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INVITED PAPER

The Pecking Order of Free Radicals and Antioxidants: Lipid Peroxidation, α-Tocopherol, and Ascorbate

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Free radicals vary widely in their thermodynamic properties, ranging from very oxidizing to very reducing. These thermodynamic properties can be used to predict a pecking order, or hierarchy, for free radical reactions. Using one-electron reduction potentials, the predicted pecking order is in agreement with experimentally observed free radical electron (hydrogen atom) transfer reactions. These potentials are also in agreement with experimental data that suggest that vitamin E, the primary lipid soluble small molecule antioxidant, and vitamin C, the terminal water soluble small molecule antioxidant, cooperate to protect lipids and lipid structures against peroxidation. Although vitamin E is located in membranes and vitamin C is located in aqueous phases, vitamin C is able to recycle vitamin E; i.e., vitamin C repairs the tocopheroxyl (chromanoxyl) radical of vitamin E, thereby permitting vitamin E to function again as a free radical chain-breaking antioxidant. This review discusses: (i) the thermodynamics of free radical reactions that are of interest to the health sciences; (ii) the fundamental thermodynamic and kinetic properties that are associated with chain-breaking antioxidants; (iii) the unique interfacial nature of the apparent reaction of the tocopherol free radical (vitamin E radical) and vitamin C; and (iv) presents a hierarchy, or pecking order, for free radical electron (hydrogen atom) transfer reactions.
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Free radicals are now known to play an important role in many areas of biology and are therefore being actively investigated in connection with various human health problems (1, 2). Free radicals are in general reactive species that can be of benefit to an organism, e.g., the radicals produced during phagocytosis (3), as well as a liability, e.g., in producing DNA damage (4) or lipid peroxidation (5). A hierarchy of free radical reactions exists, which can be exploited by organisms that generate these radicals during normal metabolic processes.

Organisms have developed many defenses to protect themselves from free radical processes. Antioxidant enzymes such as superoxide dismutase (6), catalase (7), and the glutathione peroxidases (8) are preventive antioxidants, because they eliminate species involved in the initiation of free radical chain reactions; while small molecule antioxidants, such as ascorbate (9), the tocopherols (10), $CoQH_2$ (reduced coenzyme Q_{10}[1]) (11), urate (12), and glutathione (13), are able to "repair" oxidizing radicals directly and therefore are chain-breaking antioxidants.[2] In addition, it is now apparent that ascorbate and tocopherol function together to protect membrane lipids from damage (14–17).

THERMODYNAMICS, i.e., ONE-ELECTRON REDUCTION POTENTIALS

Because of their reactivity, most radicals undergo simple first- and second-order reactions; thus at least the elementary reactions of radicals are often straightforward and predictable. To be able to predict the direction of free

[1] Abbreviations used: $AscH^-$, ascorbate monoanion; $Asc^{\bullet-}$, semidehydroascorbate radical or ascorbate free radical; CoQ, ubiquinone 10; $CoQ^{\bullet-}$, semiquinone radical of the $CoQ/CoQH_2$ system; $CoQH_2$, reduced ubiquinone 10, ubiquinol 10; Desferal, trade name for deferrioximine; DETAPAC, diethylenetriaminepentaacetic acid; DMPO, 5,5-dimethylpyrroline-1-oxide; EtOH, ethyl alcohol; FA-CoA, fatty acyl-coenzyme A; GPx, glutathione peroxidase; GSH, glutathione; $GS^\bullet$, glutathiyl radical; HRP, horseradish peroxidase; HRP-I, HRP compound I; HRP-II, HRP compound II; LDL, low density lipoprotein; PH-GPx, phospholipid hydroperoxide glutathione peroxidase; PUFA, polyunsaturated fatty acids; PUFA-H, polyunsaturated fatty acid where H represents a weakly bonded bis-allylic hydrogen; TOH, tocopherol; $TO^\bullet$, the tocopheroxyl (chromanoxyl) radical of tocopherol.

[2] Superoxide dismutase can be both a preventative and a chain-breaking antioxidant, depending on how superoxide is involved in a free radical chain reaction.

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TABLE I

Couple	$E^{o'}$/mV (pH)[a]	Ref.
$HO^\bullet$, H^+/H_2O	2310	22
$H_3CH_2C^\bullet$, H^+/CH_3CH_3	1900	38
$O_3^{\bullet -}$, $2H^+/H_2O + O_2$	1800	65
$RO^\bullet$, H^+/ROH (aliphatic alkoxyl radical)	1600	38
$N_3^\bullet/N_3^-$	1330	18
$^\bullet CH_2OH$, H^+/CH_3OH	1200	66
Fe(III)(1,10-phenanthroline)$_3$/Fe(II)(1,10-phenanthroline)$_3$	1150	22
$HOO^\bullet$, H^+/H_2O_2	1060	22
$ROO^\bullet$, $H^+/ROOH$ (alkylperoxyl radical)	1000[b]	38
HRP-II/HRP (horseradish peroxidase)	970	67
Allyl$^\bullet$, H^+/allyl-H (propene)	960	38
HRP-I/HRP-II	950	67
$O_2^{\bullet -}$, $2H^+/H_2O_2$	940	22
$RS^\bullet/RS^-$ (cysteine)	920	68, 69
$C_6H_5O^\bullet$, H^+/C_6H_5OH	900	70
$O_3/O_3^{\bullet -}$	890	65,[c] 66
Cyclopentenyl-3$^\bullet$, H^+/H-cyclopentenyl	700	38
Cyclopentadien-1,3-yl-5$^\bullet$, H^+/H-cyclopentadien-1,3-yl-5	650	38
$O_2(^1\Delta g)/O_2^{\bullet -}$	650	71
Pentadien-1,4-yl-3$^\bullet$, H^+/H-pentadien-1,4-yl-3	600	38
PUFA$^\bullet$, H^+/PUFA-H (polyunsaturated fatty acid, bis-allylic-H)	600	38
$HU^{\bullet -}$, H^+/UH_2^- (Urate)	590	12
Catechol-O$^\bullet$, H^+/catechol-OH	530	70
α-Tocopheroxyl$^\bullet$, H^+/α-tocopherol ($TO^\bullet$, H^+/TOH) (Vitamin E)	500	72–75

