The Virtual Free Radical School

Ascorbate (Vitamin C), its Antioxidant Chemistry

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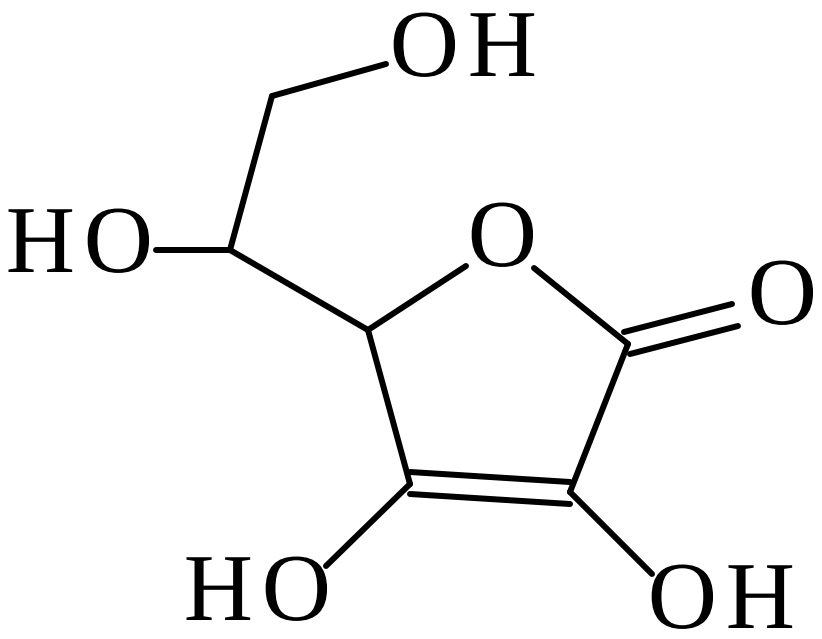
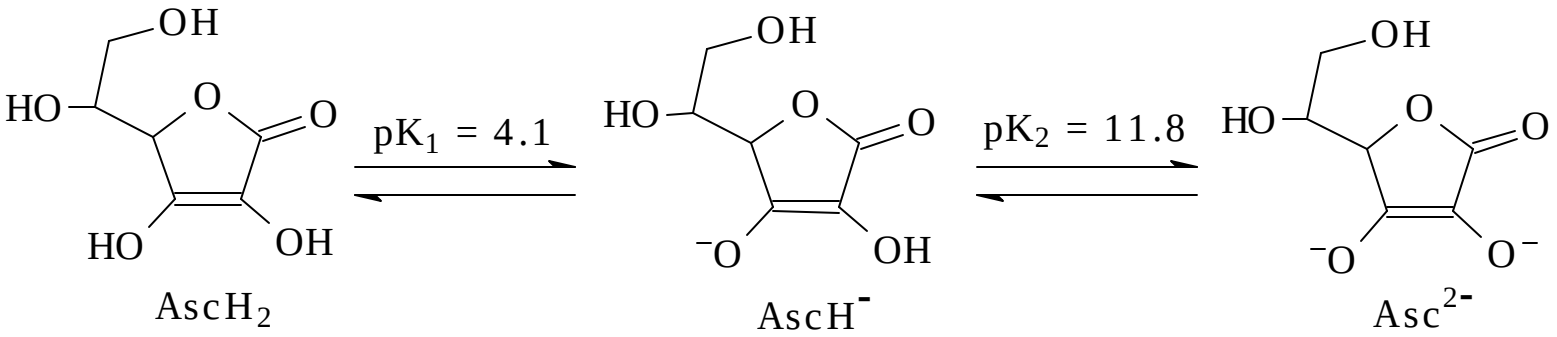
Ascorbic Acid Structure



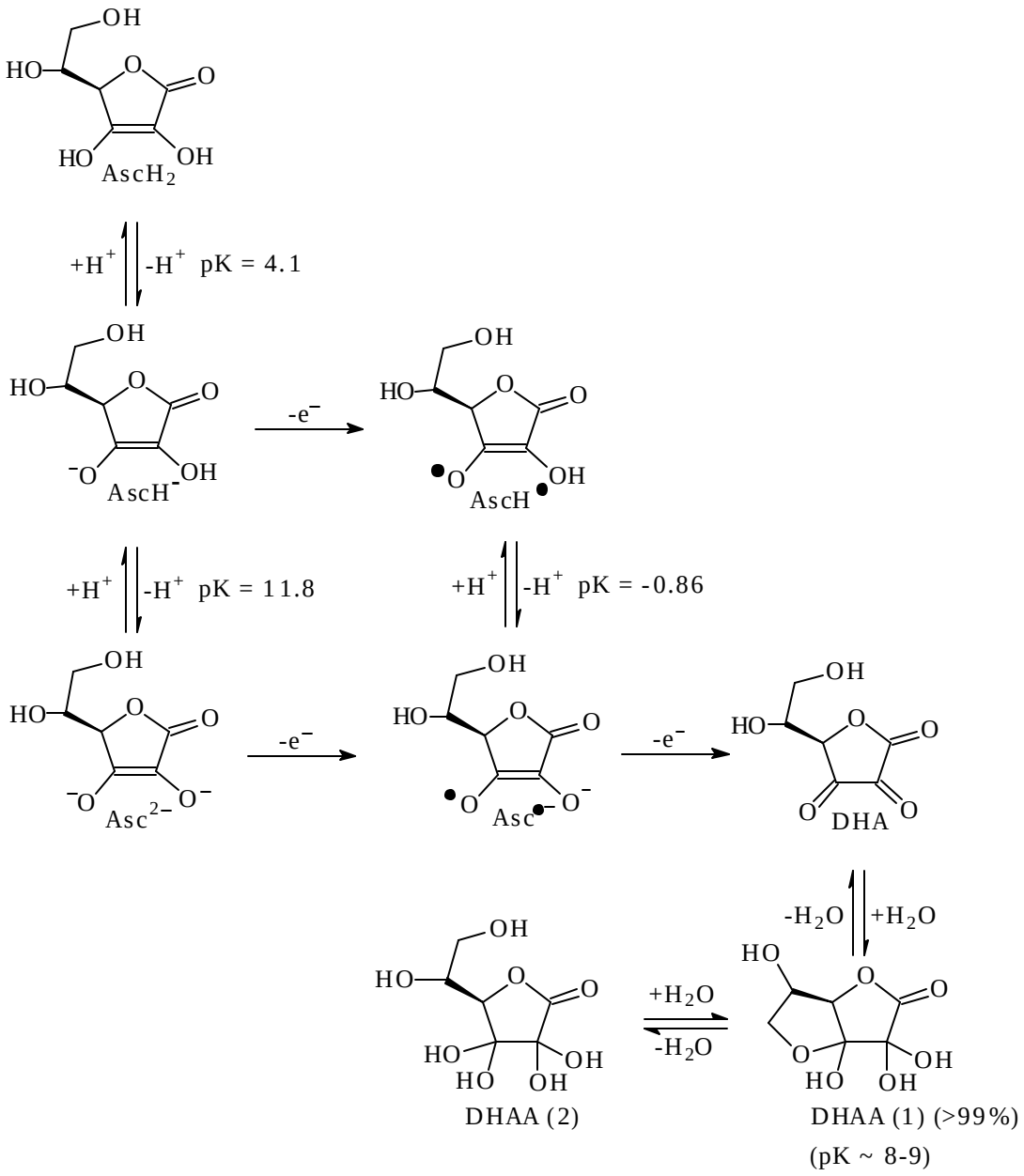
($AscH_2$)

