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# Ascorbate (Vitamin C), its Antioxidant Chemistry

**Garry R. Buettner and Freya Q. Schafer**

Free Radical and Radiation Biology Program

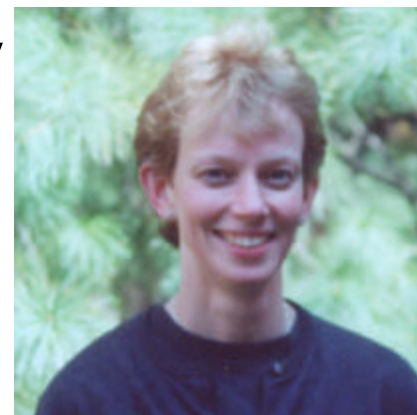


Department of Radiation Oncology

The University of Iowa

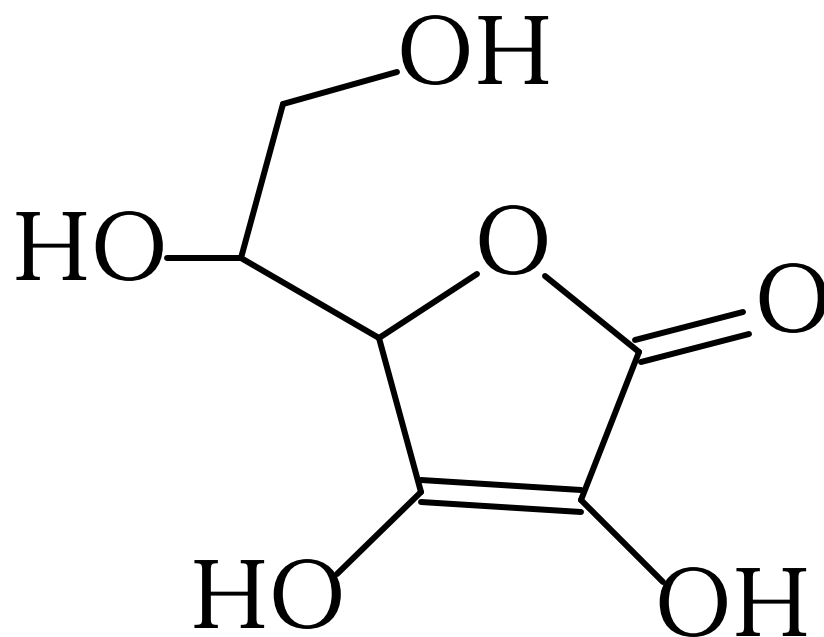
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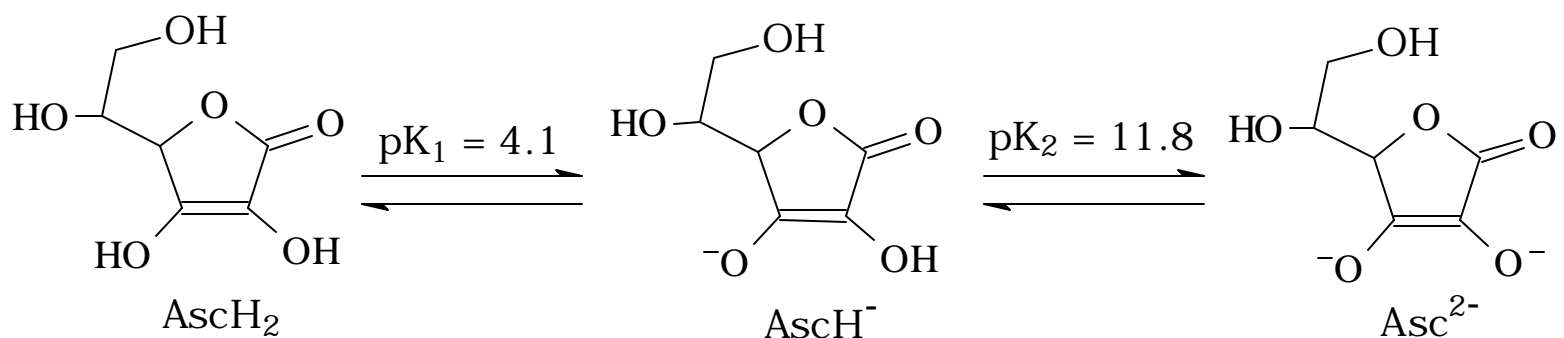
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[freya-schafer@uiowa.edu](mailto:freya-schafer@uiowa.edu)

