

# Ascorbate (Vitamin C), its Antioxidant Chemistry

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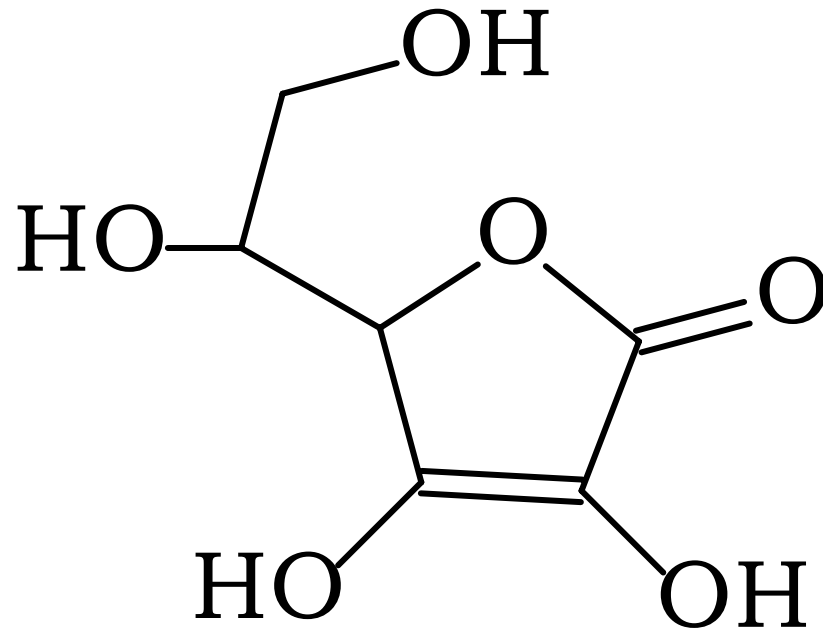
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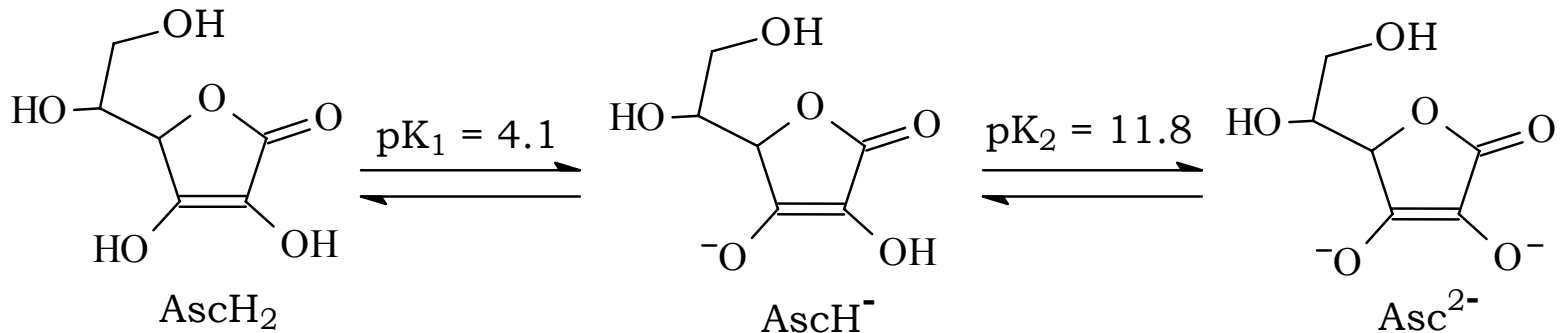
[freya-schafer@uiowa.edu](mailto:freya-schafer@uiowa.edu)

# Ascorbic Acid Structure



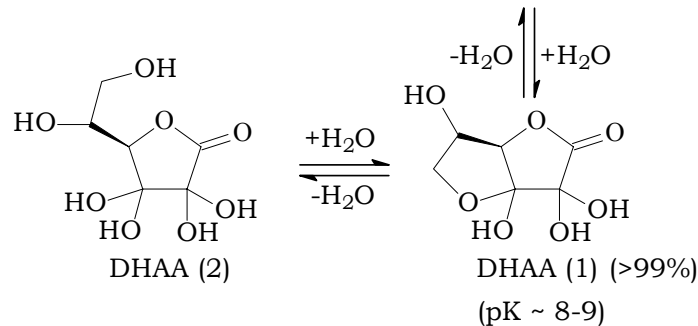
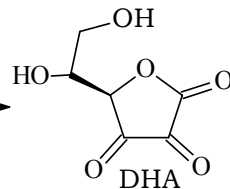
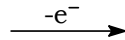
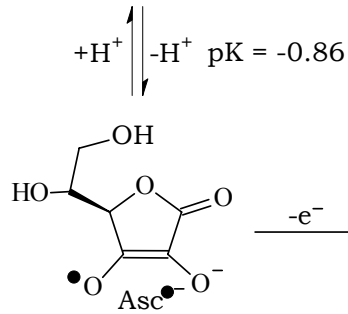
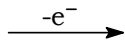
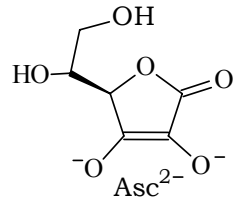
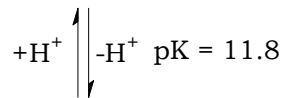
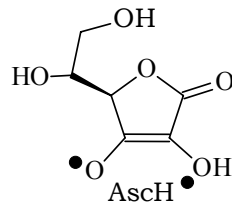
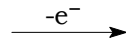
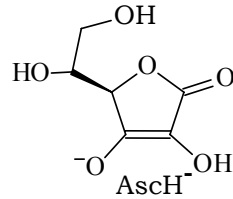
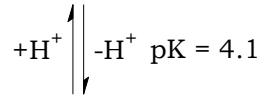
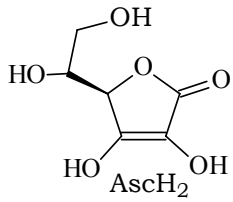
**(AscH<sub>2</sub>)**

