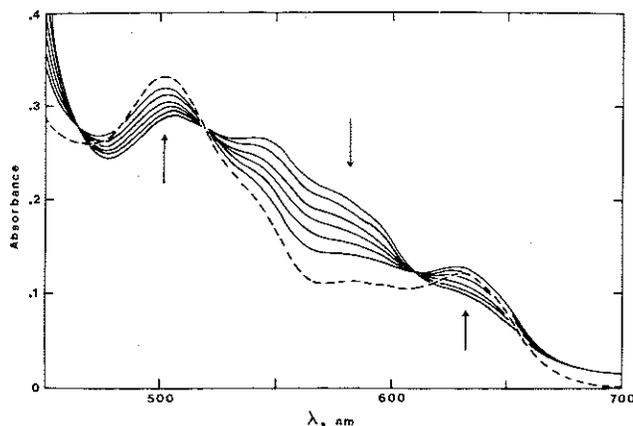


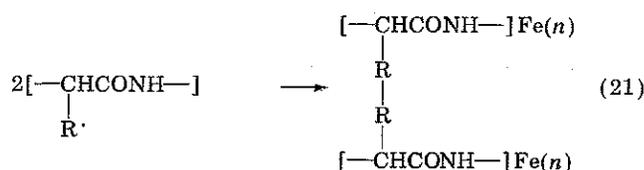
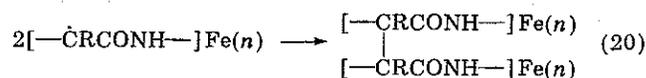
Figure 8. Spectral changes induced by 20.5 krad of γ -radiation in an N_2O -saturated $30.7 \mu M$ Ferrimyoglobin solution. Before radiolysis ---; after radiolysis —, measured 3.4, 6.4, 19.6, 34.0, 59.0 min after initial time of radiolysis. Arrows indicate direction of postirradiation absorbance changes. From reference (27).

examination of an irradiated, frozen slurry of ferrimyoglobin demonstrates the presence of protein radicals (27).

Competitive Intermolecular/Intramolecular Reactions

Myoglobin free radicals can react either intermolecularly or intramolecularly depending on thermodynamic and kinetic factors. The former pertains to the redox potentials of the protein radical moiety and the heme group in the same myoglobin molecule; the latter pertains to the disposition of the radical site relative to the heme group and the diffusivity of the myoglobin radical in a given medium. This competition among different reaction pathways can be illustrated using generalized, myoglobin peptide and adduct radicals designated by $[-\dot{C}RCONH-]Fe(n)$ and $[-CHRCONH-]Fe(n)$, respectively, where the group in brackets represents the section of the protein chain on which the radical is located, the R represents the unsaturated homocyclic or heterocyclic rings, and the n in the parenthesis represents the formal valence state of the iron in the heme group.

Intermolecular reaction will be favored if the redox couple is small, the radical site is far removed from the heme, and diffusion is not impeded. One possible consequence of this reaction is dimerization



The formation of dimeric myoglobin in the irradiation of ferrimyoglobin has been demonstrated, as Figure 9 indicates (21, 22). Another consequence is disproportionation, which in the case of the peptide radical forms an imine function ($-N=CR-$) that upon hydrolysis leads to degradation of the protein chain. If oxygen is present, the consequence is formation of a peroxy radical, which could also decay bimolecularly. Other intermolecular reactions are also possible, including combination with different radical species.