# Ascorbic Acid Structure



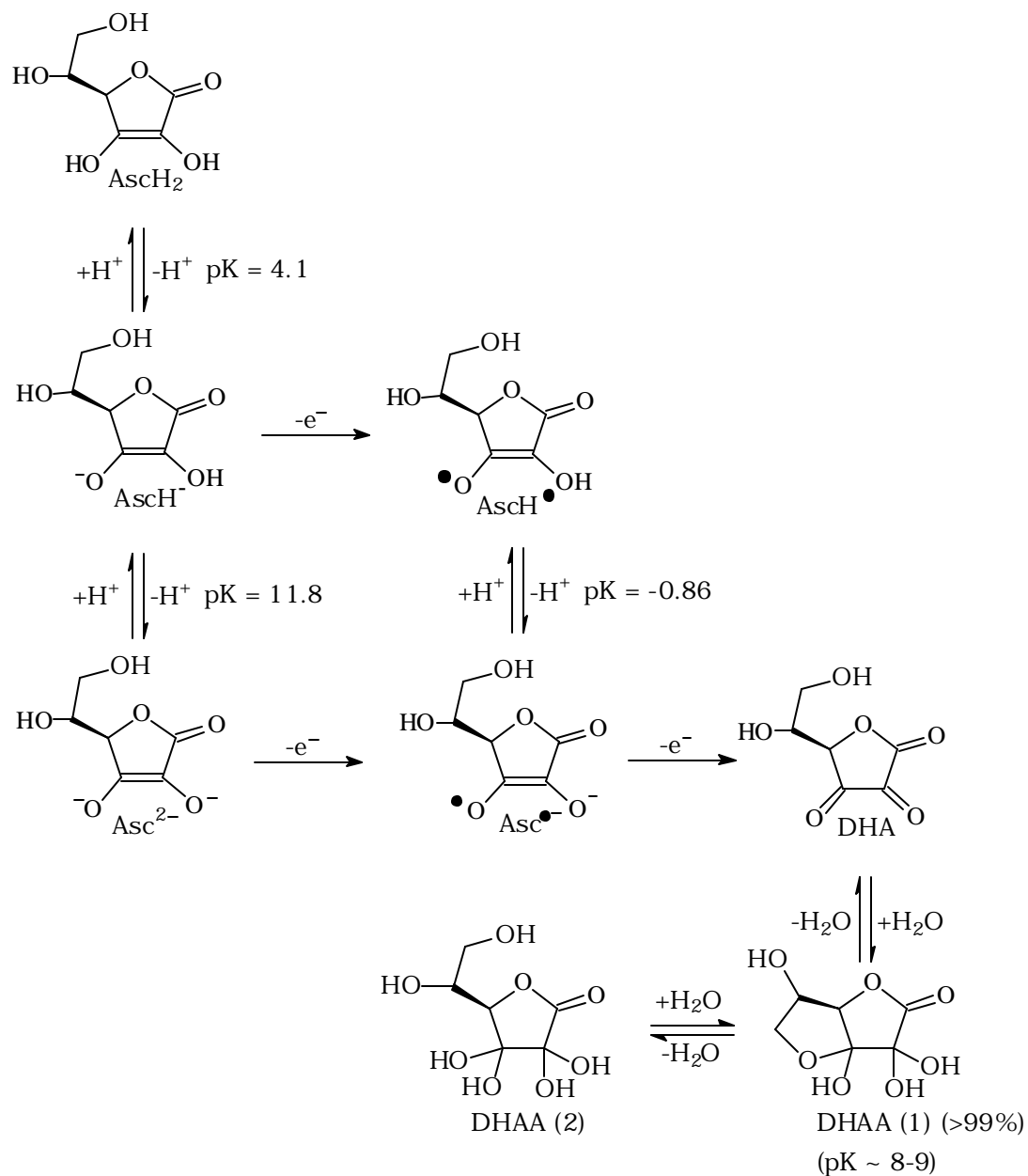
**(AscH<sub>2</sub>)**

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

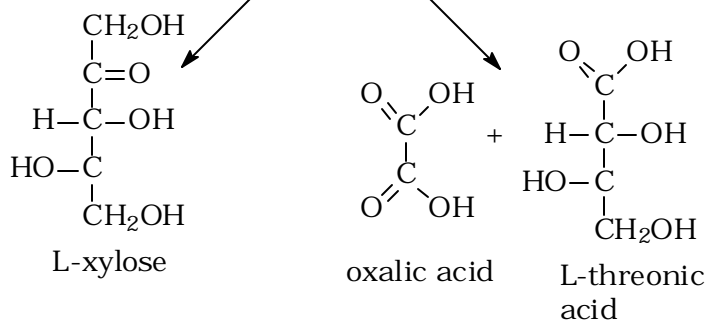
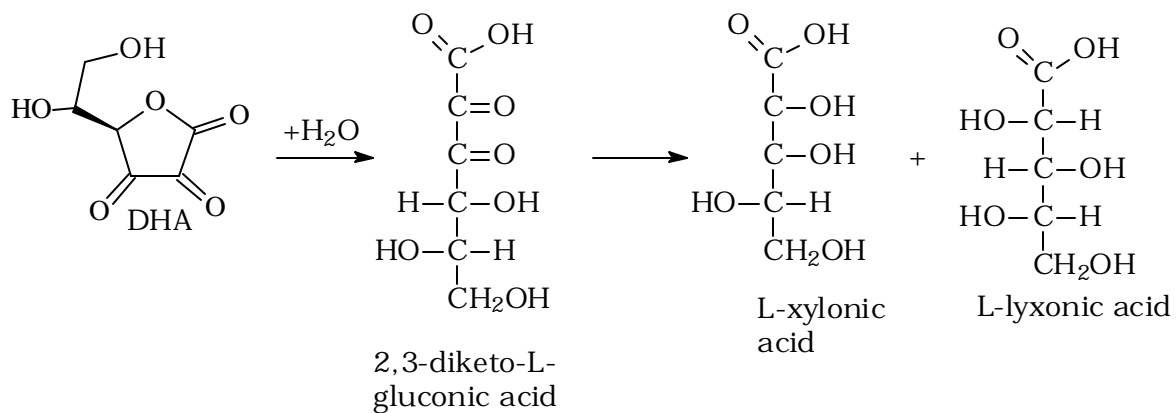
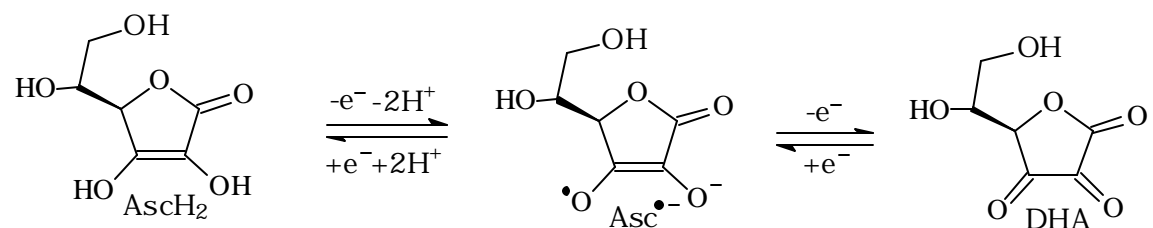


At pH 7.4, 99.95% of vitamin C will be present as AscH<sup>-</sup>; 0.05% as AscH<sub>2</sub> and 0.004% as Asc<sup>2-</sup>. Thus, the antioxidant chemistry of vitamin C is the chemistry of AscH<sup>-</sup>.