# AscH<sub>2</sub> is a Di-acid

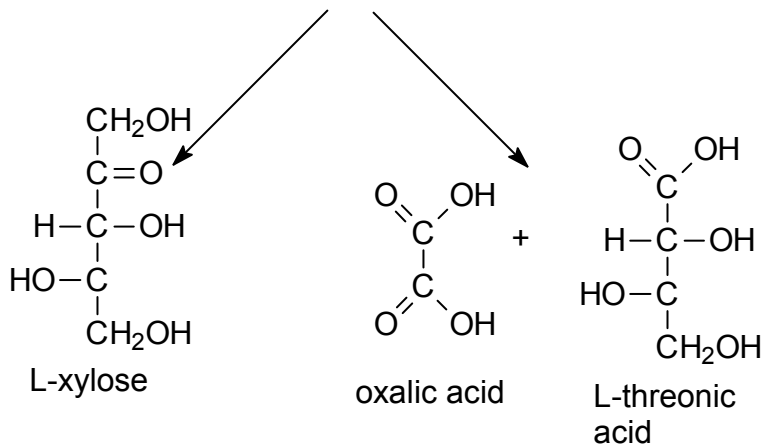
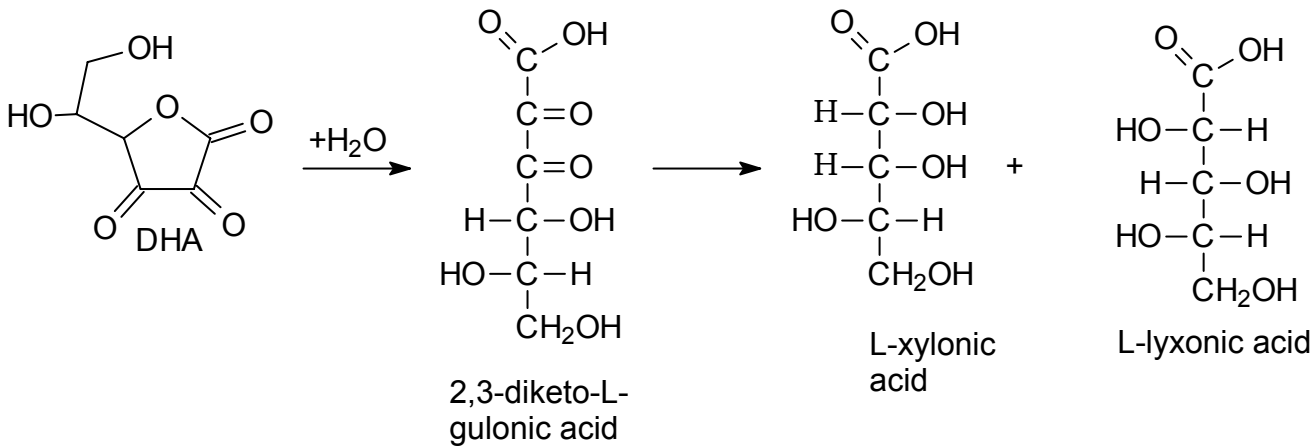
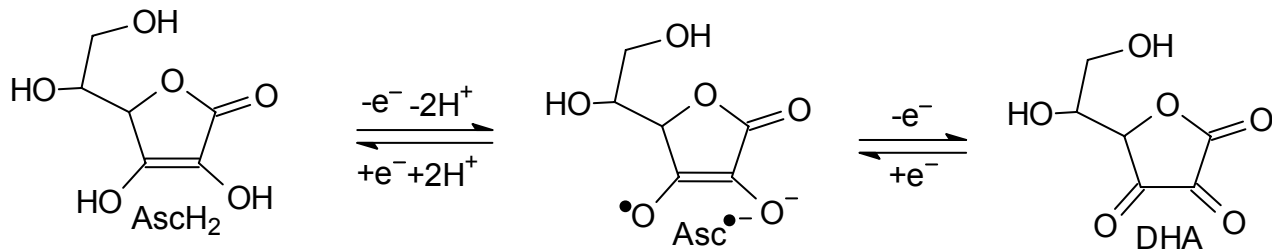


At pH 7.4, 99.95% of vitamin C will be present as AscH<sup>-</sup>; 0.05% as AscH<sub>2</sub> and 0.004% as Asc<sup>2-</sup>. Thus, the antioxidant chemistry of vitamin C is the chemistry of AscH<sup>-</sup>.