Intramolecular reaction will be favored if the redox couple is large and the radical site is close to the heme group; these factors probably overwhelm any influence of the diffusivity. Oxidation or reduction of the heme and a complementary change in the radical occur

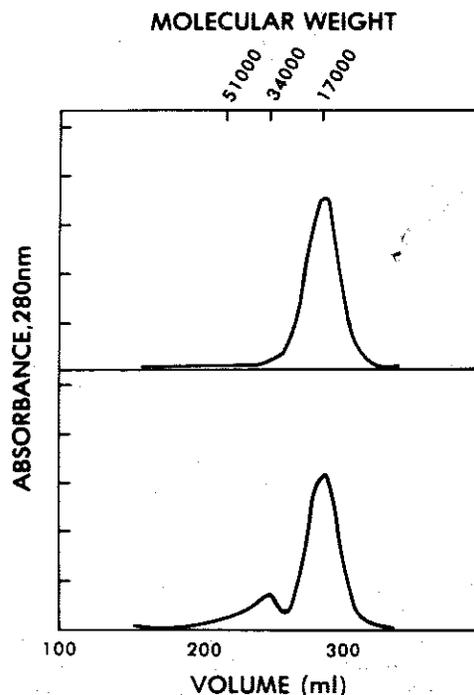
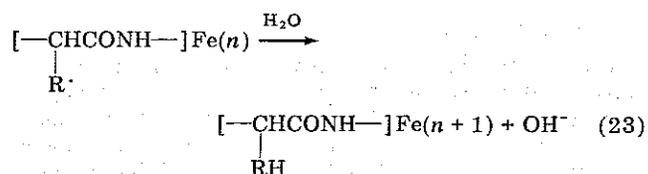
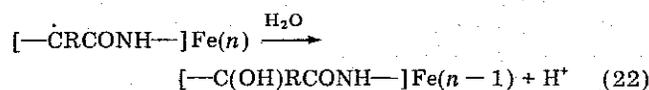
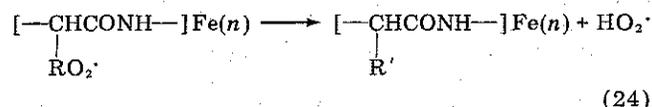


Figure 9. Chromatography on Sephadex G-100 of unirradiated and irradiated N_2O -saturated solutions of Ferrimyoglobin (0.49 mM) at pH 7.4. Upper frame: control; lower frame: γ -irradiated, 61 krad. Each ordinate interval corresponds to 0.2 absorbance unit at 280 nm. The peak in the lower frame at a molecular weight of 34,000 corresponds to a dimeric myoglobin derivative. Adapted from reference (27).



The formulas for the protein groups in the stable product are shown with a hydroxyl or hydrogen added only to indicate modification by oxidation or reduction, respectively. If the myoglobin radical first reacts intermolecularly with oxygen, then the resulting peroxy radical probably undergoes intramolecular reaction, including decomposition into a stable myoglobin derivative and HO_2 .



Fate of Ferrimyoglobin Radicals

Based on the works of Whitburn and Hoffman (24, 25), a scheme for the alternative reaction pathways for OH -induced radicals of ferrimyoglobin is presented in Figure 10. These authors determined the yield of myoglobin derivatives from the ferrimyoglobin radicals both with and without oxygen and hydrogen peroxide present (the latter being removed by reaction with catalase), and proposed several novel reactions to explain the results. As written, the scheme includes only the peptide radicals (as designated above), but pertains to the adduct radicals also. It shows that, in the absence of O_2 , the $[-\dot{C}RCONH-]Fe(III)$ radical converts intramolecularly to a modified ferromyoglobin and reacts bimolecularly to form

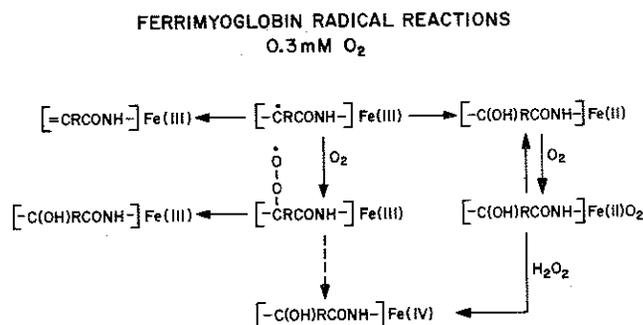


Figure 10. Proposed reaction pathways for OH-induced radicals of ferrimyoglobin, illustrated with peptide radicals. The designations for individual species are the same as in the text, except that a Roman numeral is used to indicate the formal valence of the iron. The hydroxy group in some of the nonradical species implies only that the myoglobin has been oxidatively modified. A solid arrow indicates a single reaction step; a broken arrow implies a series of steps.