Couple	$E^{o'}$/mV (pH)[a]	Ref.
Trolox C (T-O$^\bullet$, H^+/TOH)[d]	480	18, 72
H_2O_2, H^+/H_2O, $^\bullet OH$	320	66
Ascorbate$^{\bullet -}$, H^+/ascorbate monoanion (vitamin C)	282	76
Ferricytochrome c/ferrocytochrome c	260	77
Semiubiquinone, H^+/ubiquinol ($CoQ^{\bullet -}$, $2H^+/CoQH_2$)	200	79
Fe(III)EDTA/Fe(II)EDTA	120	22
Fe(III)/Fe(II) (aqueous)	110[e]	78
Fe(III)citrate/Fe(II)citrate	≈100	f
Fe(III)ADP/Fe(II)ADP	≈100	f
TEMPO ($R_2NO^\bullet$, H^+/R_2NOH)	80	80
Fe(III)DETAPAC/Fe(II)DETAPAC	30	22
Ubiquinone, H^+/Semiubiquinone ($CoQ/CoQ^{\bullet -}$)	−36	79
Dehydroascorbic/ascorbate$^{\bullet -}$	−174	76
Fe(III)ferritin, $2H^+$/Fe(II)ferritin	−190	81
Duroquinone (D=O)/D—O$^{\bullet -}$)	−264	70
Riboflavin/riboflavin$^{\bullet -}$	−317	82
$O_2/O_2^{\bullet -}$	−330	18
Adriamycin/adriamycin$^{\bullet -}$	−341	83
Fe(III)transferrin/Fe(II)transferrin	−400 (7.3)	84
Paraquat/paraquat$^{\bullet -}$	−448	18
Fe(III)Desferal/Fe(II)Desferal	−450	85, g
O_2, $H^+/HO_2^\bullet$	−460	66
RSSR/RSSR$^{\bullet -}$ (cystine or glutathione disulfide, GSSG)	−1500	31, 90[h]
$CO_2/CO_2^{\bullet -}$	−1800	66
H_2O/e^-_{aq}	−2870	86

[a] pH, if other than 7.0.

[b] Peroxyl radicals have a wide range of reduction potentials, $0.77 < E^o < 1.44$ V.

[c] The value for this reaction was overestimated in Ref. (65) and has recently been updated (66).

[d] Trolox C is 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid, alias 3,5-dihydro-6-hydroxy-2,5,7,8-tetramethylbenzo pran-2-carboxylic acid, a water soluble analogue of vitamin E.

[e] It is surprising how often the Fe (III)/Fe (II) (aqueous) reduction potential of E^o = +770 mV (pH 0) rather than $E^{o'}$ = +110 mV (pH 7) is used as some apparently meaningful benchmark to compare processes occurring at pH 7.

[f] This reduction potential has not yet been accurately determined at neutral pH. However, preliminary data suggest that $E^{o'}$ is similar to that of the FeEDTA couple, approximately 0.1 V (W.H. Koppenol, 1992, personal communication).

[g] G.R. Buettner, unpublished observations.

[h] Reference (90) adjusts experimental values to pH 7.

radical processes, it is necessary to understand the thermodynamics of free radical reactions. A key thermodynamic property to consider is the reduction potential, from which predictions can be made (18). With the advent of flash photolysis and pulse radiolysis (19, 20), methods have been developed to measure the one-electron reduction potential of transient species, such as free radicals. The work of the last three decades in this area has produced a wealth of information (18).

Table I is a summary of standard one-electron reduction potentials that are of interest for predicting the course of free radical processes. They are listed in order from highly oxidizing to highly reducing. Making a prediction is straightforward: Each oxidized species is capable of stealing an electron (or hydrogen atom) from any reduced species listed below it, or viewed another way, each reduced species is willing to donate an electron (or hydrogen atom) to any oxidized species listed above.[3] This table focuses on electron/hydrogen atom transfer reactions; other reactions may be possible or even preferred. For example, singlet oxygen in principle could react with PUFA to form $O_2^{\bullet -}$ and PUFA$^\bullet$. However, the kinetically preferred reaction is not electron transfer, but rather an addition reaction of singlet oxygen to the double bonds of PUFA to

[3] For a spontaneous process, the change in the Gibbs free energy must be negative, $\Delta G < 0$. But, $\Delta G = -\eta F \Delta E$; thus, the change in the reduction potential must be positive for a spontaneous reaction.

form lipid peroxides (21). Thus, it must be kept in mind that a reaction that is *thermodynamically possible* may not be *kinetically feasible;* i.e., the rate constant for the reaction may be too small for the reaction to be biologically significant.