# 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. [\[PDF\]](#)

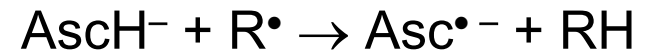


# Ascorbate Falling Apart

# Kinetics of AscH<sup>-</sup> Reactions

Radical	$k_{\text{obs}}/\text{M}^{-1}\text{s}^{-1}$ (pH 7.4)
HO <sup>•</sup>	$1.1 \times 10^{10}$
RO <sup>•</sup> ( <i>tert</i> -butyl alkoxy radical)	$1.6 \times 10^9$
ROO <sup>•</sup> (alkyl peroxy radical, e.g. CH <sub>3</sub> OO <sup>•</sup> )	$1\text{-}2 \times 10^6$
Cl <sub>3</sub> COO <sup>•</sup>	$1.8 \times 10^8$
GS <sup>•</sup> (glutathiy radical)	$6 \times 10^8$ (5.6)
UH <sup>•-</sup> (Urate radical)	$1 \times 10^6$
TO <sup>•</sup> (Tocopheroxyl radical)	$2 \times 10^5$ <sup>b</sup>
Asc <sup>•-</sup> (dismutation)	$2 \times 10^5$
CPZ <sup>•+</sup> (Clorpromazine radical action)	$1.4 \times 10^9$ (5.9)
Fe(III)EDTA/ Fe(II)EDTA	$\approx 10^2$
O <sub>2</sub> <sup>•-</sup> / HO <sub>2</sub> <sup>•</sup>	$2.7 \times 10^5$
Fe(III)Desferal <sup>®</sup> / Fe(II)Desferal <sup>®</sup>	Very slow

These rate constants are for the reaction:



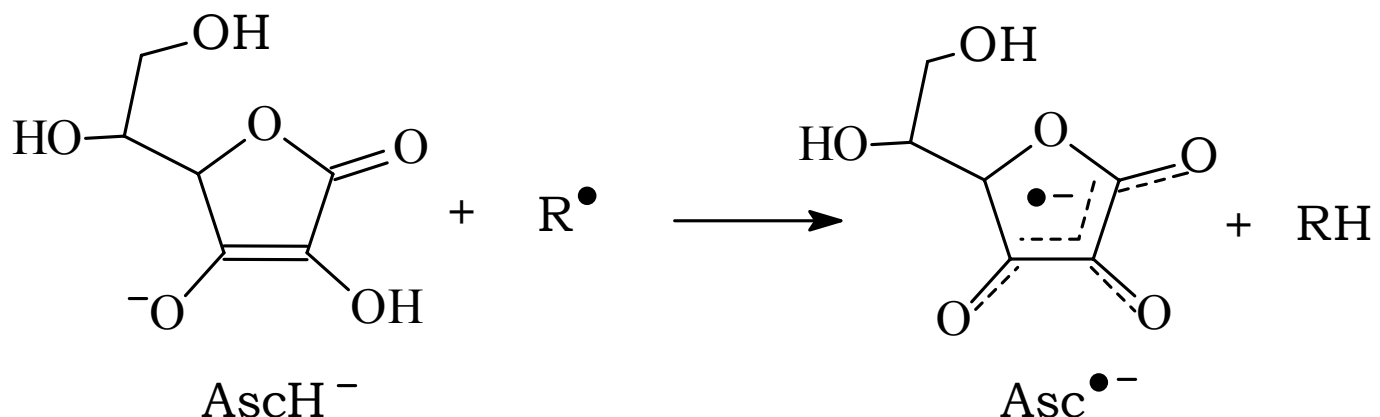
AscH<sup>-</sup> reacts rapidly with these and similar oxidants making it an outstanding donor antioxidant.

<sup>a</sup> Estimated  $k_{\text{obs}}$  for TO<sup>•</sup> when in a biological membrane.

<sup>b</sup>  $k$  is pH dependent, thus this is  $k_{\text{obs}}$  at pH 7.4.

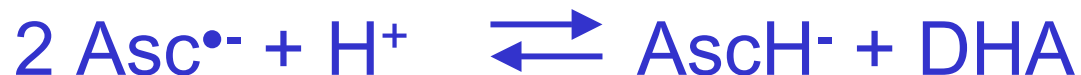
Adapted from: Buettner GR, Jurkiewicz BA. (1996) Catalytic metals, ascorbate, and free radicals: combinations to avoid. *Rad Research* **145**:532-541. [[PDF](#)]

# AscH<sup>-</sup> is a Donor Antioxidant



AscH<sup>-</sup> donates a hydrogen atom (H<sup>•</sup> or H<sup>+</sup> + e<sup>-</sup>) to an oxidizing radical to produce the resonance-stabilized tricarbonyl ascorbate free radical. AscH<sup>•</sup> has a pK<sub>a</sub> of -0.86; thus, it is not protonated in biology and will be present as Asc<sup>•-</sup>.