# 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.

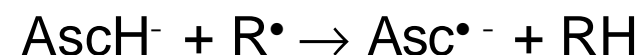


# Ascorbate Falling Apart

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

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

These rate constants are for the reaction:



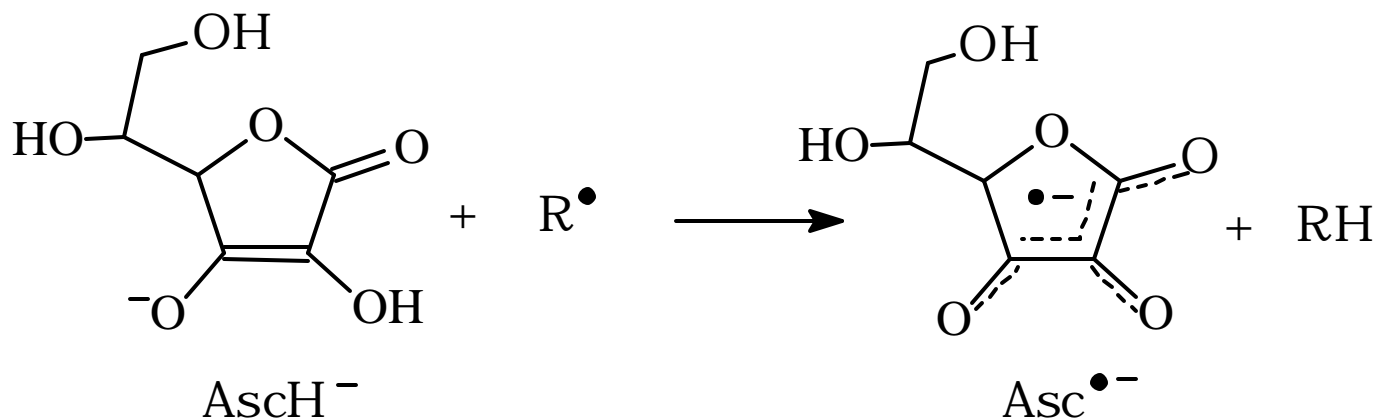
AsCH<sup>-</sup> reacts rapidly with these and similar oxidants making it an outstanding donor antioxidant.