$AscH_2$ is a Di-acid

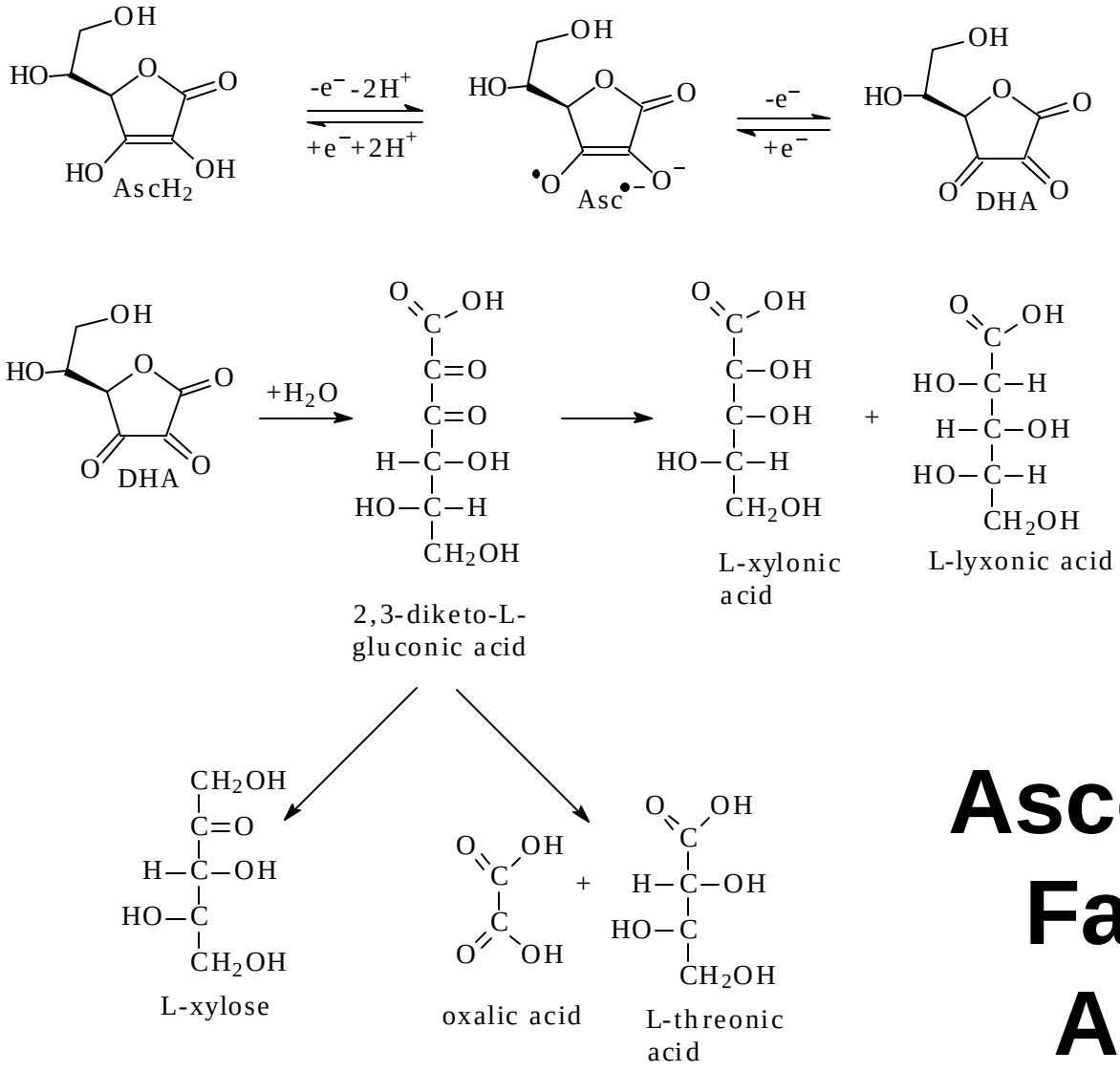


At pH 7.4, 99.95% of vitamin C will be present as $AscH^-$; 0.05% as $AscH_2$ and 0.004% as Asc^{2-}. Thus, the antioxidant chemistry of vitamin C is the chemistry of $AscH^-$.