# Dismutation of Ascorbate Radical



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

**This rate constant increases by a factor of  $\approx 10$  when phosphate is present.\***

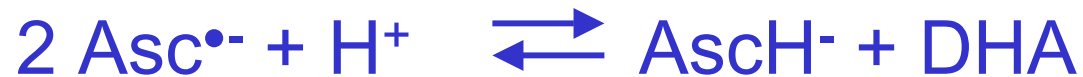
This dismutation reaction is the principal route to the elimination of the  $\text{Asc}^{\bullet-}$  *in vitro*. However, *in vivo* it is thought that reducing enzymes are involved in the removal of this radical, resulting in the recycling of ascorbate.\*\*

\*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. [[PDF](#)]

\*\*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

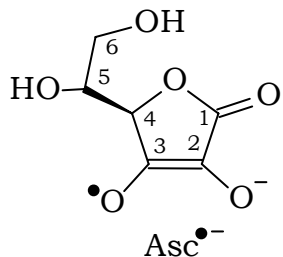


$$K = \frac{[\text{DHA}] [\text{AscH}_2]_{\text{total}}}{[\text{Asc}^{\bullet-}]^2 [\text{H}^+] [1 + \text{H}^+ / K_{\text{AscH}_2}]} = 5 \times 10^{14} \text{ M}^2$$

Because of this equilibrium reaction,  $\text{Asc}^{\bullet-}$  can be formed if both, DHA and  $\text{AscH}^-$  are present. Note that the equilibrium constant,  $K$ , for this dismutation reaction is pH-dependent. Thus, the higher the pH the more  $\text{Asc}^{\bullet-}$  would be formed; however, at higher pH DHA is more unstable. ( $K_{\text{AscH}_2}$  is the first ionization constant of ascorbic acid.)

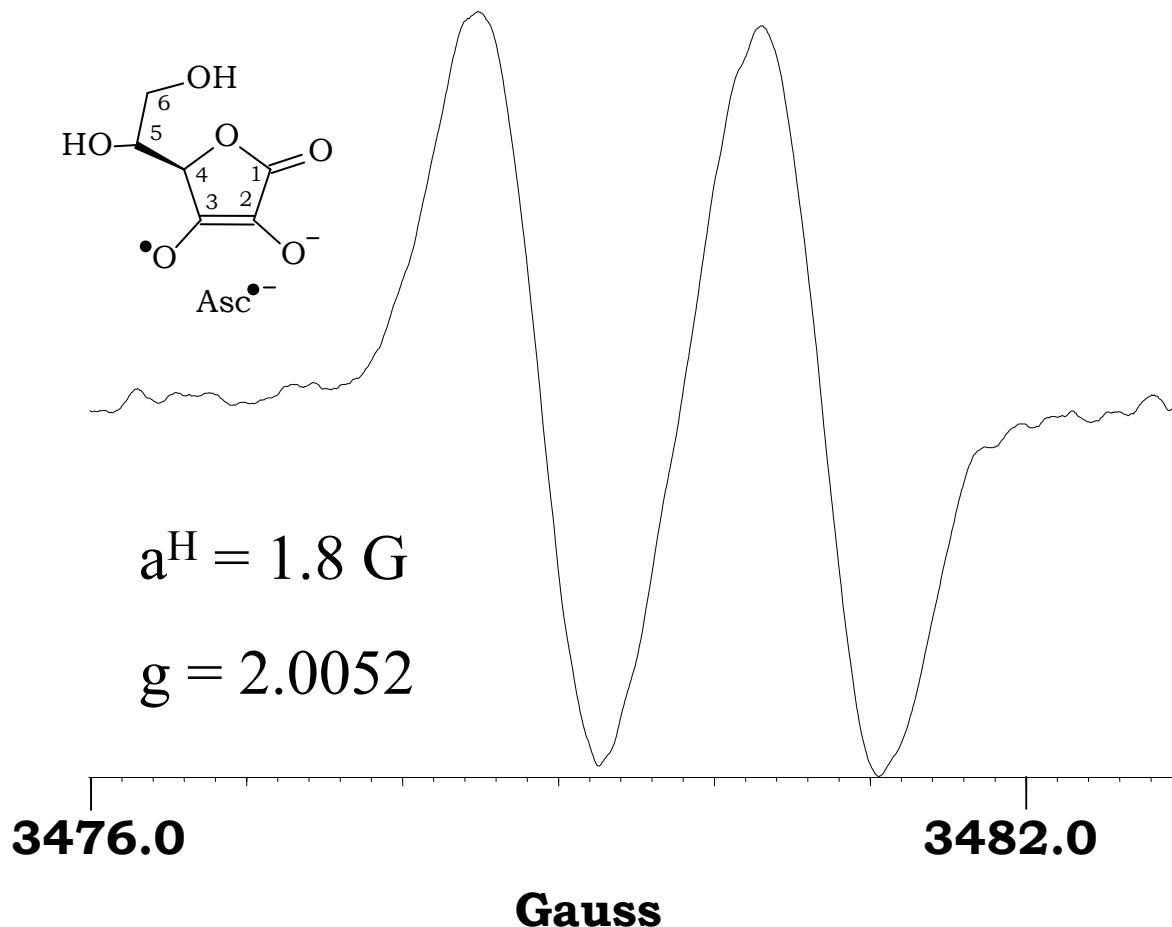
Reviewed in: Bors W, Buettner GR. (1997) The vitamin C radical and its reactions in *Vitamin C in Health and Disease*, ed. by L. Packer and J. Fuchs, Marcel Dekker, Inc., New York, Chapter 4, pp75-94. [[PDF](#)]

# EPR Detection of Asc<sup>•-</sup>



$$a^H = 1.8 \text{ G}$$

$$g = 2.0052$$



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

The intensity of the EPR spectrum of Asc<sup>•-</sup> can be used as an indicator of oxidative stress *in vitro* and *in vivo*.