modified ferrimyoglobin products, including most likely the dimer (not shown) and probably the disproportionation product indicated. These products cannot be distinguished spectrally from the unmodified myoglobin derivatives, since the heme group primarily determines the spectrum. It also shows that in the presence of O₂ the radical converts primarily to the peroxy radical, which in turn converts primarily to a modified ferrimyoglobin (shown here with an oxidized amino acid moiety) and to a minor extent, through a set of unspecified reactions, to a ferrilymyoglobin derivative. In a solution 0.3 mM in O₂, 96% of the radical reacts with O₂ and 90% of the consequent peroxy radical converts to modified ferrimyoglobin. A small fraction of the radical still converts intramolecularly to a modified ferromyoglobin, which reacts with O₂ to give the modified oxymyoglobin derivative, which in the presence of H₂O₂ becomes the ferrilymyoglobin derivative.

Fate of Oxymyoglobin Radicals

A scheme for the decay of oxymyoglobin radicals in solutions with differing concentrations of O₂ is shown in Figure 11, which also is based on the work of Whitburn and Hoffman (25). Here again, they determined the yield of myoglobin derivatives formed from the different oxymyoglobin radicals. For the sake of illustration, the scheme specifies the radical as that formed upon OH[•] addition to the phenylalanine ring, but could apply to other ring or peptide radicals as well. It shows that the adduct radical of oxymyoglobin could react intermolecularly with the O₂ in solution forming a peroxy radical of modified oxymyoglobin, or intramolecularly with the O₂ bound to (or trapped within) the oxymyoglobin structure to form a peroxy radical of modified ferromyoglobin, or through a series of intramolecular electron transfers to yield a modified ferrimyoglobin and H₂O₂. Both types of peroxy radicals apparently decompose by eliminating HO₂[•] and giving the respective, modified ferromyoglobin or oxymyoglobin. As the [O₂] is increased, the pathway from radical to peroxy radical of oxymyoglobin to modified oxymyoglobin and HO₂[•] predominates.

Implication for Food

These radical reactions are relevant to myoglobin in meats and in poultry. Upon irradiation, curing, or autoxidation of such foods, myoglobin free radicals could be formed. Irrespective of whether they are formed through reaction by hydroxyl radicals, by nitrite-derived radicals, or by alkoxy or peroxy radicals of lipids, their modes of decay and the resultant products will be influenced by the considerations just described. In irradiated cooked beef, the ferrimyoglobin is reduced to ferromyoglobin, primarily by a sequence initiated by electrons (22). In meats containing peroxidizing lipids,

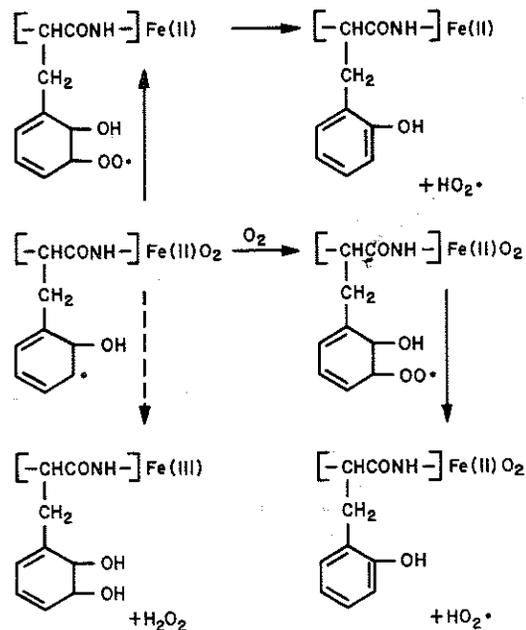


Figure 11. Proposed reaction pathways for OH-induced radicals of oxymyoglobin, illustrated with the ortho adduct radical of phenylalanine. The designations for individual species are the same as in the text, except that a Roman numeral is used to indicate the formal valence of the iron. A solid arrow indicates a single reaction step; a broken arrow implies a series of steps.