Forms of Ascorbate



From Bors W, Buettner GR. (1997) The vitamin C radical and its reactions in *Vitamin C in Health and Disease*, ed. by L. Packer and J. Fuchs, Marcel Dekker, Inc., New York, Chapter 4, pp75-94.



Ascorbate Falling Apart

Kinetics of $AscH^-$ Reactions

Radical	$k_{obs}/M^{-1}s^{-1}$ (pH 7.4)
$HO^\bullet$	1.1×10^{10}
$RO^\bullet$ (*tert*-butyl alkoxyl radical)	1.6×10^{9}
$ROO^\bullet$ (alkyl peroxyl radical, *e.g.* $CH_3OO^\bullet$)	$1\text{-}2 \times 10^{6}$
$Cl_3COO^\bullet$	1.8×10^{8}
$GS^\bullet$ (glutathiyl radical)	6×10^{8} (5.6)
$UH^{\bullet -}$ (Urate radical)	1×10^{6}
$TO^\bullet$ (Tocopheroxyl radical)	2×10^{5} [b]
$Asc^{\bullet -}$ (dismutation)	2×10^{5}
$CPZ^{\bullet +}$ (Clorpromazine radical action)	1.4×10^{9} (5.9)
Fe(III)EDTA/ Fe(II)EDTA	$\approx 10^{2}$
$O_2^{\bullet -}/HO_2^\bullet$	2.7×10^{5}
Fe(III)Desferal®/ Fe(II)Desferal®	Very slow

These rate constants are for the reaction:

$AscH^- + R^\bullet \rightarrow Asc^{\bullet -} + RH$

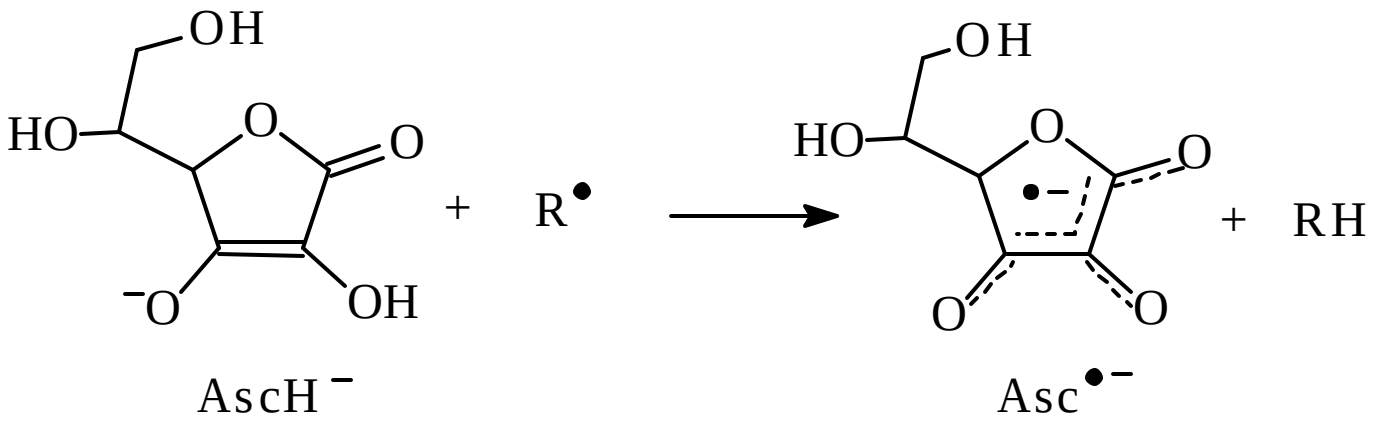
$AscH^-$ reacts rapidly with these and similar oxidants making it an outstanding donor antioxidant.

[a] Estimated k_{obs} for $TO^\bullet$ when in a biological membrane.

[b] k is pH dependent, thus this is kobs at pH 7.4.

Adapted from: Buettner GR, Jurkiewicz BA. (1996) Catalytic metals, ascorbate, and free radicals: combinations to avoid. *Rad Research* **145:**532-541.

$AscH^-$ is a Donor Antioxidant



$AscH^-$ donates a hydrogen atom ($H^\bullet$ or $H^+ + e^-$) to an oxidizing radical to produce the resonance-stabilized tricarbonyl ascorbate free radical. $AscH^\bullet$ has a pK_a of -0.86; thus, it is not protonated in biology and will be present as $Asc^{\bullet-}$.

Dismutation of Ascorbate Radical

$$2\ Asc^{\bullet -} + H^{+} \rightleftharpoons AscH^{-} + DHA$$

$$k_{obs}\ (7.4) = 1.4 \times 10^{5}\ M^{-1}\ s^{-1}$$

This rate constant increases by a factor of »10 when phosphate is present.*

This dismutation reaction is the principal route to the elimination of the $Asc^{\bullet -}$ *in vitro.* However, *in vivo* it is thought that reducing enzymes are involved in the removal of this radical, resulting in the recycling of ascorbate.**

*Reviewed in: Bors W, Buettner GR. (1997) The vitamin C radical and its reactions in *Vitamin C in Health and Disease*, ed. by L. Packer and J. Fuchs, Marcel Dekker, Inc., New York, Chapter 4, pp75-94.

Hossain MA, Asada K. (1985) Monodehydroascorbate reductase from cucumber is a flavin adenine dinucleotide enzyme. *J Biol Chem.* **260:12920-12926.