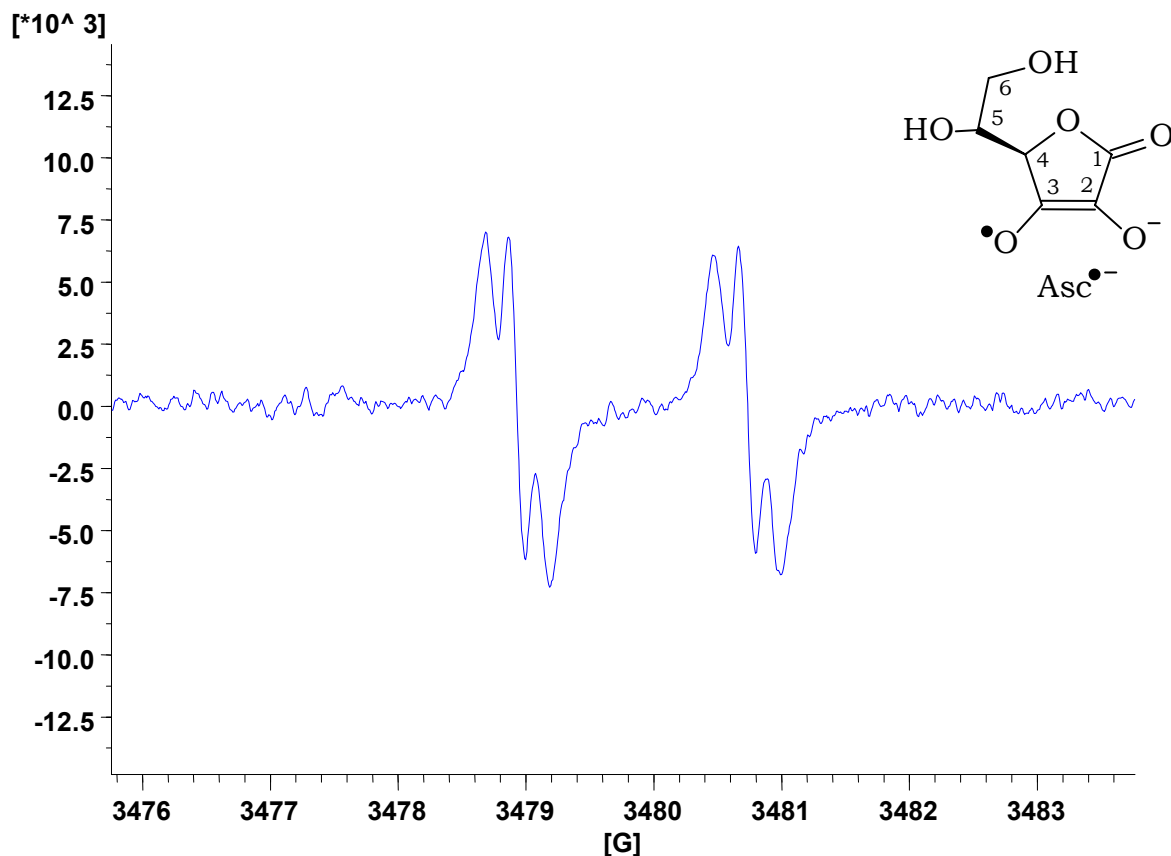
# Higher Resolution EPR

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

$$a^{\text{H}}_4 (1) = 1.76 \text{ G}$$

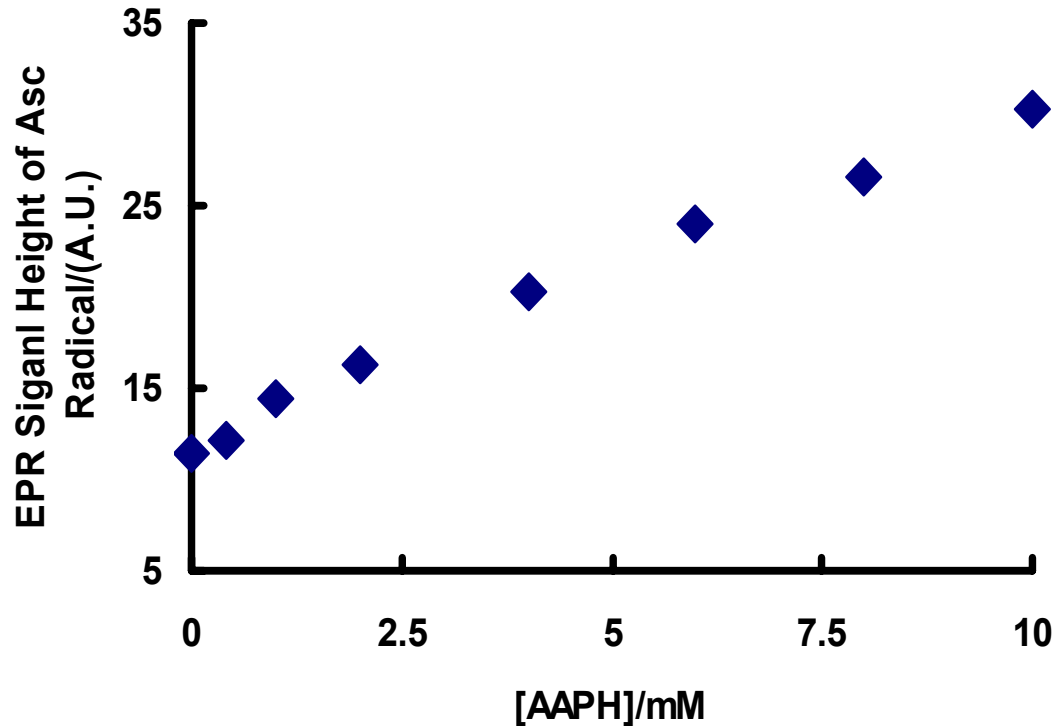
$$a^{\text{H}}_5 (1) = 0.07 \text{ G}$$

$$a^{\text{H}}_6 (2) = 0.19 \text{ G}$$



# Asc<sup>•-</sup>, Real Time Marker of Oxidative Stress

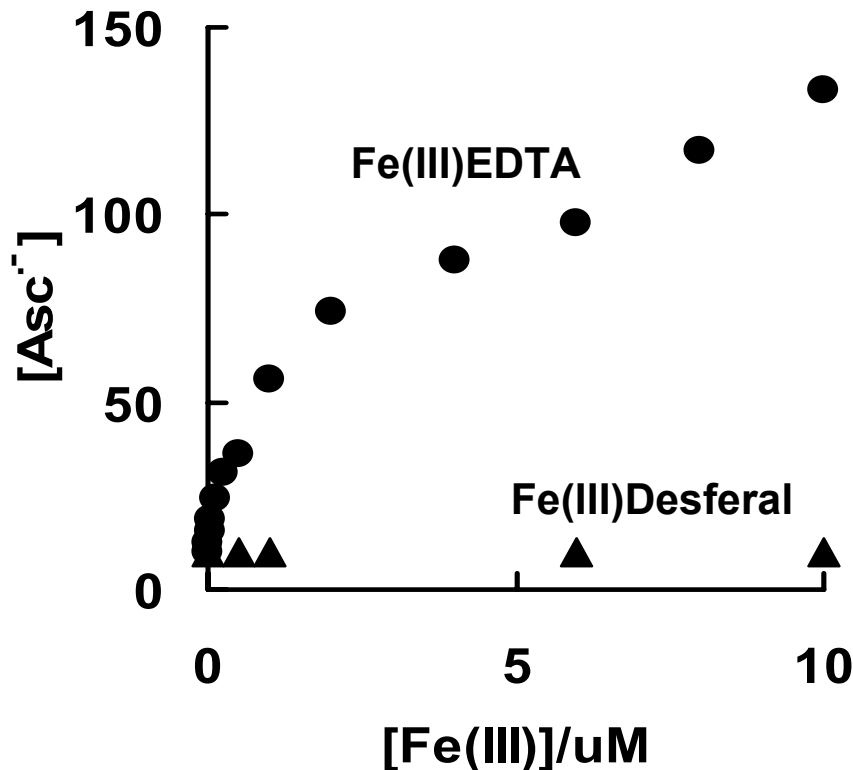
## Ascorbate Radical in Plasma



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

$[\text{Asc}^{\bullet-}]_{\text{ss}}$  in plasma is directly proportional to oxidative flux: EPR signal height of Asc<sup>•-</sup> (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. [[PDF](#)][[DOI](#)]

# Asc<sup>•-</sup>, as an indicator for adventitious transition metals



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

EPR as well as UV-Vis spectroscopy ( $\epsilon_{265} = 14,500 \text{ M}^{-1} \text{ cm}^{-1}$  for Asch<sup>-</sup>) 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. [\[PDF\]](#)

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 [\[PDF\]](#)

# Thermodynamics of Ascorbate

The unpaired electron of  $\text{Asc}^{\bullet-}$  resides in the  $\pi$ -system that includes the tri-carbonyl moiety of ascorbate. This results in a weakly oxidizing and weakly reducing radical. Due to its  $\pi$ -character  $\text{Asc}^{\bullet-}$  does not react with oxygen to form dangerously oxidizing peroxy 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. [[PDF](#)][[DOI](#)]

Buettner GR. (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation,  $\alpha$ -tocopherol, and ascorbate. *Arch Biochem Biophys*. **300**:535-543. [[PDF](#)]

# The Pecking Order

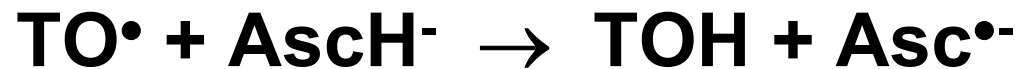
Note that the donor antioxidants are found in the middle of the “pecking order”.

Buettner GR. (1993) The pecking order of free radicals and antioxidants: Lipid peroxidation,  $\alpha$ -tocopherol, and ascorbate. *Arch Biochem Biophys.* **300**:535-543. [[PDF](#)]