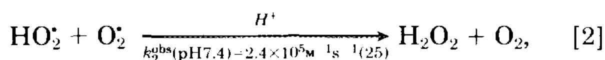
THE PECKING ORDER

The most oxidizing radical that is likely to arise in a biological system is the hydroxyl radical, $HO^{\bullet}$. Being at the top of the pecking order, it can oxidize all the reduced species below it, as listed in Table I. The most often cited source of $HO^{\bullet}$ in a biochemical or biological system is the reductive cleavage of H_2O_2 by a reduced metal, such as Fe(II), i.e., the Fenton reaction (22–24):

$$\text{Fe(II)complex} + H_2O_2 \rightarrow \text{Fe(III)complex} + OH^- + HO^{\bullet} \quad [1]$$

From Table I we see that this reaction has $\Delta E^{O'} > 0$ for Fe(II)EDTA (−120 mV for Fe(II)EDTA oxidation and +320 mV for H_2O_2 reduction yields $\Delta E^{O'} = +200$ mV), Fe(II)DETAPAC ($\Delta E^{O'} = +290$ mV), Fe(II)Desferal ($\Delta E^{O'} = +720$ mV), and ferrocytochrome c ($\Delta E^{O'} = +60$ mV), but not for Fe(II)(1,10-phenanthroline)$_3$ ($\Delta E^{O'} = -830$ mV). For biologically relevant iron complexes, the Fenton reaction has $\Delta E^{O'} > 0$ for Fe(II)citrate ($\Delta E^{O'} \approx +200$ mV), Fe(II)ADP ($\Delta E^{O'} \approx 200$ mV), Fe(II)-ferritin ($\Delta E^{O'} \approx 520$ mV), and Fe(II)-transferrin ($\Delta E^{O'} \approx 720$ mV). Thus, the presence of an Fe(II)complex and H_2O_2 can lead to $HO^{\bullet}$, an oxidant that will indiscrimately initiate a cascade of oxidizing free radical reactions.

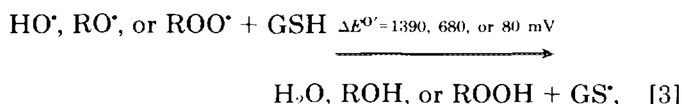
The source of H_2O_2 can be from a direct two-electron reduction of oxygen as well as from a one-electron reduction, which produces superoxide. The one-electron reduction appears to be a more dangerous route, because it will not only result in the production of H_2O_2 via superoxide dismutation,



$$HO_2^{\bullet} + O_2^{\bullet-} \xrightarrow[k_2^{obs}(\text{pH}7.4)=2.4\times10^5\text{M}^{-1}\text{s}^{-1}(25)]{H^+} H_2O_2 + O_2, \quad [2]$$

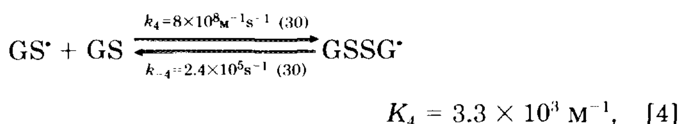
but the presence of $O_2^{\bullet-}$, a reducing species, can convert an Fe(III)complex to an Fe(II)complex, thereby providing all the components for the Fenton reaction, Eq. [1] (26, 27). The formation of $O_2^{\bullet-}$ is often associated with the presence of even stronger reductants, such as the paraquat radical (28) or reduced anthracycline antibiotics such as adriamycin (29). These one-electron reducing species not only reduce O_2, forming $O_2^{\bullet-}$ but they also can efficiently reduce Fe(III) complexes, thereby producing the reduced metal required for the Fenton reaction (29).

The remarkable aspect of free radical electron transfer reactions is that not only can oxidants beget oxidants, but also that oxidants can beget reductants and reductants can beget oxidants. For example, glutathione can react with various highly oxidizing species



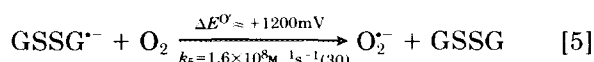
$$HO^{\bullet}, RO^{\bullet}, \text{ or } ROO^{\bullet} + GSH \xrightarrow{\Delta E^{O'}=1390,\ 680,\ \text{or}\ 80\ \text{mV}} H_2O, ROH, \text{ or } ROOH + GS^{\bullet}, \quad [3]$$

generating the glutathiyl radical, $GS^{\bullet}$, which is less oxidizing. However, $GS^{\bullet}$ can react rapidly with another GSH, most efficiently via GS^-



$$GS^{\bullet} + GS^- \underset{k_{-4}=2.4\times10^5\text{s}^{-1}\ (30)}{\overset{k_4=8\times10^8\text{M}^{-1}\text{s}^{-1}\ (30)}{\rightleftharpoons}} GSSG^{\bullet-} \qquad K_4 = 3.3 \times 10^3\ \text{M}^{-1}, \quad [4]$$

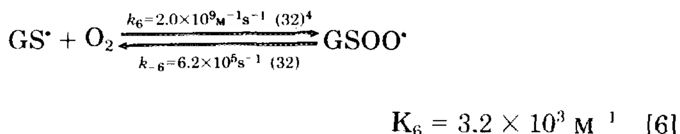
yielding a strongly reducing species, $GSSG^{\bullet-}$ Table I. The very negative potential of the $GSSG/GSSG^{\bullet-}$ couple ($E^{O'} \approx -1500$ mV (31, 90)) makes $GSSG^{\bullet-}$ probably the most reducing species that can arise in a biological setting (except for e^-_{aq} from ionizing radiation). This species can reduce metals as well as produce $O_2^{\bullet-}$



$$GSSG^{\bullet-} + O_2 \xrightarrow[k_5=1.6\times10^8\text{M}^{-1}\text{s}^{-1}(30)]{\Delta E^{O'}\approx+1200\text{mV}} O_2^{\bullet-} + GSSG \quad [5]$$

The possibility of reaction [5] has led to the proposal that superoxide dismutase and GSH in combination are an integral component of cellular antioxidant defense (13, 89).