The Dismutation of Ascorbate Radical is an Equilibrium Reaction

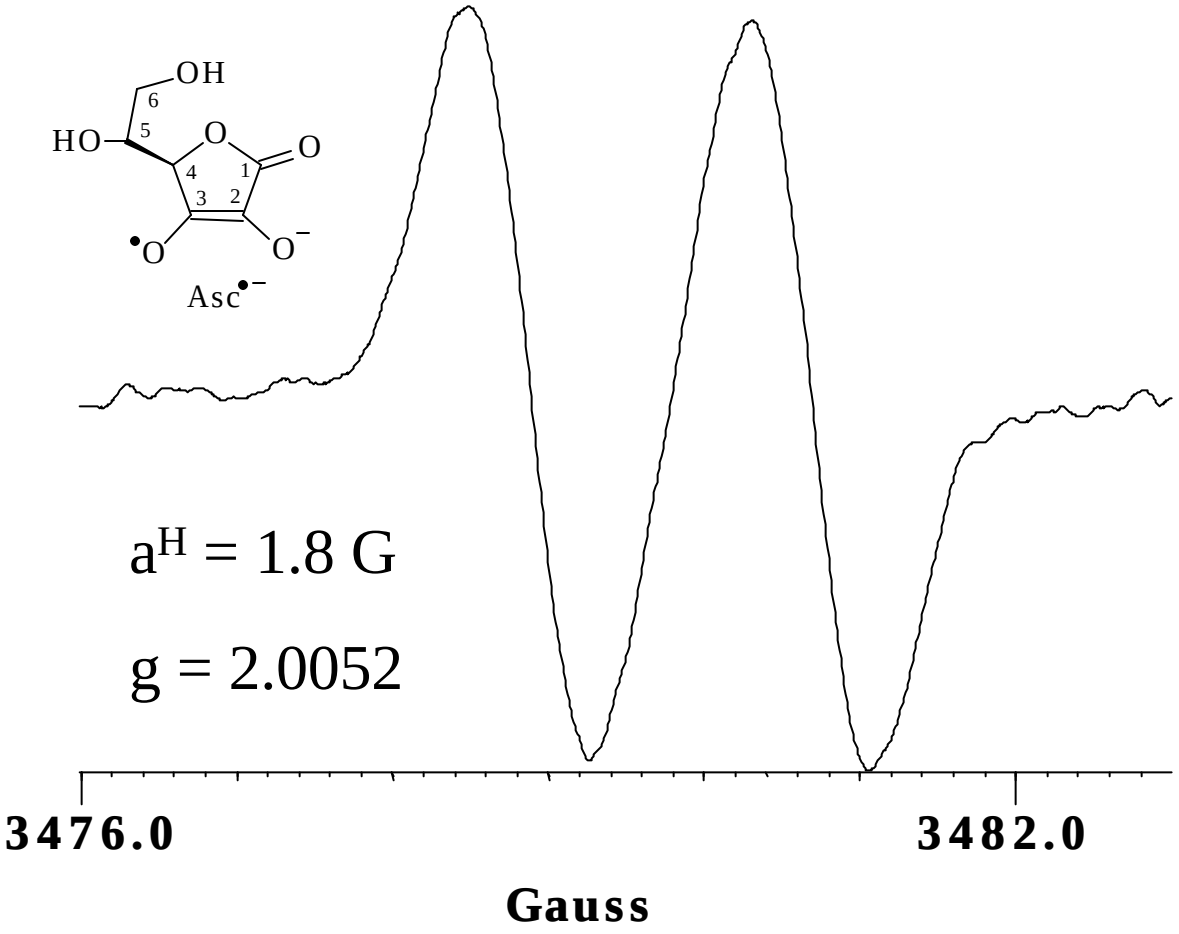
$$2\ Asc^{\bullet -} + H^{+} \rightleftharpoons AscH^{-} + DHA$$

$$K = \frac{[DHA]\,[AscH_2]_{total}}{[Asc^{\bullet -}]^2\,[H^{+}]\,[1 + H^{+}/K_{AscH_2}]} = 5 \times 10^{14}\ M^2$$

Because of this equilibrium reaction, $Asc^{\bullet -}$ can be formed if both, DHA and $AscH^-$ are present. Note that the equilibrium constant, *K*, for this dismutation reaction is pH-dependent. Thus, the higher the pH the more $Asc^{\bullet -}$ would be formed; however, at higher pH DHA is more unstable. (K_{AscH2} is the first ionization constant of ascorbic acid.)

Reviewed in: Bors W, Buettner GR. (1997) The vitamin C radical and its reactions in *Vitamin C in Health and Disease*, ed. by L. Packer and J. Fuchs, Marcel Dekker, Inc., New York, Chapter 4, pp75-94.

EPR Detection of $Asc^{\bullet -}$



The ascorbate radical is usually observed as a simple doublet species by EPR.

The intensity of the EPR spectrum of $Asc^{\bullet -}$ can be used as an indicator of oxidative stress *in vitro* and *in vivo*.