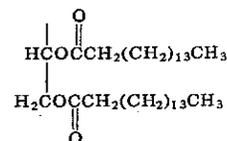
Karel and coworkers (28) have shown that dimer formation increases with the extent of lipid oxidation. In other systems in which myoglobin acts as an antioxidant, it could be assumed that free radical intermediates are involved and affected by the physical and chemical conditions of the system.

Ethyl Palmitate and Tripalmitin Radicals

Free radicals are formed from palmitate-containing constituents of lipids during thermal treatment, irradiation, and autoxidation. Some of these radicals have been detected directly and others are inferred from analyses of their stable end products. A few radicals tend to be common to many lipid systems. The types of radicals and radical reactions described here for palmitates can be considered representative for other saturated fatty acid esters and triglycerides. Palmitate is designated here as



where R is —CH₃ for the ethyl ester and



for the triglyceride.

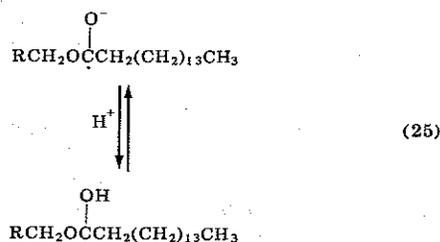
Major Radical Species

Among the major radicals proven or presumed to be formed from palmitate are: various alkyl radicals, carbonyl radicals, and acyl and acyloxy radicals. Of the alkyl radicals formed, the abstraction radical, in which the hydrogen is lost from the carbon alpha to the carbonyl group, predominates



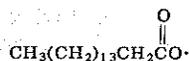
The ESR spectrum of the ethyl palmitate radical determined by Sevilla is shown in Figure 12. This slightly asymmetric, five-line spectrum (reflecting the interaction of the unpaired electron with the single hydrogen as well as with the two hydrogens on the adjacent carbon) is typical for abstraction radicals (5, 29) found in the other saturated fatty acid derivatives and found, to a limited extent, in some unsaturated fatty acid derivatives. Another alkyl radical of significance is the one formed at the carbon of the ester linkage; this radical corresponds to the ethyl radical in the case of the simple ester and to the propane dioldipalmitate radical in the case of the triglyceride.

The carbonyl radical, formed by electron attachment, can be in either an anionic or protonated form

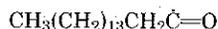


The ESR spectrum of irradiated tripalmitin powder at low temperature (the lower frame of Figure 13) shows a significant contribution by the carbonyl anion radical (3). Depending on the temperature, its spectrum is characterized by a broad single line with a partial splitting due to interaction with a hydrogen on the neighboring carbon.

The acyloxy radical



can be formed through the decomposition of excited palmitate moieties, and the acyl radical



can be formed by mechanisms involving both excited state and electron attachment reactions. ESR spectra with contributions by the acyl radical have been obtained recently by Halliday (30) for irradiated saturated and unsaturated fatty acids at temperatures near -50°C . A spectrum that is almost exclusively due to this radical was determined for irradiated

PALMITIC ACID, ETHYL ESTER

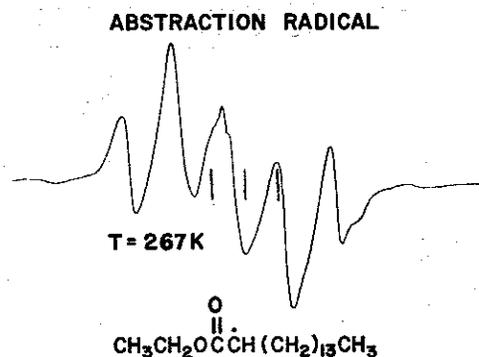
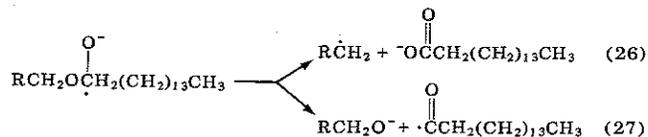