<sup>a</sup> Estimated k<sub>obs</sub> for TO• when in a biological membrane.

<sup>b</sup> k is pH dependent, thus this is k<sub>obs</sub> 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.

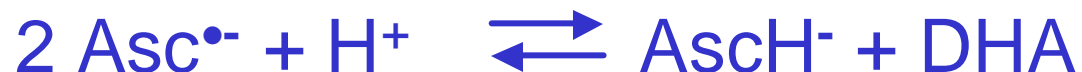
# AscH<sup>-</sup> is a Donor Antioxidant



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



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

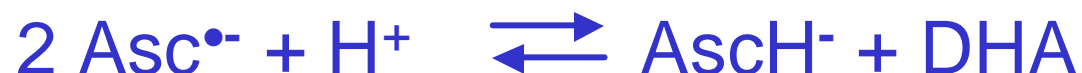
**This rate constant increases by a factor of »10 when phosphate is present.\***

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.

\*\*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

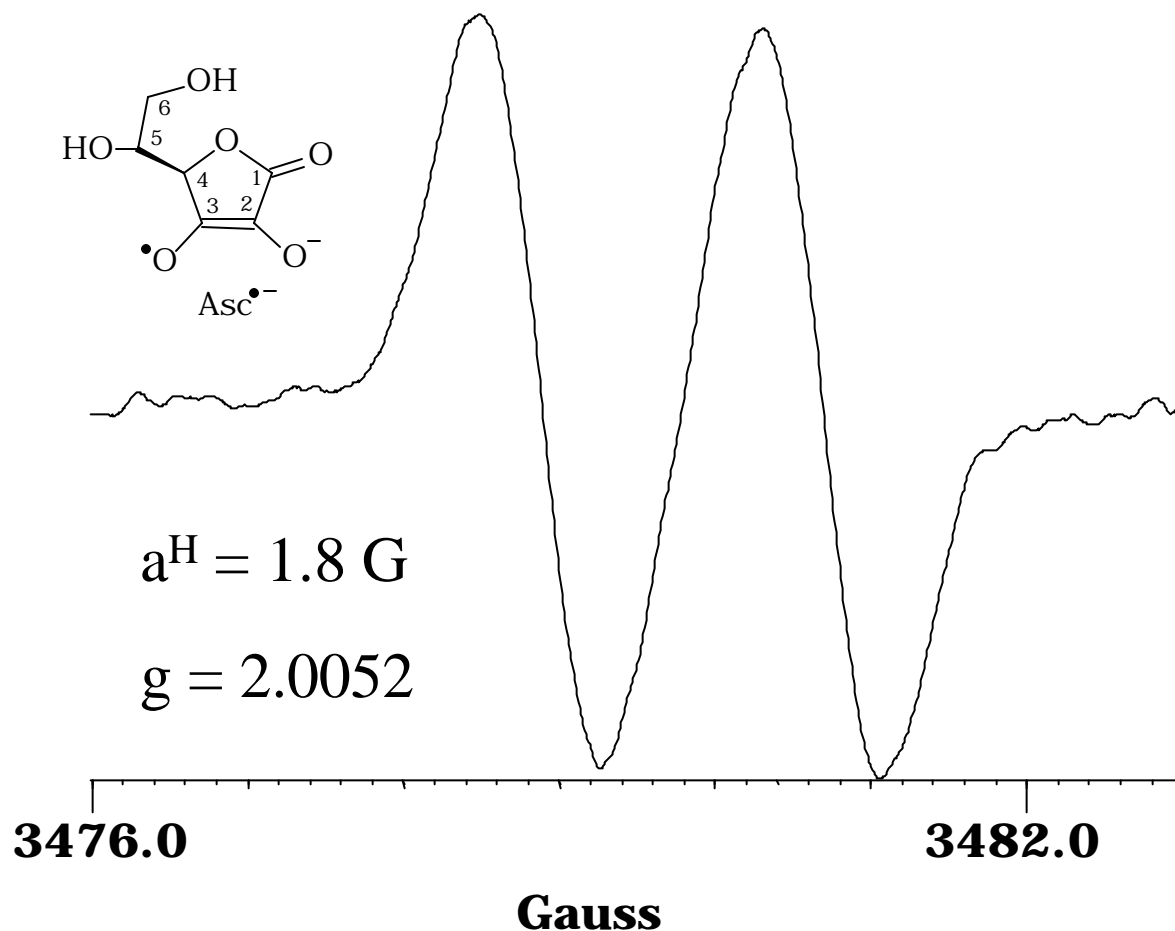


$$K = \frac{[\text{DHA}] [\text{AscH}_2]_{\text{total}}}{[\text{Asc}^{\bullet-}]^2 [\text{H}^+] [1 + \text{H}^+/K_{\text{AscH}_2}]} = 5 \times 10^{14} \text{ M}^2$$

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.