If oxygen is present, the glutathiyl radical can be converted to its thiol peroxyl radical

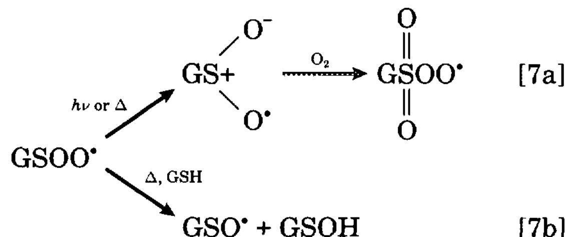


$$GS^{\bullet} + O_2 \underset{k_{-6}=6.2\times10^5\text{s}^{-1}\ (32)}{\overset{k_6=2.0\times10^9\text{M}^{-1}\text{s}^{-1}\ (32)^4}{\rightleftharpoons}} GSOO^{\bullet} \qquad K_6 = 3.2 \times 10^3\ \text{M}^{-1} \quad [6]$$

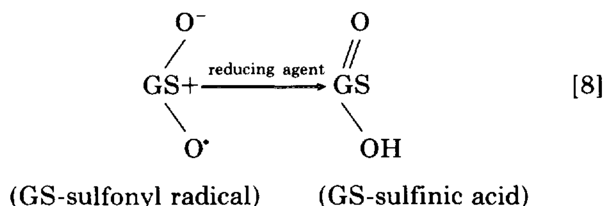
This thiol peroxyl radical is quite unstable toward reorganization. Low temperature EPR studies have demonstrated (33, 34) that visible light will bring about the isomerization of $GSOO^{\bullet}$. There is also evidence that $GSOO^{\bullet}$ will undergo thermal isomerization at 300 K,

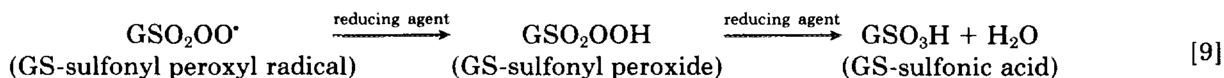
[4] Direct kinetic measurement of k_6 poses many problems, principally from the lack of a strong absorption band for both the $GS^{\bullet}$ and $GSOO^{\bullet}$ radicals. Thus, not all measurements are in agreement. While a reversible reaction has been proposed, reaction [−6] (30, 32), and generally accepted by the research community, other researchers have proposed a nonreversible addition of O_2 to $GS^{\bullet}$ with $k_6 = 3.0 \times 10^7\ \text{M}^{-1}\ \text{s}^{-1}$ and $k_{-6} \approx 0$ (35). Using $K_6 = 3.2 \times 10^3\ \text{M}^{-1}$, we can estimate the bond strength ($\approx \Delta H_f$) of the S-O bond in $GSOO^{\bullet}$ using $\Delta G = -RT \ln K_6 = \Delta H - T\Delta S$. Because the loss of O_2 will dominate the ΔS term, we can estimate ΔS as ≈ -21 e.u. This leads to $\Delta H_f \approx -11$ kcal mol^{-1}, which is in remarkable agreement with -11.7 ± 0.9 kcal mol^{-1} found for formation of this bond in $CH_3SOO^{\bullet}(g)$ (91). Thus, the equilibrium results seem appropriate.

yielding the sulfonyl radical; ab initio calculations, using $CH_3SOO^{\bullet}$ as a model, suggest that $GS^{+}(O^{-})(O^{\bullet})$ would be more thermodynamically stable than $GSOO^{\bullet}$ (150 kJmol^{-1} for the model compound) (34).

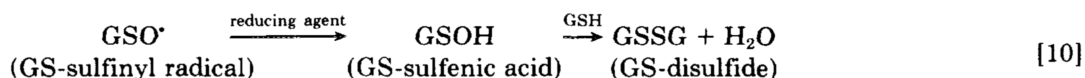


Thus, the glutathiyl peroxyl radical will lead to additional oxidizing intermediates, which can in turn react as follows (34):





and



Glutathione disulfide, glutathione sulfinic acid, and glutathione sulfonic acid are the stable end products observed in glutathione oxidation. In each of the reactions [8]–[10], a reducing agent is shown. If this agent is GSH, then $GS^{\bullet}$ is a possible product. Although the mechanism for reaction [9] has not been investigated, $GSO_2OO^{\bullet}$ would be a very strong hydrogen abstractor; even stronger (higher on the pecking order) than an alkyl peroxyl radical, $ROO^{\bullet}$ (34). Thus, $GS^{\bullet}$ would be a likely product, and as suggested by Sevilla *et al.* (34), $GS^{\bullet}$ would then serve as the chain-carrying radical in glutathione autoxidation. Among the various glutathione-oxygen radicals, the predicted pecking order for hydrogen atom abstraction appears to be (34)

$$\text{(Best) } GSO_2OO^{\bullet} > ROO^{\bullet} \gg GSOO^{\bullet} > GSO_2^{\bullet} \approx GSO^{\bullet} \text{ (Poorest).}$$

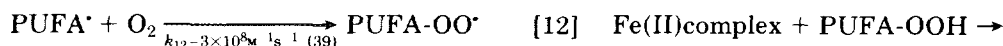
The intracellular concentration of GSH is $\approx$1 mM (or more) while mitochondrial respiration keeps $[O_2] \approx$ 0–10 μM in the cell. Thus, kinetics suggest that nearly all ($\approx$99%) $GS^{\bullet}$ formed should react with GSH, yielding $GSSG^{\bullet -}$, reaction [4] rather than reaction [6], with subsequent formation of GSSG and $O_2^{\bullet -}$ reaction [5]. This shows the importance of superoxide dismutase to keep $[O_2^{\bullet -}]_{ss}$ low (13, 89, 90).