Redox Couple (one-electron reductions)	$E^{\circ}/\text{mV}$
$\text{HO}^{\bullet}, \text{H}^+/\text{H}_2\text{O}$	+ 2310
$\text{RO}^{\bullet}, \text{H}^+/\text{ROH}$ (aliphatic alkoxy radical)	+ 1600
$\text{ROO}^{\bullet}, \text{H}^+/\text{ROOH}$ (alkyl peroxy radical)	+ 1000
$\text{GS}^{\bullet}/\text{GS}^-$ (glutathione)	+ 920
$\text{PUFA}^{\bullet}, \text{H}^+/\text{PUFA-H}$ ( <i>bis</i> -allylic-H)	+ 600
<b><math>\text{TO}^{\bullet}, \text{H}^+/\text{TOH}</math> (tocopherol)</b>	<b>+ 480</b>
$\text{H}_2\text{O}_2, \text{H}^+/\text{H}_2\text{O}, \text{HO}^{\bullet}$	+ 320
<b><math>\text{Asc}^{\bullet-}, \text{H}^+/\text{AscH}^-</math> (Ascorbate)</b>	<b>+ 282</b>
$\text{CoQ}^{\bullet-}, 2\text{H}^+/\text{CoQH}_2$	+ 200
$\text{Fe(III) EDTA}/\text{Fe(II) EDTA}$	+ 120
$\text{CoQ}/\text{CoQ}^{\bullet-}$	- 36
$\text{O}_2/\text{O}_2^{\bullet-}$	- 160
$\text{Paraquat}/\text{Paraquat}^{\bullet-}$	- 448
$\text{Fe(III)DFO}/\text{Fe(II)DFO}$	- 450
$\text{RSSR}/\text{RSSR}^{\bullet-}$ (GSH)	- 1500
$\text{H}_2\text{O}/\text{e}^-_{\text{aq}}$	- 2870

# C and E as Co-antioxidants

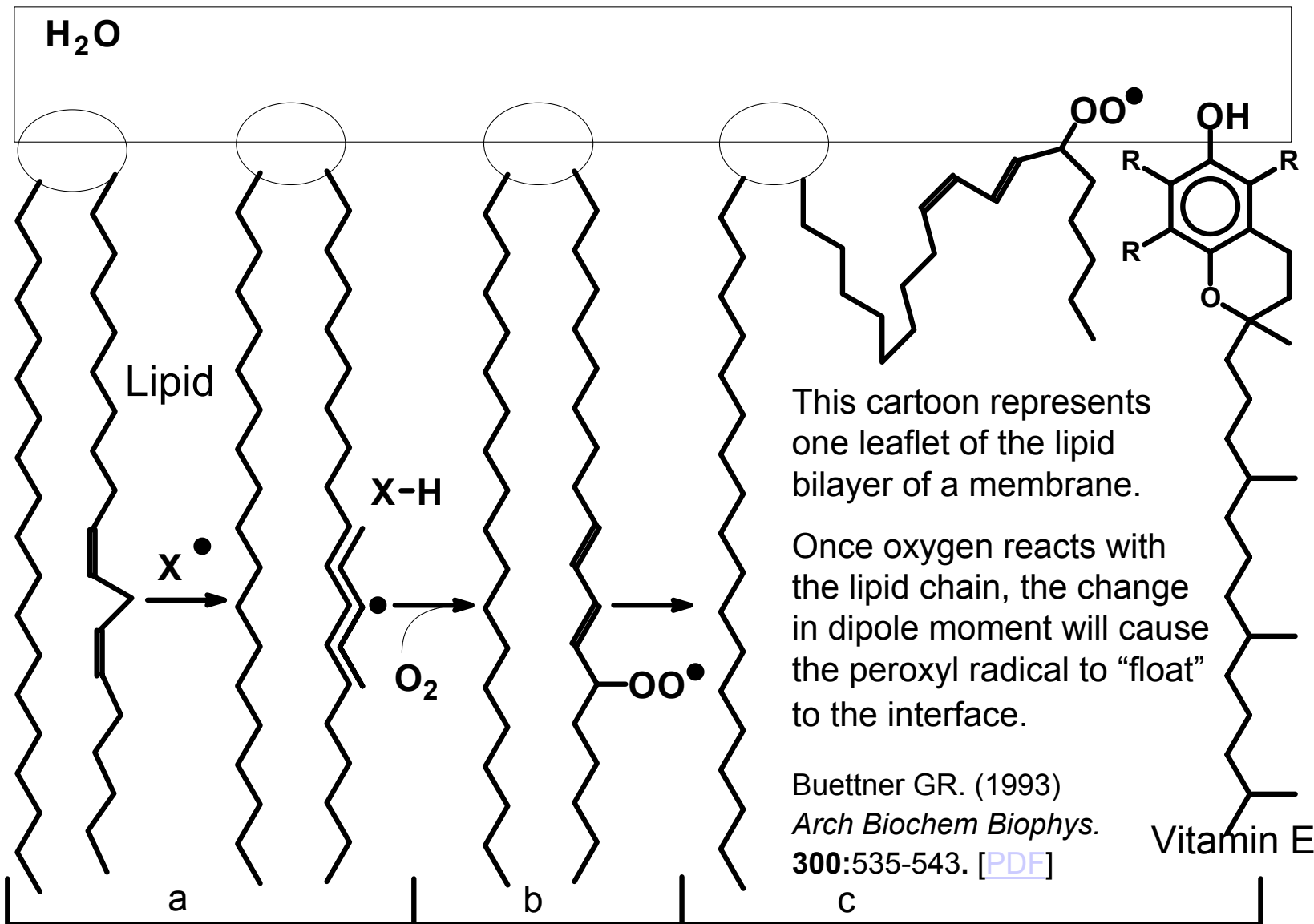
As seen in the thermodynamic pecking order above, the tocopherol radical,  $\text{TO}^\bullet$ , is more oxidizing than  $\text{Asc}^{\bullet-}$ . It is thought that ascorbate contributes to the recycling of  $\text{TO}^\bullet$  back to  $\text{TOH}$ .