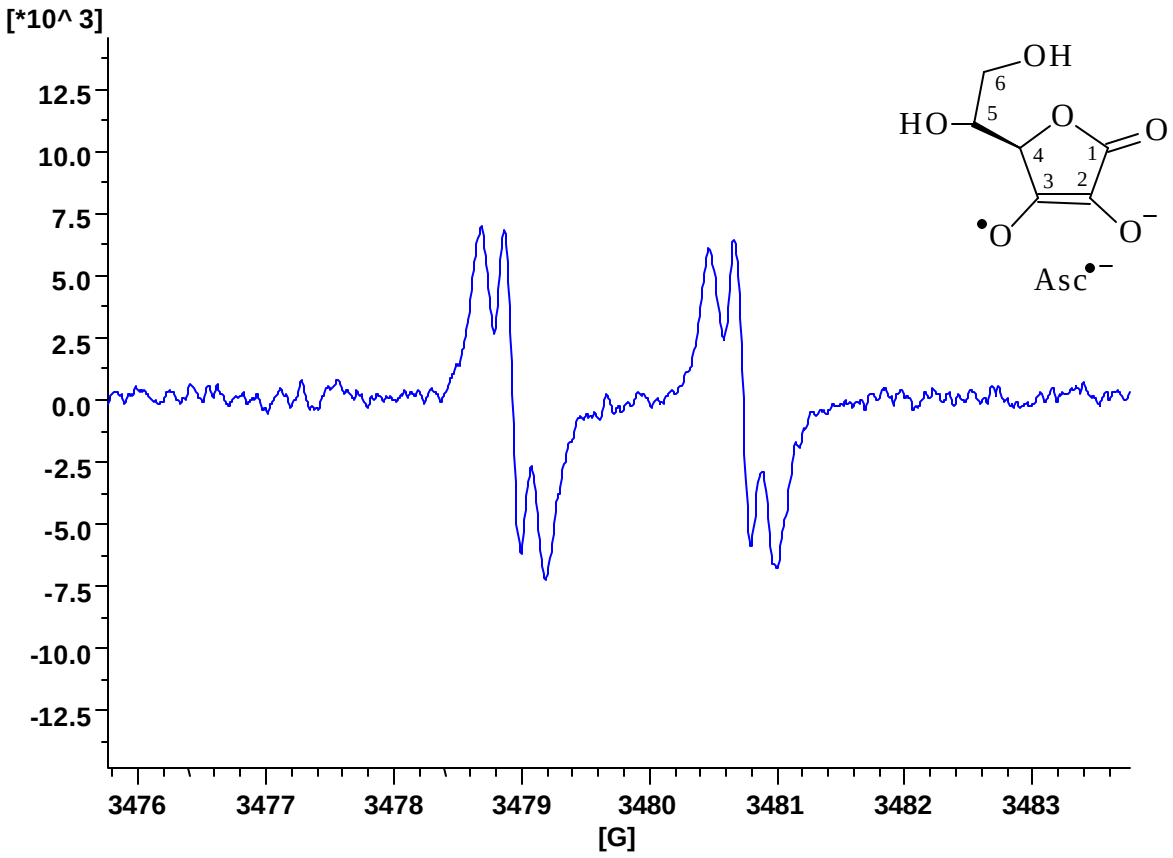
Higher Resolution EPR

With appropriate instrument settings a more detailed spectrum can be observed by EPR.