Radicals that have acid/base properties can also have different reactivities that depend on their state of protonation. For example, the perhydroxyl radical ($HOO^{\bullet}$, the conjugate acid of superoxide, $O_2^{\bullet -}$ pK_a = 4.7 (25)) is able to initiate lipid peroxidation, whereas $O_2^{\bullet -}$ is ineffective (36–40). The pecking order suggests two possible pathways for the direct involvement of $HOO^{\bullet}$ in lipid peroxidation: (i) the formation of $PUFA^{\bullet}$ by abstraction of a weakly bonded bis-allylic hydrogen ($\Delta E^{o\prime}$ = +460 mV, reaction [11] below, with $X^{\bullet}$ = $HOO^{\bullet}$) and/or (ii) the formation of PUFA-$OO^{\bullet}$ from PUFA-OOH ($\Delta E^{o\prime}$ = +60 mV). The kinetically preferred site for $HOO^{\bullet}$ attack appears to be PUFA-OOH (36).

LIPID PEROXIDATION ENERGETICS

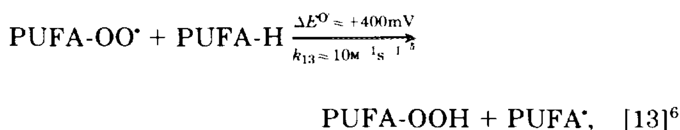
The free radical oxidation of polyunsaturated membrane lipids is the focus of many biological free radical studies. PUFA-H are especially susceptible to peroxidation because of the easily oxidizable bis-allylic hydrogens. The $PUFA^{\bullet}$, H^+/PUFA-H couple has $E^{o\prime}$ = +600 mV (38) (compared to $\approx$1900 mV for an aliphatic hydrogen); thus, any oxidizing species above PUFA-H in Table I can in principle bring about initiation of lipid peroxidation:

Propagation of this chain reaction is both thermodynamically and kinetically favored because $PUFA^{\bullet}$, the pentadienyl radical, reacts rapidly with O_2:



$$\text{PUFA}^{\bullet} + \text{O}_2 \xrightarrow[k_{12} = 3\times10^{8}\text{M}^{-1}\text{s}^{-1}\ (39)]{} \text{PUFA-OO}^{\bullet} \quad [12]$$

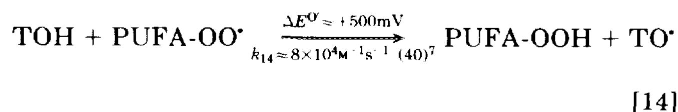
The peroxyl radical formed is higher on the pecking order than PUFA$^{\bullet}$ and thus is able to propagate the chain reaction:



$$\text{PUFA-OO}^{\bullet} + \text{PUFA-H} \xrightarrow[k_{13} \approx 10\text{M}^{-1}\text{s}^{-1}\ ^{5}]{\Delta E^{0'} \approx +400\text{mV}} \text{PUFA-OOH} + \text{PUFA}^{\bullet}, \quad [13]^{6}$$

thereby bringing about the oxidation of other unsaturated lipids.

This low rate constant for the propagation step (Eq. [13]) is a blessing, because it permits vitamin E, a lipid soluble small-molecule antioxidant, to outcompete the propagation reactions to "repair" PUFA-OO$^{\bullet}$, forming PUFA-OOH.



$$\text{TOH} + \text{PUFA-OO}^{\bullet} \xrightarrow[k_{14} \approx 8\times10^{4}\text{M}^{-1}\text{s}^{-1}\ (40)^{7}]{\Delta E^{0'} \approx +500\text{mV}} \text{PUFA-OOH} + \text{TO}^{\bullet} \quad [14]$$

Thus, when comparing the rates for the competing reactions for PUFA-OO$^{\bullet}$,

$$\frac{\text{rate (PUFA-OO}^{\bullet} + \text{TOH)}}{\text{rate (PUFA-OO}^{\bullet} + \text{PUFA-H)}} = \frac{8 \times 10^{4}\ \text{M}^{-1}\ \text{s}^{-1}\ [\text{TOH}][\text{PUFA-OO}^{\bullet}]}{10\ \text{M}^{-1}\ \text{s}^{-1}\ [\text{PUFA-H}][\text{PUFA-OO}^{\bullet}]}, \quad [15]$$

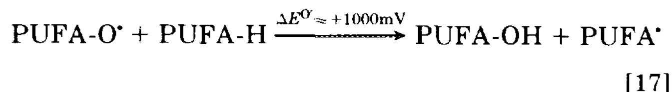
if TOH is to repair 90% of the PUFA-OO$^{\bullet}$ produced, then [TOH]:[PUFA-H] must be $\approx$1:1000, i.e, one tocopherol is able to protect $\approx$1000 lipid molecules from the chain propagation step, reaction [13]. Indeed, the experimentally observed level of TOH in membranes appears to be on the order of 1:1000 (14).

The lipid hydroperoxides produced, PUFA-OOH, in reactions [13] and [14] can undergo reductive cleavage by reduced metals, such as Fe(II), in a process parallel to reaction [1].

Fe(II)complex + PUFA-OOH →

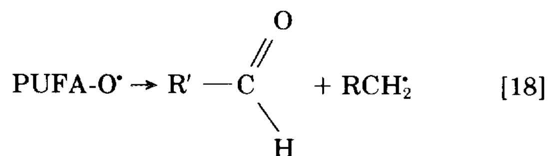
$$\text{Fe(III)complex} + \text{OH}^{-} + \text{PUFA-O}^{\bullet} \quad [16]$$

The lipid alkoxyl radical produced, PUFA-O$^{\bullet}$, is higher on the pecking order than PUFA-H and PUFA-OOH and thus can initiate additional chain reactions, e.g.,



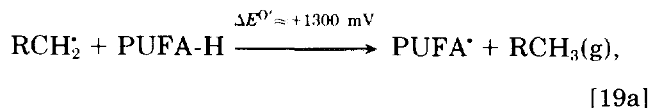
$$\text{PUFA-O}^{\bullet} + \text{PUFA-H} \xrightarrow{\Delta E^{0'} \approx +1000\text{mV}} \text{PUFA-OH} + \text{PUFA}^{\bullet} \quad [17]$$

In addition, PUFA-O$^{\bullet}$ can undergo a β-scission reaction that will produce a short chain alkyl radical such as the ethyl or pentyl radical (92).