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



# C and E as Co-Antioxidants (1)



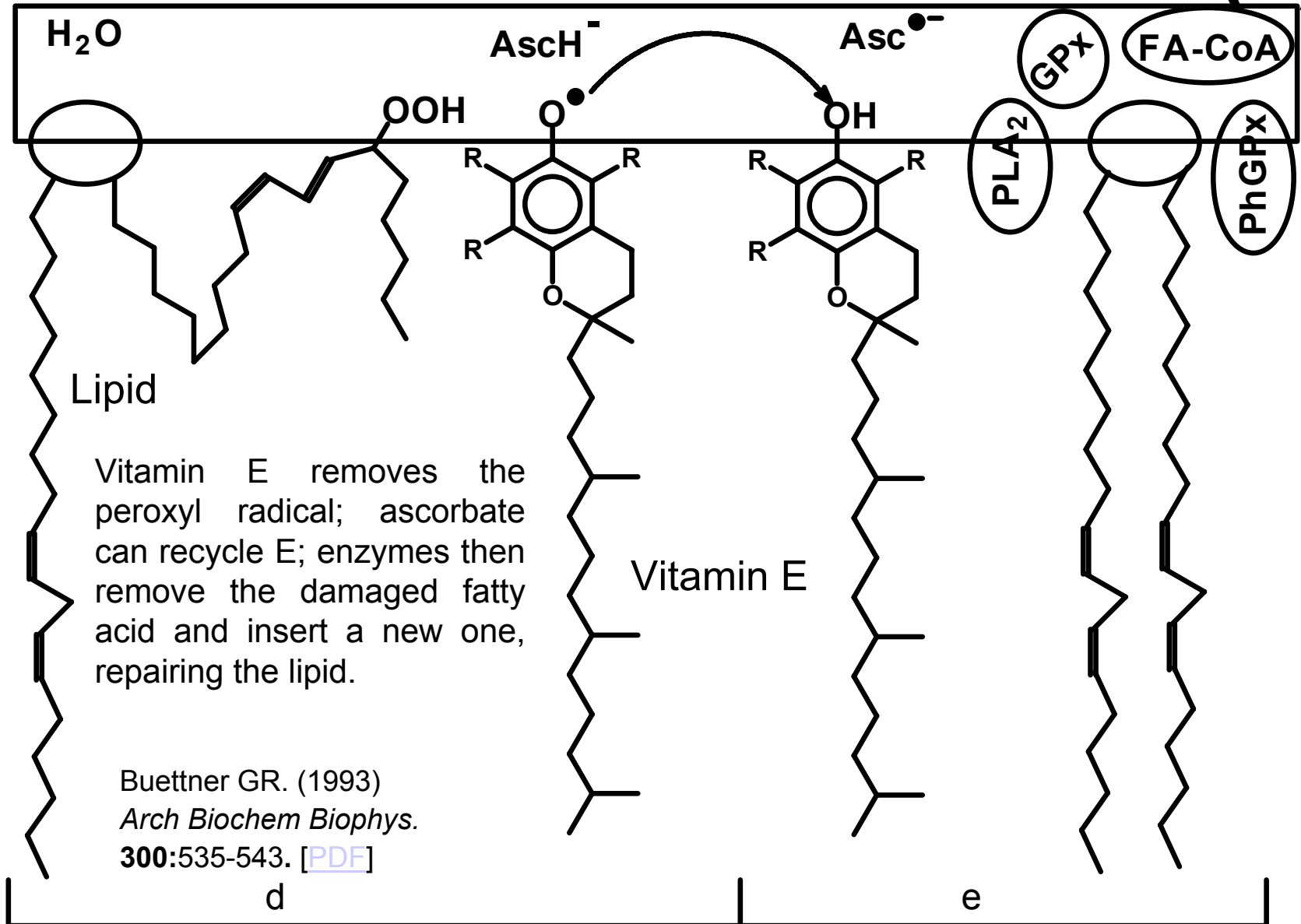
This cartoon represents one leaflet of the lipid bilayer of a membrane.

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

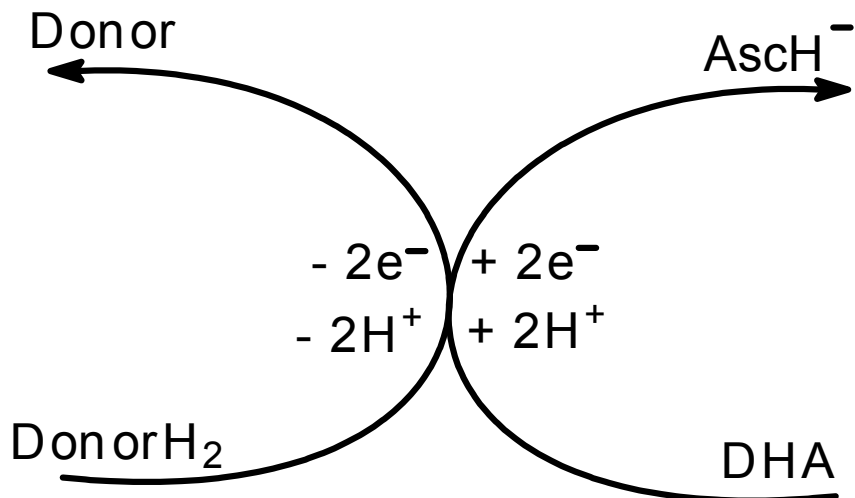
Buettner GR. (1993)  
*Arch Biochem Biophys.*  
**300**:535-543. [[PDF](#)]

Vitamin E

# 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.