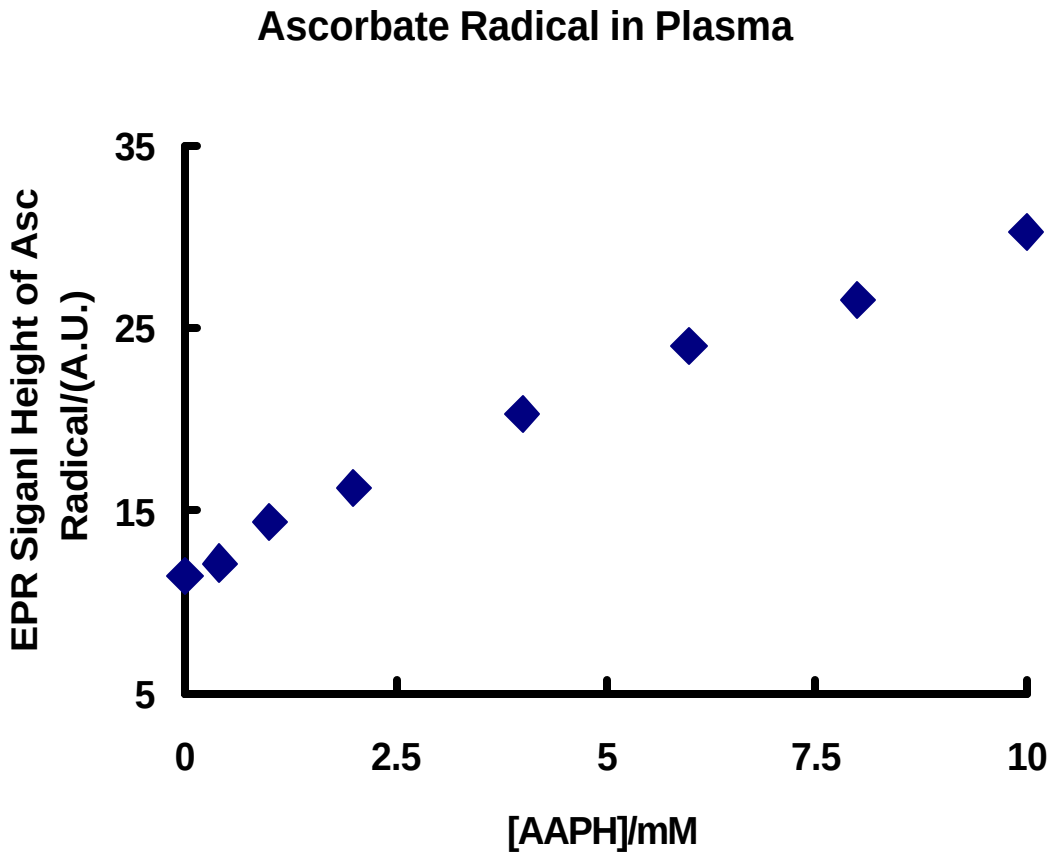
a^{H}_{4} (1) = 1.76 G

a^{H}_{5} (1) = 0.07 G

a^{H}_{6} (2) = 0.19 G



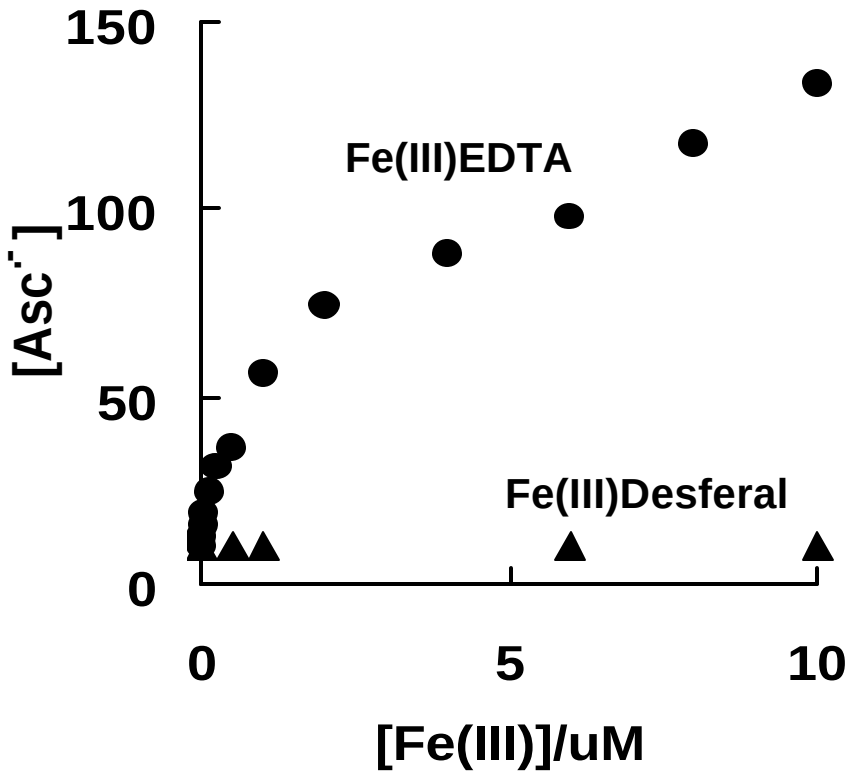
$Asc^{\cdot -}$, Real Time Marker of Oxidative Stress



$[Asc^{\bullet -}]_{ss}$ is proportional to the rate of ascorbate oxidation.

$[Asc^{\bullet -}]_{ss}$ in plasma is directly proportional to oxidative flux: EPR signal height of $Asc^{\bullet -}$ (arbitrary units) *versus* AAPH concentration. The solutions contained 58 µM ascorbate in plasma and various amounts of the free radical-generator AAPH. From: Buettner GR, Jurkiewicz BA. (1993) The ascorbate free radical as a marker of oxidative stress: An EPR study. *Free Radic Biol Med* **14:** 49-55.

$Asc^{\cdot -}$, as an indicator for adventitious transition metals



The oxidation of ascorbate is very slow in the absence of catalytic metals. This plot shows $[Asc^{\bullet -}]_{SS}$ with varying [Fe(III)] in two different chelating environments. Because the iron in EDTA is freely accessible to reductants, Fe(III)EDTA is an excellent catalyst for $AscH^-$ oxidation. In contrast Fe(III) in Desferal is not accessible to reductants and thus Fe(III)Desferal does not catalyze $AscH^-$ oxidation.

EPR as well as UV-Vis spectroscopy (ε_{265} = 14,500 $M^{-1}\,cm^{-1}$ for $AscH^-$) have been used to determine the metal content of buffer solutions.

Buettner GR. (1988) In the absence of catalytic metals, ascorbate does not autoxidize at pH 7: Ascorbate as a test for catalytic metals. *J Biochem Biophys Meth* **16:** 20-40.

Buettner GR. (1990) Ascorbate oxidation: UV absorbance of ascorbate and ESR spectroscopy of the ascorbyl radical as assays for iron. *Free Rad Res Comm* **10:** 5-9\

Thermodynamics of Ascorbate

The unpaired electron of $Asc^{\bullet-}$ resides in the π-system that includes the tri-carbonyl moiety of ascorbate. This results in a weakly oxidizing and weakly reducing radical. Due to its π-character $Asc^{\bullet-}$ does not react with oxygen to form dangerously oxidizing peroxyl radicals. Thermodynamically, it is relatively unreactive with a one-electron reduction potential of only +282 mV. It is considered to be a terminal, small-molecule antioxidant.

Buettner GR, Jurkiewicz BA. (1993) The ascorbate free radical as a marker of oxidative stress: An EPR study. *Free Radic Biol Med* **14:** 49-55.

Buettner GR. (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophy.* **300:**535-543**.**

The Pecking Order

Note that the donor antioxidants are found in the middle of the "pecking order".

Redox Couple (one-electron reductions)	E°'/mV
$HO^{\bullet}$, H^{+}/H_2O	+ 2310
$RO^{\bullet}$, H^{+}/ROH (aliphatic alkoxyl radical)	+ 1600
$ROO^{\bullet}$, $H^{+}/ROOH$ (alkyl peroxyl radical)	+ 1000
$GS^{\bullet}/GS^{-}$ (glutathione)	+ 920
$PUFA^{\bullet}$, H^{+}/PUFA-H (*bis*-allylic-H)	+ 600
$TO^{\cdot}$, H^{+}/TOH (tocopherol)	**+ 480**
H_2O_2, H^{+}/H_2O, $HO^{\bullet}$	+ 320
$Asc^{\cdot -}$, H^{+}/AscH^{-} (Ascorbate)	**+ 282**
$CoQ^{\bullet -}$, $2H^{+}/CoQH_2$	+ 200
Fe(III) EDTA/Fe(II) EDTA	+ 120
$CoQ/CoQ^{\bullet -}$	- 36
$O_2/O_2^{\bullet -}$	- 160
Paraquat/Paraquat$^{\bullet -}$	- 448
Fe(III)DFO/Fe(II)DFO	- 450
$RSSR/RSSR^{\bullet -}$ (GSH)	- 1500
H_2O/e^{-}_{aq}	- 2870

Buettner GR. (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys.* **300:**535-543.