Figure 12. ESR spectrum of the abstraction radical of ethyl palmitate at 267°K in a neat system, under vacuum, irradiated to 0.2 Mrad at 77°K . This radical, $\text{CH}_3\text{CH}_2\text{OOC}\dot{\text{C}}\text{H}(\text{CH}_2)_{13}\text{CH}_3$, the most stable in the system, is a consequence of several radiolytic reactions, including a series that starts with electron attachment at 77°K and continues upon subsequent annealing at higher temperatures (23). Spectrum was taken for this paper by M. D. Sevilla.

stearoyl chloride at temperatures in the -140°C region. It is characterized by a sharp singlet with a very slight doublet splitting due to a weak interaction with one hydrogen on the neighboring carbon.

Main Pathways for Reaction

The carbonyl radicals can react in two distinct ways. Aside from protonating as shown in reaction (25), the anionic radical can undergo two possible decomposition reactions



Reaction (26) corresponds to the conversion of the anion radical to the propane dioldipalmitate radical in the case of tripalmitin, which is reflected in the complex spectrum in the middle frame of Figure 13. The protonated radical has a pronounced abstracting capability (31) and reacts with the substrate (taken here as the parent compound) to give the alpha abstraction radical and a hemiacetal (which is relatively unstable)

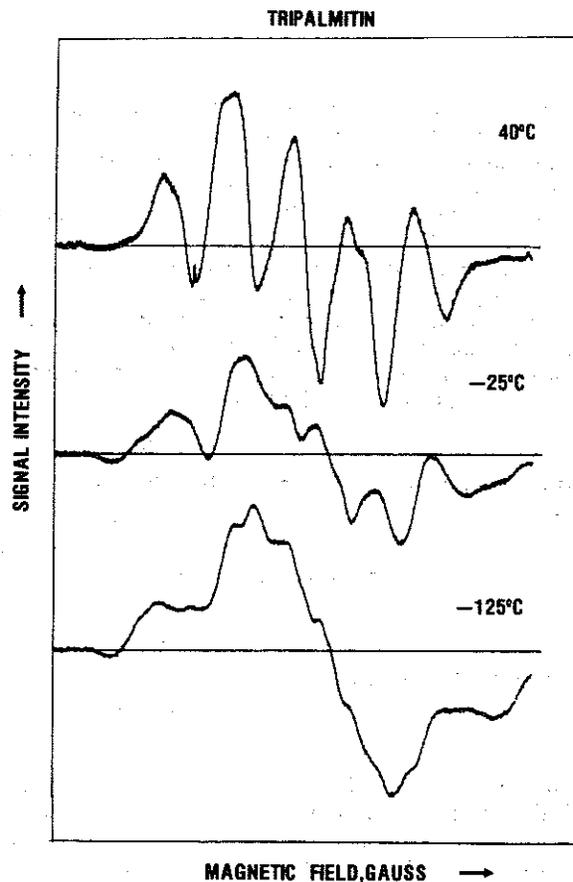
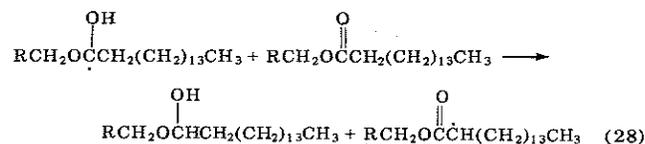
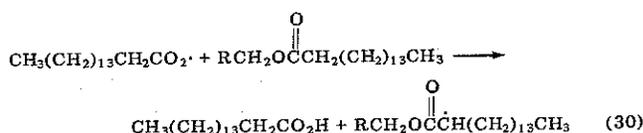
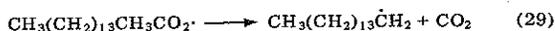
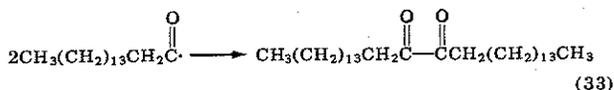
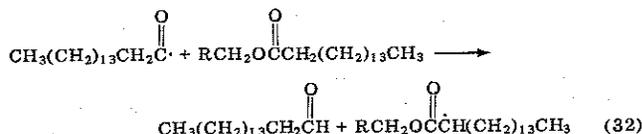
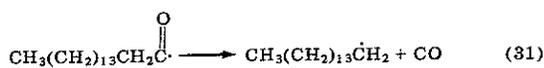