# EPR Detection of Asc<sup>•-</sup>



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*.

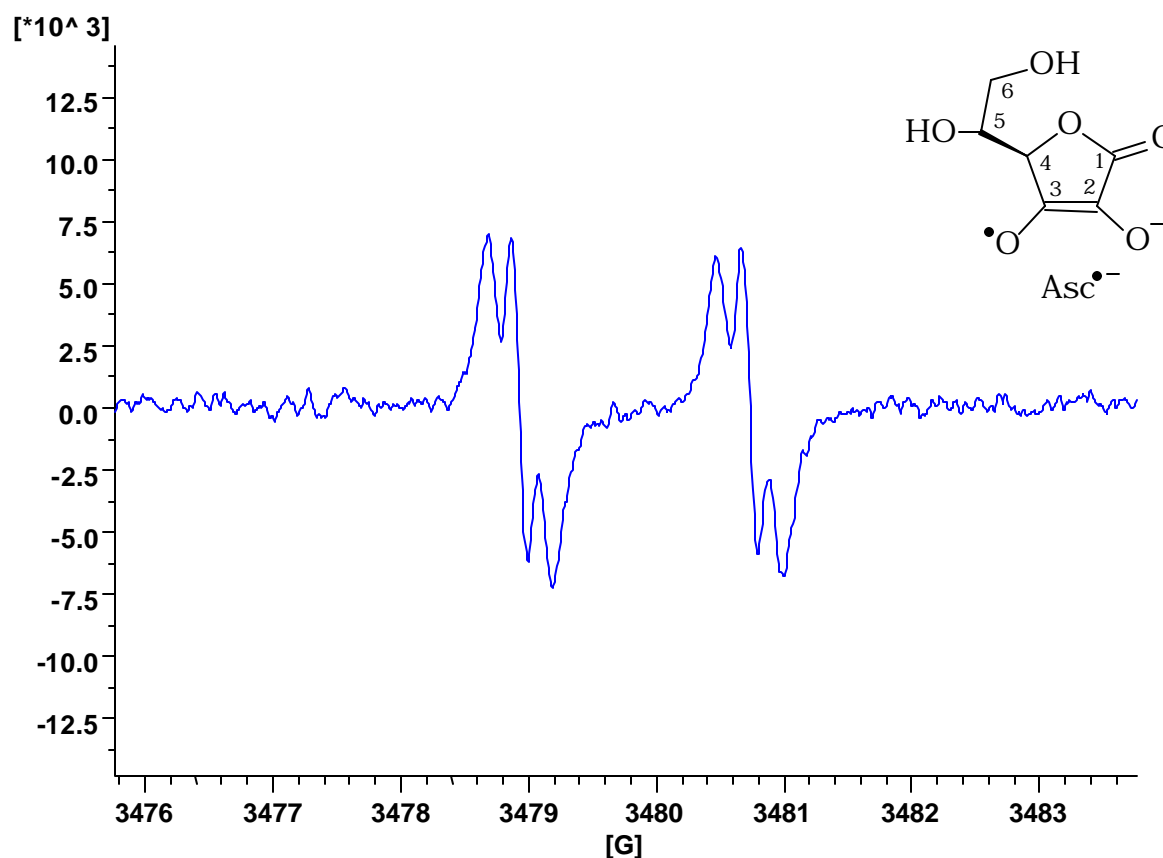
# Higher Resolution EPR

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

$$a^H_4 (1) = 1.76 \text{ G}$$

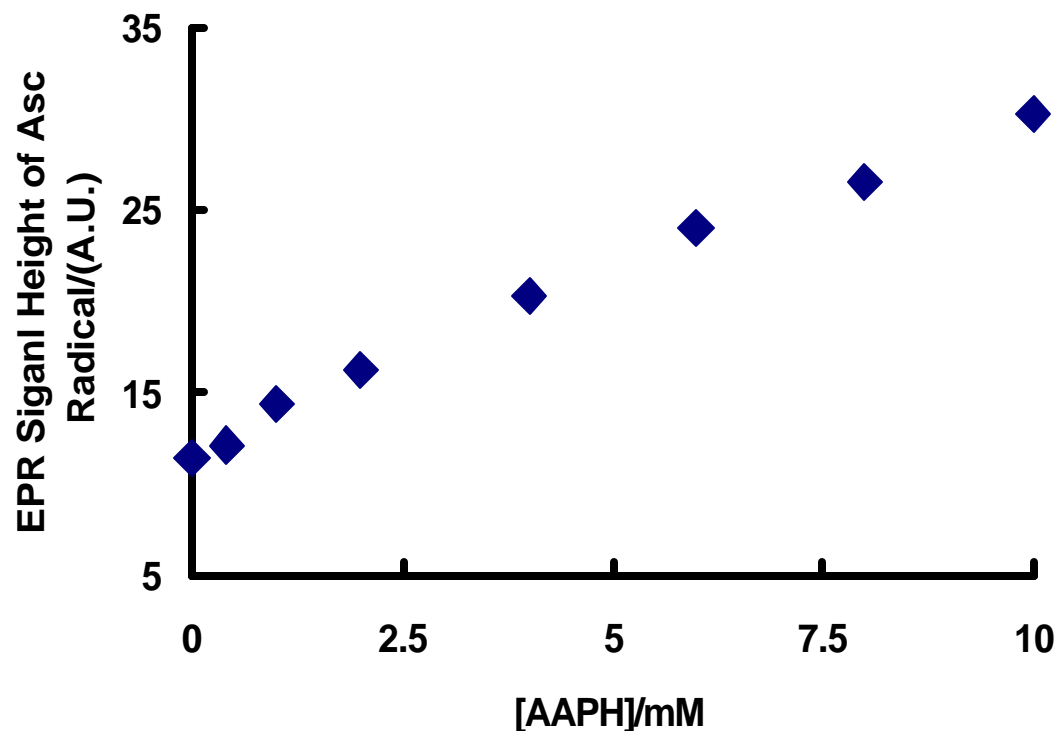
$$a^H_5 (1) = 0.07 \text{ G}$$

$$a^H_6 (2) = 0.19 \text{ G}$$



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

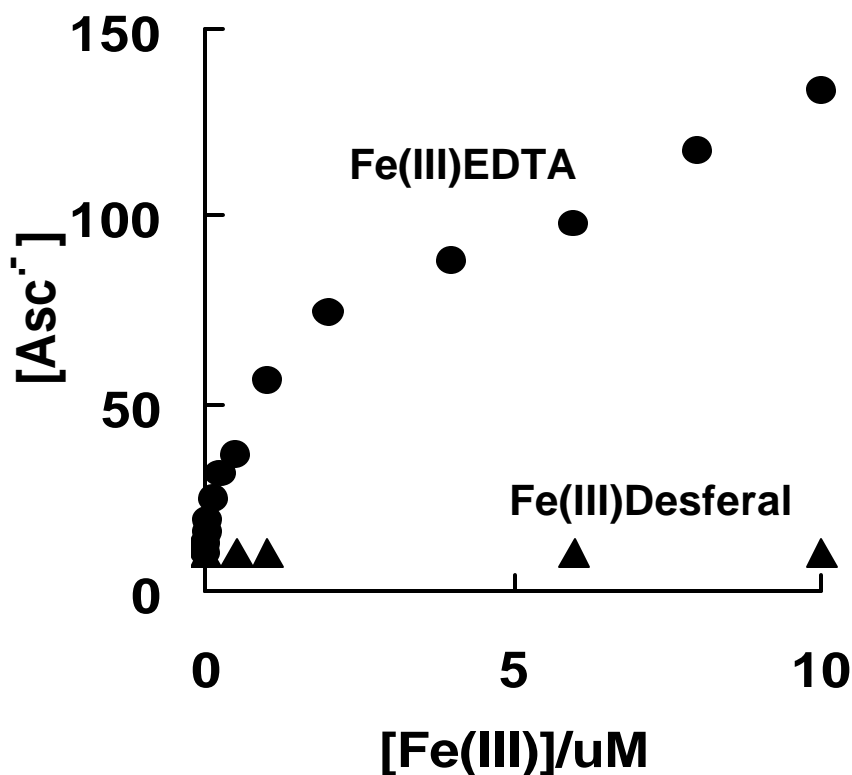
## Ascorbate Radical in Plasma



$[\text{Asc}\bullet^-]_{\text{ss}}$  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  $\text{Asc}\bullet^-$  (arbitrary units) versus AAPH concentration. The solutions contained 58  $\mu\text{M}$  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<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  $[\text{Asc}^{\bullet-}]_{ss}$  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 Ascorbic acid oxidation. In contrast Fe(III) in Desferal is not accessible to reductants and thus Fe(III)Desferal does not catalyze Ascorbic acid oxidation.

EPR as well as UV-Vis spectroscopy ( $\epsilon_{265} = 14,500 \text{ M}^{-1} \text{ cm}^{-1}$  for Ascorbic acid) 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  $\text{Asc}^{\bullet-}$  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.