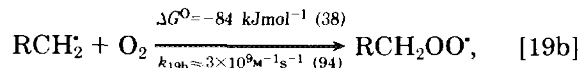
$$\text{PUFA-O}^{\bullet} \rightarrow \text{R}'\text{—C(=O)H} + \text{RCH}_2^{\bullet} \quad [18]$$

This alkyl radical is also higher on the pecking order than PUFA-H or PUFA-OOH and could also initiate additional chain reactions,



$$\text{RCH}_2^{\bullet} + \text{PUFA-H} \xrightarrow{\Delta E^{0'} \approx +1300\text{ mV}} \text{PUFA}^{\bullet} + \text{RCH}_3\text{(g)}, \quad [19a]$$

forming ethane or pentane, end products observed in lipid peroxidation of ω-3 or ω-6 fatty acids (92, 93). Also, the alkyl radicals can easily react with O_2 to form peroxyl radicals,



$$\text{RCH}_2^{\bullet} + \text{O}_2 \xrightarrow[k_{19b} \approx 3\times10^{9}\text{M}^{-1}\text{s}^{-1}\ (94)]{\Delta G^{0} = -84\ \text{kJmol}^{-1}\ (38)} \text{RCH}_2\text{OO}^{\bullet}, \quad [19b]$$

which will initiate further oxidations.

ASCORBATE AND TOCOPHEROL AS ANTIOXIDANTS

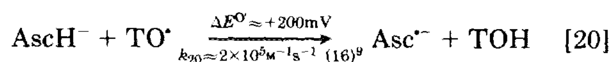
Each tocopherol can donate two electrons as a chain-breaking antioxidant, and then it is "consumed." To be an effective antioxidant, each oxidized tocopherol must be recycled (reduced back to TOH), but, at present, enzyme systems capable of reducing oxidized tocopherol have not been described.[8] The current thinking is that ascorbate recycles tocopherol via TO$^{\bullet}$, producing the ascorbate radical (9, 15–17).

[5] A rate costant of 40 $\text{M}^{-1}\ \text{s}^{-1}$ has been found for the propagation step for linoleate in miceller solution (41, 42). The value of 10 $\text{M}^{-1}\ \text{s}^{-1}$ is an estimate for the propagation step in a biological membrane, this lower value reflecting the more restricted motion of the fatty acid chains in a biological membrane.

[6] Numbering this reaction as [13] was no accident.

[7] McKenna *et al.* (43) have determined a value for the inhibition rate constant $k_{14} = 5.0 \times 10^{-2}$ mol%$^{-1}$ s^{-1} in erythrocyte ghosts. The term mol% is the moles of antioxidant per total moles of organic phosphorus. Barclay *et al.* (41) have reported a value of $5.8 \times 10^{3}\ \text{M}^{-1}\ \text{s}^{-1}$ for the inhibition rate constant for α-tocopherol in dilinoleoylphosphatidylcholine bilayers. This much slower rate constant is more difficult to rationalize with the observed membrane levels of α-tocopherol, see Eq. [15].

[8] Evidence for a glutathione-dependent factor, presumably an enzyme, that cycles the tocopheroxyl radical back to tocopherol has been observed in hepatic endoplasmic reticulum (44) and human platelets (45).



$$\mathrm{AscH^- + TO^\bullet \xrightarrow[k_{20} \approx 2\times10^5 M^{-1}s^{-1}\ (16)^9]{\Delta E^{\circ\prime} \approx +200 mV} Asc^{\bullet-} + TOH} \quad [20]$$

$Asc^{\bullet-}$ can be removed by dismutation, yielding $AscH^-$ and dehydroascorbate. Both dehydroascorbate and $Asc^{\bullet-}$ can be reduced by enzyme systems that use NADH or NADPH as sources of reducing equivalents (46); thus, ascorbate is recycled. A possible problem with this model is that ascorbate being water soluble and tocopherol being lipid soluble are in different phases. But it has been demonstrated that in a membrane, the phenolic OH group of tocopherol will be at the membrane–water interface, i.e., near the polar head groups of the phospholipids that compose the bilayer, and the phytyl tail of tocopherol lies parallel to the phospholipid fatty acyl chains, Fig. 1 (47, 48). Thus, the peroxidation and antioxidant processes will proceed as follows:

1. Initiation, reaction [11], will produce the pentadienyl radical. Its very fast reaction with O_2, reaction [12], and the physical separation of the radical site on the PUFA and the "OH" of tocopherol preclude any significant reaction between $PUFA^\bullet$ and TOH, Fig. 1.
2. However, once the lipid peroxyl radical is formed, a significant dipole is present, ≈2.6 Debye, (49, 50) that will allow the peroxyl radical moiety on the lipid chain to "float" (partition) to the membrane–water interface, Fig. 1.
3. This yields the proximity required for the repair of PUFA-$OO^\bullet$ by TOH, reaction [14], thereby preventing chain propagation, reaction [13].
4. The relatively stable tocopheroxyl radical formed, $TO^\bullet$, is at the membrane–water interface, allowing water soluble ascorbate access to membrane-bound $TO^\bullet$ for the repair reaction, reaction [20], thereby recycling the tocopherol, Fig. 1.
5. The potentially dangerous lipid hydroperoxide can be cleaved by phospholipase A_2, allowing GPx to detoxify the hydroperoxide to a fatty acid alcohol (51), or it may be converted directly to an alcohol by PH-GPx (52).