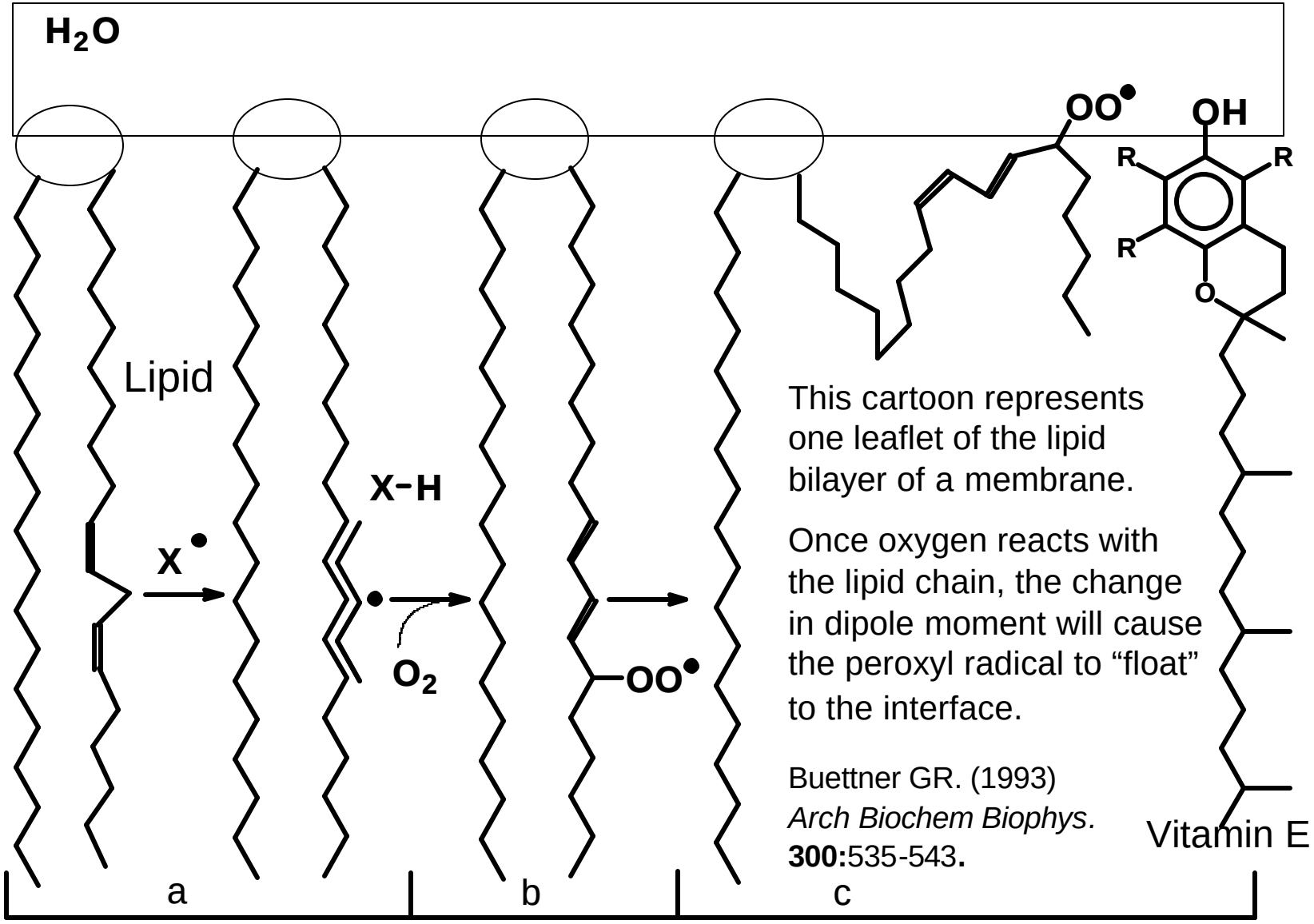
C and E as Co-antioxidants

As seen in the thermodynamic pecking order above, the tocopherol radical, $TO^{\bullet}$, is more oxidizing then $Asc^{\bullet -}$. It is thought that ascorbate contributes to the recycling of $TO^{\bullet}$ back to TOH.

$$\mathbf{TO^{\cdot} + AscH^{-} \rightarrow TOH + Asc^{\cdot -}}$$

This mechanism is clearly important in protecting LDL from unwanted oxidations, because LDL lacks enzymes that could recycle $TO^{\bullet}$. But its importance in cells and tissues is still being debated.

C and E as Co-Antioxidants (1)