Figure 13. ESR spectra of irradiated tripalmitin powder: Upper frame—irradiated to 3.0 Mrads at 40°C and measured at 40°C ; middle frame—irradiated to 3.0 Mrads at -130°C , annealed to -25°C , and measured at -130°C ; lower frame—irradiated to 3.0 Mrads at -125°C and measured at -125°C . Samples were in the shape of cylindrical sticks made by compacting the powder, and required no containers for irradiation or examination (3).

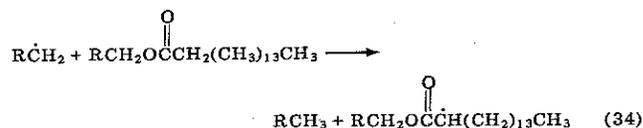
The acyloxy and acyl radicals also have several competitive pathways for reaction. Two possibilities for the former are decomposition and abstraction



The acyl radical can react similarly, as well as by dimerization

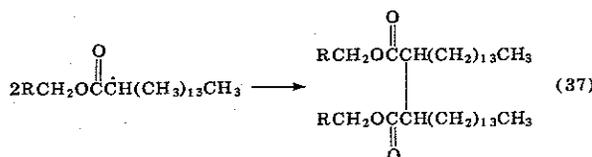
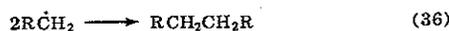
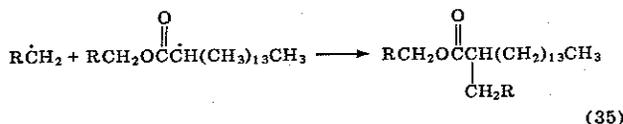


All of the alkyl radicals could react by abstraction. The abstraction reaction



is driven by the formation of a stronger C—H bond at the expense of the weaker C—H bond at the position alpha to the carbonyl group; it leads to the most stable radical in the system, the alpha abstraction radical. The spectrum in the upper frame of Figure 13 shows the five-line spectrum attributable solely to the presence of the alpha abstraction radical of tripalmitin at 40°C.

The remaining fate for those radicals that do not unimolecularly decompose or bimolecularly react with nonradicals (to abstract or to add) is to react with other radicals by combination or disproportionation. For the alkyl radical and the alpha abstraction radical, the combination between two dissimilar or similar species is illustrated as follows



Presumably, the very slow decay of the alpha abstraction radical of tripalmitin at room temperature ($\tau_{1/2} \sim 4$ h) is attributable to the slow migration of this large species in a solid matrix before combining according to reaction (37).

Evidence from Stable Products

Chromatographic and mass spectrometric analyses of irradiated ethyl palmitate and tripalmitin confirm the involvement of the radicals and their reactions described above. Merritt and coworkers (32, 33) have used both size exclusion

and gas chromatographic techniques to separate the stable products formed, including those with molecular weights higher than the parent compounds; they have also used both conventional mass spectrometry and fast atom bombardment (FAB) mass spectrometry (especially necessary for high-molecular-weight, low-volatility products) to identify conclusively the structure of the products. On the basis of these analyses, the yields of several relevant products relative to the yield of palmitic acid in tripalmitin are given in Table 4.