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

# 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.

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



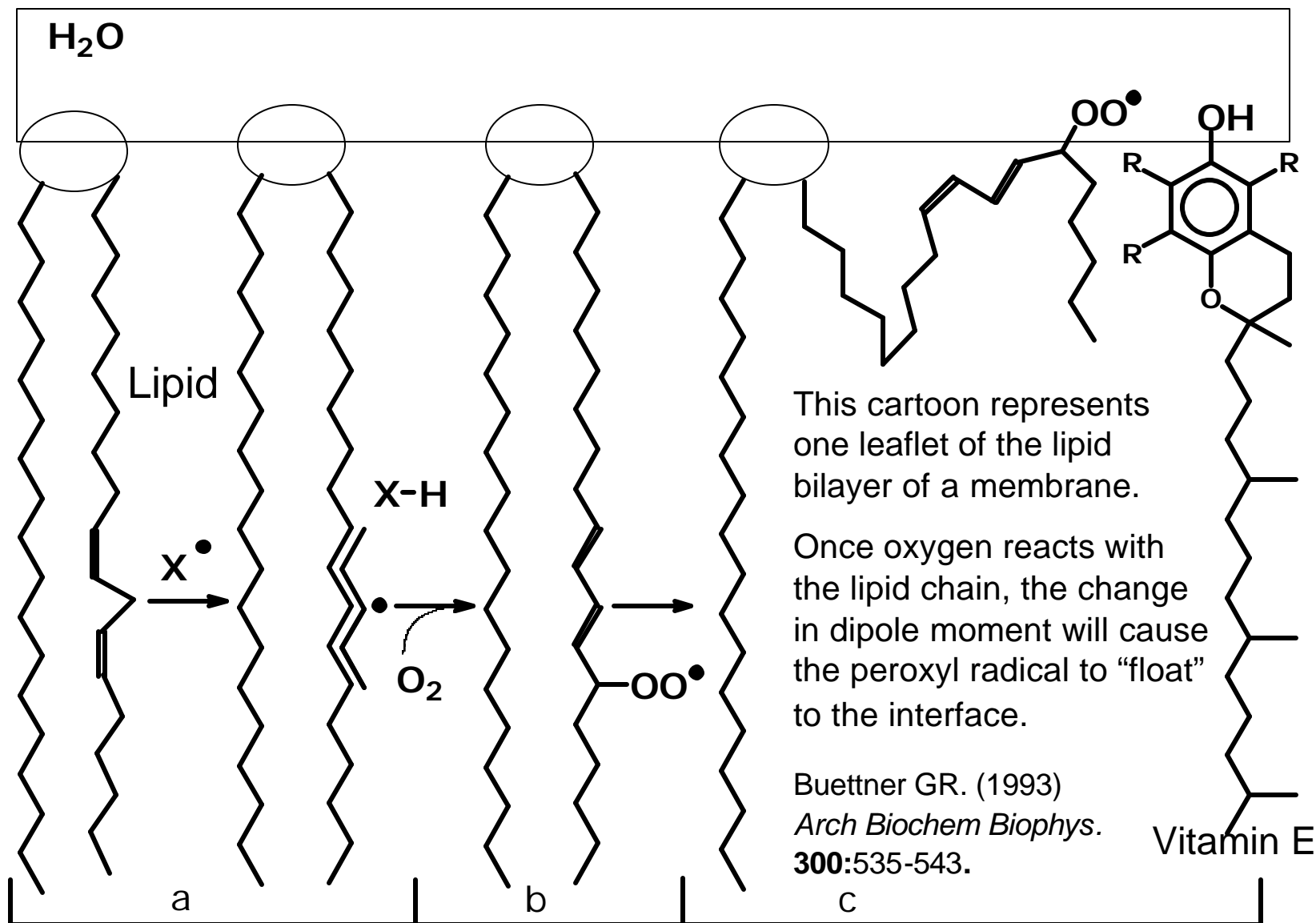
# C and E as Co-antioxidants

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  $\text{TOH}$ .