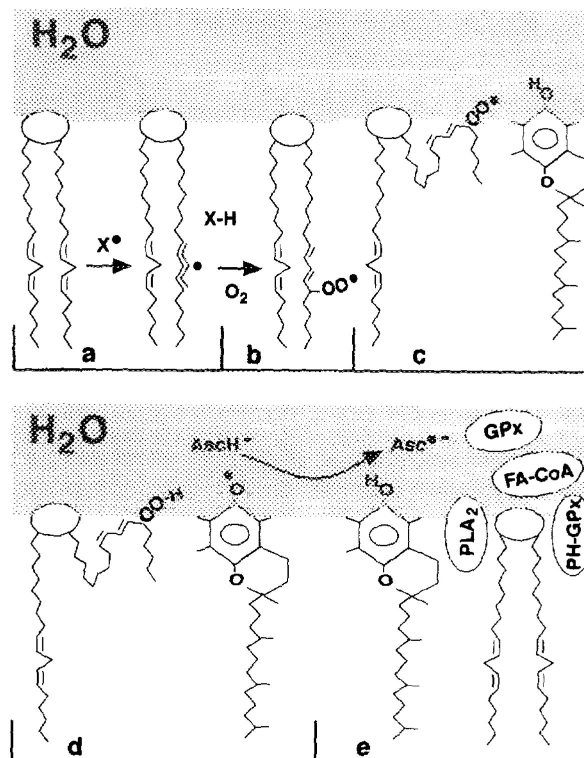


FIG. 1. Membrane lipid peroxidation. Only one leaflet of the bilayer is represented. (a) Initiation of the peroxidation process by an oxidizing radical, $X^\bullet$, by abstraction of a bis-allylic hydrogen, thereby forming a pentadienyl radical. (b) Oxygenation to form a peroxyl radical and a conjugated diene. (c) The peroxyl radical moiety partitions to the water–membrane interface where it is poised for repair by tocopherol. (d) The peroxyl radical is converted to a lipid hydroperoxide, and the resulting tocopheroxyl radical can be repaired by ascorbate. (e) Tocopherol has been recycled by ascorbate; the resulting ascorbate radical can be recycled by enzyme systems. The enzymes phospholipase A_2 (PLA_2), phospholipid hydroperoxide glutathione peroxidase (PH-GPx), glutathione peroxidase (GPx), and fatty acyl-coenzyme A (FA-CoA), cooperate to detoxify and repair the oxidized fatty acid chain of the phospholipid. This cartoon cannot show the dynamic aspects of this process. TOH in the membrane will undoubtly be bobbing "up and down" so that the position of the "OH" is variable. In addition, TOH and $TO^\bullet$ may have somewhat differing positions at the interface. The chemical aspects of this process will undoubtedly also describe the free radical oxidation of LDL.

Ascorbate and tocopherol are both well-suited to serve as small molecule chain-breaking donor antioxidants in biological systems because:

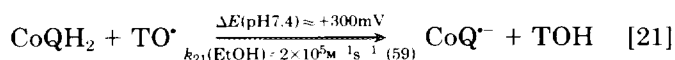
1. They are thermodynamically at the bottom of the pecking order for oxidizing radicals, Table I.
2. Their radicals are *relatively* harmless, being neither strongly oxidizing nor strongly reducing.
3. Their radicals react poorly with oxygen, producing very little, if any, superoxide via electron transfer or peroxyl radical by O_2 addition.
4. Their kinetic properties require that only relatively small amounts be present for them to serve as effective antioxidants.
5. They can be recycled, directly or indirectly, by enzyme systems.

[9] The value of k_{20} is dependent on the solvent and environment. In a water/2-propanol/acetone homogeneous solution, $k_{20} = 1.55 \times 10^6\ M^{-1}\ s^{-1}$ (15). However, in positively charged hexadecyltrimethylammonium chloride micelles, with α-tocopherol in the micelle while ascorbate is in the aqueous phase, $k_{20} = 7.2 \times 10^7\ M^{-1}\ s^{-1}$ (62). In negatively charged sodium dodecyl sulfate micelles, $k_{20} = 3.8 \times 10^4\ M^{-1}\ s^{-1}$ (62); while in phosphatidylcholine liposomes, $k_{20} \approx 2 \times 10^5\ M^{-1}\ s^{-1}$ (16). In Triton X-100 micelles, this rate constant drops to $3.2 \times 10^2\ M^{-1}\ s^{-1}$ (63), but Triton X-100 may not be a good model for α-tocopherol in a membrane bilayer, because the oxygen present in the Triton X-100 chains will undoubtedly alter the position of tocopherol in the micelle. This range of values suggests that $2 \times 10^5\ M^{-1}\ s^{-1}$ is probably a good estimate for k_{20} when tocopherol is in a biological membrane.

OTHER ANTIOXIDANTS

Carotenoids such as β-carotene are now considered to have lipid antioxidant properties, but very little information is available on the mechanism of this antioxidant activity (53–55). It has been hypothesized that β-carotene acts not as a chain-breaking *donor* antioxidant, such as ascorbate and tocopherol, but rather as a radical trap whereby the chain-carrying peroxyl radicals add covalently to the conjugated system of β-carotene, thus breaking the chain reaction of lipid peroxidation, reaction [13] (53). β-Carotene appears to be an unusual antioxidant in that it is effective only at low oxygen tensions (53). The first evidence has recently been presented which demonstrates that β-carotene can act synergistically with α-tocopherol as a radical-trapping antioxidant in membranes (55).