Palmitic acid, the major product in both systems, is primarily a consequence of reaction (26). Some of the yield could be derived from reaction (30). The large yield of the acid relative to palmityl aldehyde suggests that reaction (26) predominates over reaction (28), since the hemiacetal formed in the latter reaction would decompose to the aldehyde.

The yields of CO in ethyl palmitate and of CO and CO₂ in both systems are consistent with reactions (29) and (31). Since so much more CO than the aldehyde is produced in ethyl palmitate, reaction (32) appears to be insignificant; the absence of positive evidence for the diketone suggests that reaction (33) may also be insignificant.

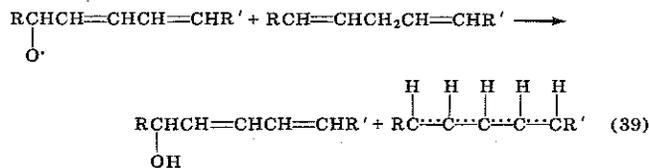
The reasonably high yields of ethane and propane dioldipalmitate are consistent with reaction (26) followed by reaction (34) in the respective ethyl palmitate and tripalmitin systems. Similarly, the high yields of pentadecane in both systems are consistent with the formation of pentadecyl radicals in reactions such as (29) and (31) followed by reaction (34). Judging from the low yields of products derived from the cross combination of ethyl or pentadecyl radicals with the abstraction radical in ethyl palmitate, reaction (34) must be favored over the others because of the mobility of the smaller radicals and the overwhelming excess of substrate molecules.

The high yield of the dimer of ethyl palmitate is consistent with reactions leading to the formation of the abstraction radical and its decay by reaction (37). The positive identification of the propane dioldipalmitate tripalmitin adduct, the propane dioldipalmitate dimer (which is hexanetetraol-tetrapalmitate), and the tripalmitin dimer substantiates reactions (35), (36), and (37). A representative FAB spectrum of a high molecular weight fraction of products extracted from irradiated tripalmitin is shown in Figure 14. Parent ion peaks and other fragment ion peaks are consistent with the structures given for these products. The larger yield of the tripalmitin dimer is consistent with the smaller radical controlling the lifetime of the larger, less mobile radical, until all of the smaller radical is gone.

Table 4. Relative Yield of Products in the Radiolysis of Model Lipid Compounds^a

Product	Ethyl Palmitate	Tripalmitin
Palmitic acid	0.56	1.00
Palmityl aldehyde	0.03	0.03
Pentadecane	0.24	0.36
Ethane	0.44	
Propanedioldipalmitate		0.23
Carbon monoxide	0.11	
Carbon dioxide	0.11	0.25
Ethyl palmitate α, α' -dimer	0.38	
Ethyl α -ethyl palmitate	0.04	
Ethyl α -pentadecyl palmitate	0.01	
Tripalmitin α, α' -dehydrodimer		0.08
Hexanetetraol-tetrapalmitate		0.11
Propanedioldipalmitate tripalmitin adduct		0.31

^a Both compounds irradiated to 25 Mrads; ethyl palmitate at 30°C and tripalmitin at 25°C.



Many other possibilities exist, including the involvement of other myoglobin derivatives and of radicals derived from myoglobin, and they should be considered in future work on iron-catalyzed autoxidation.

In general, more information is needed on specific reaction mechanisms and the absolute values for the corresponding rate constants before a self-consistent explanation of free radical chemistry in food systems can be considered established. Fortunately, appropriate experimentation is being pursued to obtain the relevant data.

Acknowledgment

The author is grateful to John Halliday, Morton Z. Hoffman, Charles Merritt, Jr., Michael D. Sevilla, and Kevin D. Whitburn for helpful discussions on the mechanistic basis for the chemistry of ascorbic acid, myoglobin, and palmitate in certain food systems. The original ESR information made available by Halliday and Sevilla and the manuscripts on myoglobin made available in advance of publication by Hoffman and Whitburn were especially appreciated.

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