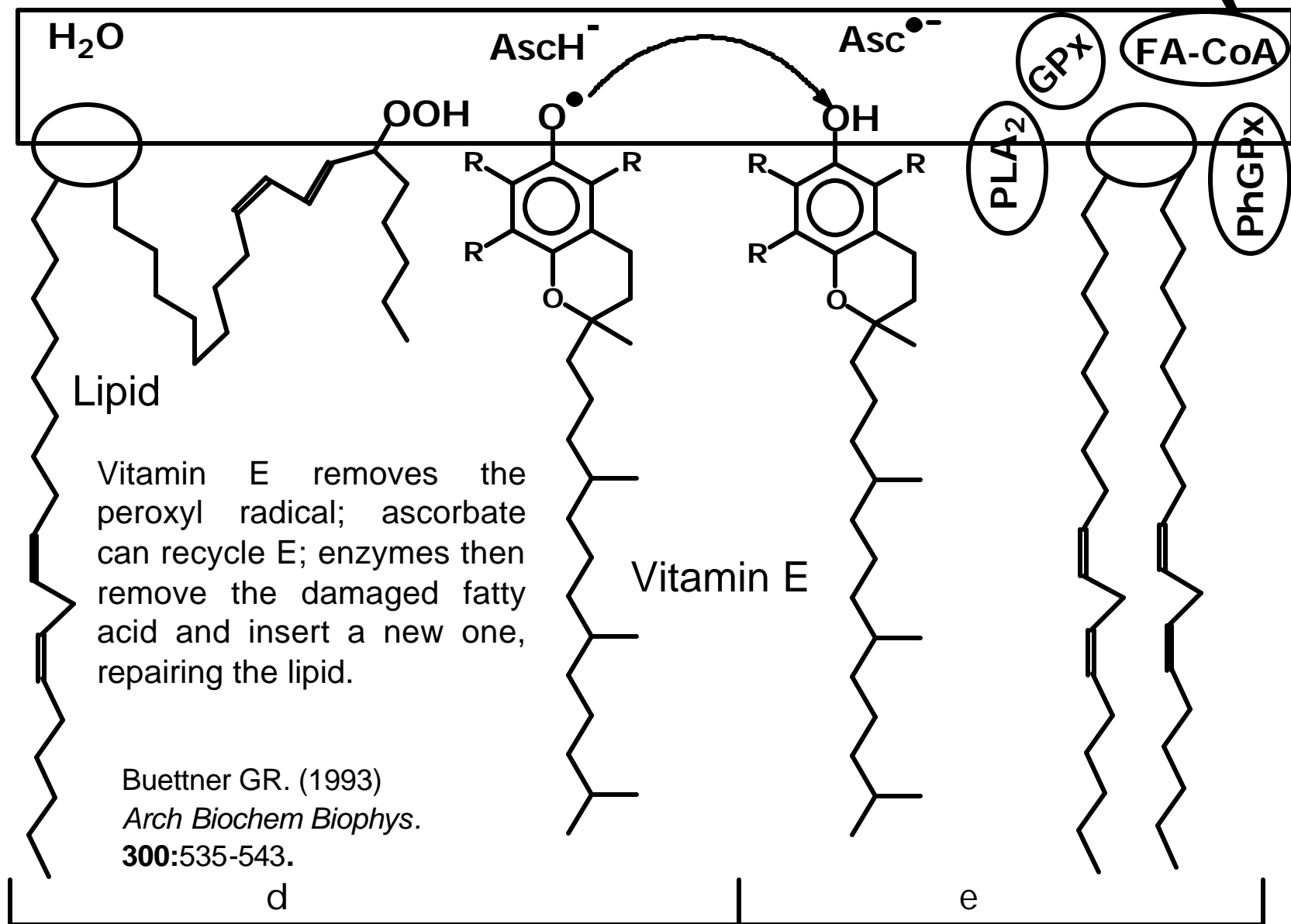


This mechanism is clearly important in protecting LDL from unwanted oxidations, because LDL lacks enzymes that could recycle  $\text{TO}^\bullet$ . But its importance in cells and tissues is still being debated.

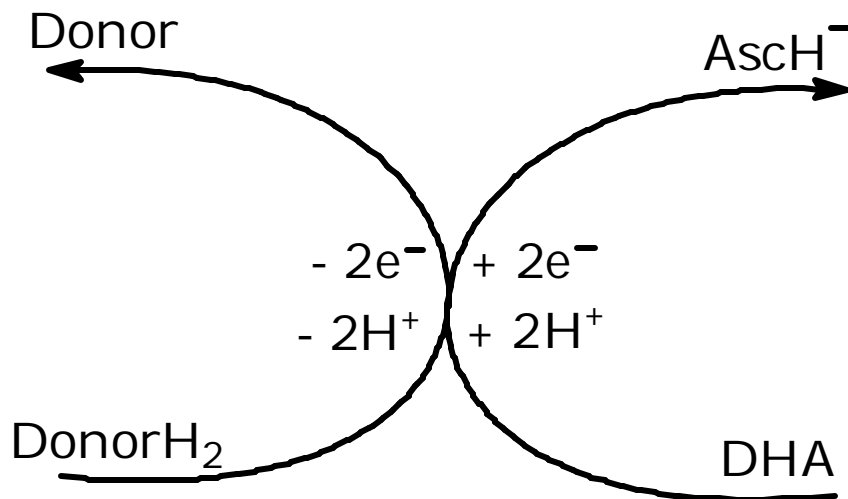
# C and E as Co-Antioxidants (1)



# 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.