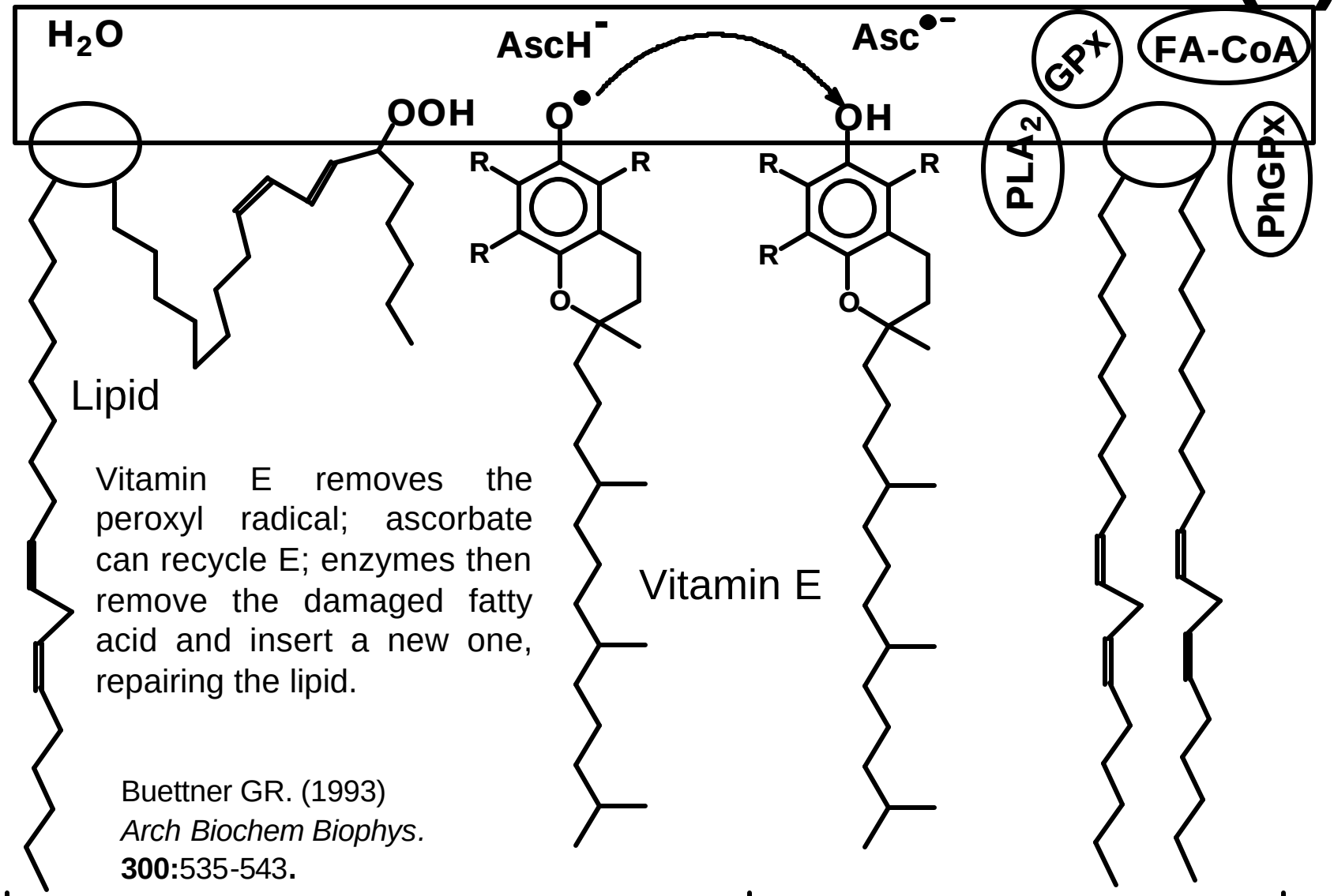


This cartoon represents one leaflet of the lipid bilayer of a membrane.

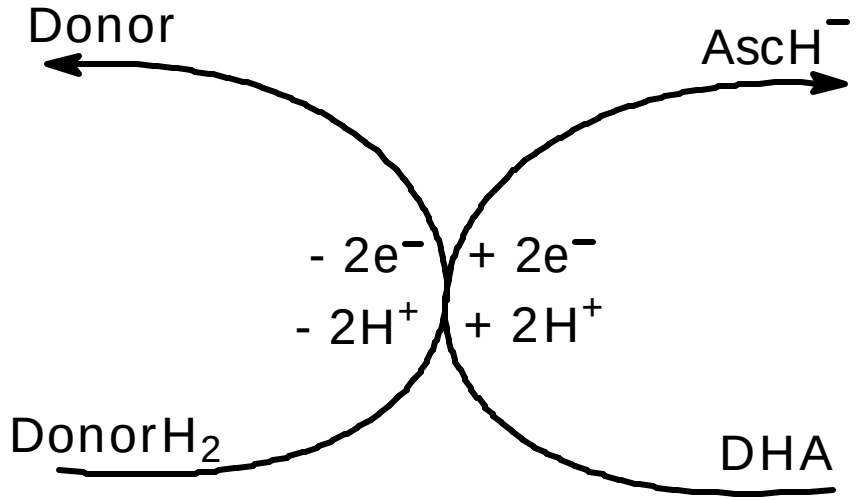
Once oxygen reacts with the lipid chain, the change in dipole moment will cause the peroxyl radical to "float" to the interface.

Buettner GR. (1993) *Arch Biochem Biophys.* **300:**535-543**.**

C and E as Co-Antioxidants (2)



Recycling of Ascorbate



The recycling of ascorbate appears to be an enzyme-dependent process. The two electrons required can come from GSH.

May JM, Qu Z. Li X, (2001) Requirement for GSH in **recycling** of ascorbic acid in endothelial cells. *Biochemical Pharmacology. 62(7):873-81.*

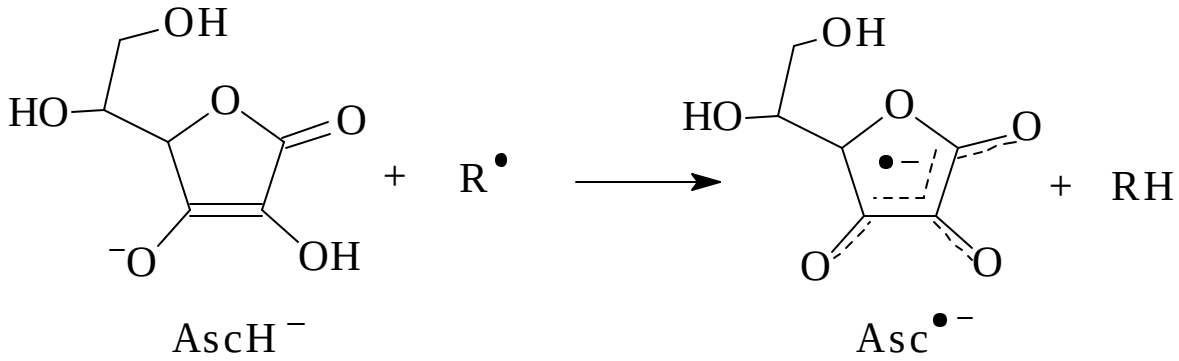
Vethanayagam JG, Green EH,. Rose RC, Bode AM. (1999) Glutathione-dependent **ascorbate recycling** activity of rat serum albumin. *Free Radical Biology & Medicine. 26:1591-8.*

Mendiratta S, Qu ZC, May JM. (1998) Enzyme-dependent **ascorbate recycling** in human erythrocytes: role of thioredoxin reductase. *Free Radical Biology & Medicine. 25:221-8.*

Ascorbate, Summary

Ascorbate is a versatile, water soluble, donor, antioxidant.

Thermodynamically, it can be considered to be the terminal small molecule antioxidant.



AscH⁻ Asc•⁻