Reduced CoQ, $CoQH_2$, has been proposed to have a role as a free radical chain-breaking antioxidant (11). $CoQH_2$ has been shown to protect against lipid peroxidation in liposomes (56, 57) as well as in LDL (58), but its most important antioxidant activity is probably in the mitochondrial membrane (11). In fact, it appears that as an antioxidant, $CoQH_2$ is sacrificed before α-tocopherol (57, 58). Consistent with this is the observation that $CoQH_2$ is able to regenerate tocopherol (59)



$$CoQH_2 + TO^{\bullet} \xrightarrow[k_{21}(EtOH) = 2\times10^{5} M^{-1}s^{-1}\ (59)]{\Delta E(pH 7.4) \approx +300 mV} CoQ^{\bullet -} + TOH \quad [21]$$

These observations are consistent with the relative positions of tocopherol and $CoQH_2$ in the pecking order, Table I.

Some nitroxide radicals have recently been shown to have SOD activity (60), and this has generated interest in their use as antioxidants in acute episodes of $O_2^{\bullet -}$ production. However, a new mode of antioxidant action for nitroxides has been revealed, i.e., they can serve as chain-breaking *acceptor* antioxidants (61). The presence of Fe(II) in a system where active oxygen species, e.g., $O_2^{\bullet -}$ and H_2O_2, are being produced can generate $HO^{\bullet}$, the ultimate oxidizing free radical, Eq. [1]. However, nitroxides are able to oxidize Fe(II) complexed with DNA, thereby preventing $HO^{\bullet}$ formation and subsequent DNA damage.

$$Fe(II)DNA + R_1R_2NO^{\bullet} + H^{+} \rightarrow Fe(III)DNA + R_1R_2NOH \quad [22]$$

The hydroxylamine formed is relatively unreactive and in fact could serve as a chain-breaking donor antioxidant. Neither the nitroxide radical nor the hydroxylamine will react significantly with oxygen to produce damaging radicals; thus nitroxides are a safe place to put an electron.

Nitroxides serving as a "safe" way to oxidize Fe(II) to Fe(III) is reminiscent of ceruloplasmin in that it oxidizes Fe(II) by a "safe" mechanism, i.e., there is no evidence for the formation of peroxides or superoxide as the reducing equivalents from Fe(II) are passed to dioxygen, forming water (87, 88).

NONSTANDARD CONDITIONS

The standard thermodynamic conditions for $E^{O'}$ imply that all reactants are 1 M (actually, unit activity). However, the vast majority of free radicals could never be at a concentration of 1 M, thus $\Delta E^{O'}$ should only serve as a guideline. The larger $\Delta E^{O'}$ is, the safer the prediction; the smaller $\Delta E^{O'}$ is, the more important the actual concentrations become for a prediction. To correct the standard redox potentials for the actual concentrations encountered in a one-electron free radical reaction, the Nernst equation is used (18):

$$\Delta E = \Delta E^{O'} - 60 \text{ mV} \log_{10} \frac{[\text{Products}] \times \cdots}{[\text{Reactants}] \times \cdots}. \quad (23)$$

As an example, consider the ESR spin trapping observation that HRP can oxidize N_3^- to $N_3^{\bullet}$ (64); briefly, the proposed reactions are:

$$HRP + H_2O_2 \rightarrow HRP\text{-}I \quad [24]$$

$$HRP\text{-}I + N_3^- \rightarrow HRP\text{-}II + N_3^{\bullet} \quad [25]$$

$$HRP\text{-}II + N_3^- \rightarrow HRP + N_3^{\bullet} \quad [26]$$

$$DMPO + N_3^{\bullet} \rightarrow DMPO/N_3^{\bullet}, \quad [27]$$

where DMPO is the spin trapping agent. For reaction [25], $\Delta E^{O'} = -380$ mV, (950 mV for HRP-I) − (1330 mV for $N_3^{\bullet}$), a thermodynamically uphill reaction. However, applying the Nernst equation and reasonable estimates for the steady-state concentration of the reactants and products for reaction [25], we have

$$\Delta E = -380 \text{ mV} - 60 \text{ mV} \log_{10} \frac{[\text{HRP-II}][N_3^{\bullet}]}{[\text{HRP-I}][N_3^-]}. \quad [28]$$

If the ratio of [HRP-II]:[HRP-I] is $\approx$1:1 and $[N_3^{\bullet}]_{ss} = 10^{-9}$ M while $[N_3^-] = 10^{-2}$ M, then $\Delta E = +100$ mV, i.e., the reaction can be pulled to the right because the steady-state concentration of the very reactive $N_3^{\bullet}$ is extremely low.

CONCLUSIONS

Knowledge of the one-electron reduction potentials for free radical reactions is invaluable in helping to understand experimental results of free radical processes. However, it must be kept in mind that reactions other than electron/hydrogen atom transfer are possible. In addition, $\Delta E^{O'} < 0$ does not make a reaction impossible;

equilibria may be shifted due to the disappearance of product by further reactions, thereby pulling a reaction. These potentials also could be invaluable in the design of new pharmacological antioxidants for use in acute episodes of high free radical flux.

The hierarchy of free radical electron transfer reactions presented here allow us to predict the cascade of free radical reactions that will occur after initiation. In general, each reaction in the sequence will generate less energetic radicals, with antioxidants producing the least energetic (most stable) radicals. However, the presence of oxygen and catalytic metals, such as iron, can result in reactions that produce *more* reactive, rather than less reactive, radicals; for example, reactions [1], [12], and [16]. It is these reactions that make unchecked free radical oxidation processes dangerous to an organism.

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