

The Virtual Free Radical School

Ascorbate (Vitamin C), its Antioxidant Chemistry

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Free Radical and Radiation Biology Program

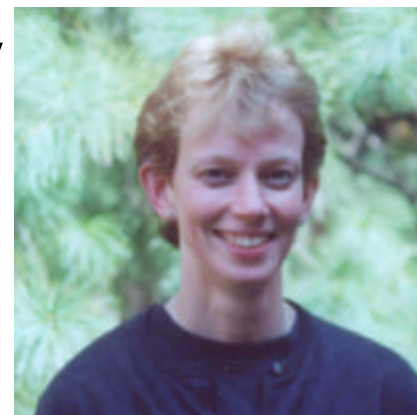


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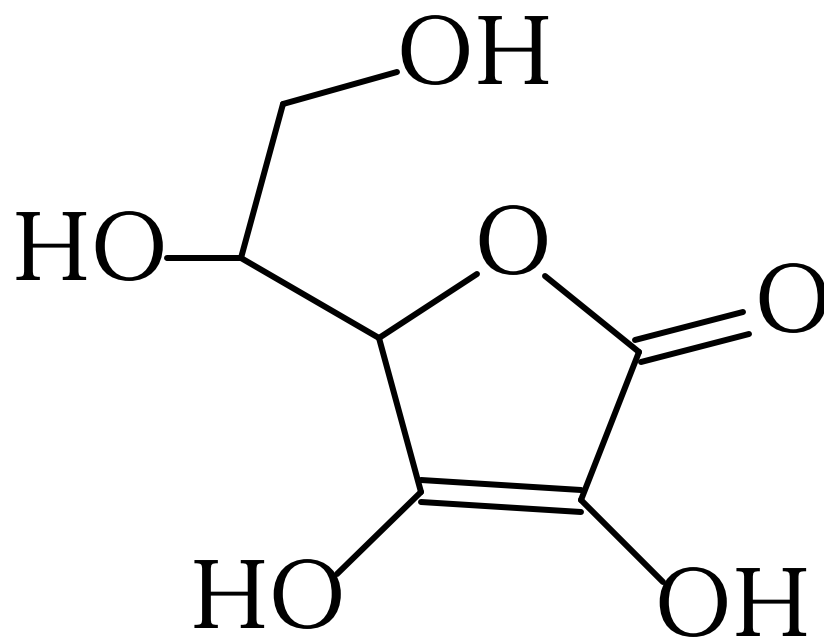
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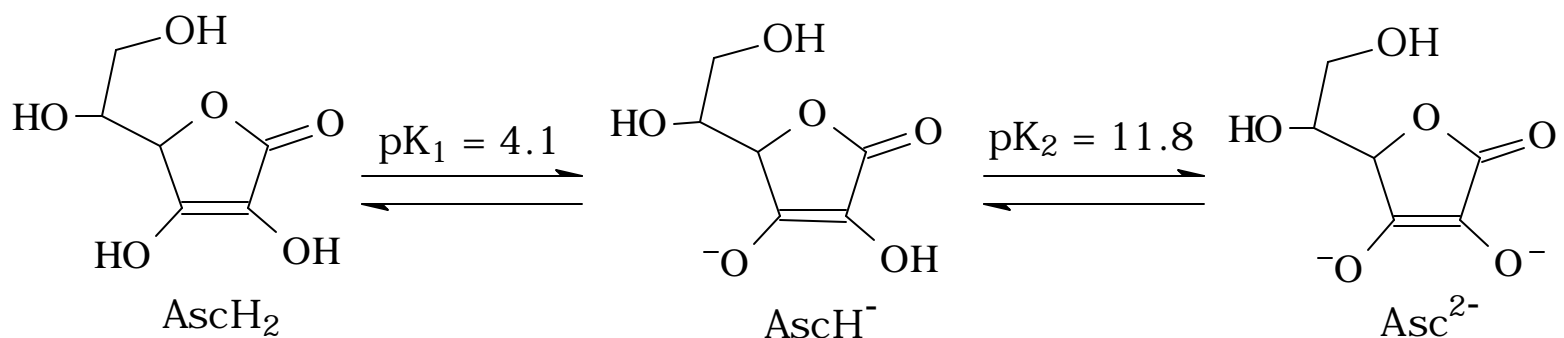
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Ascorbic Acid Structure



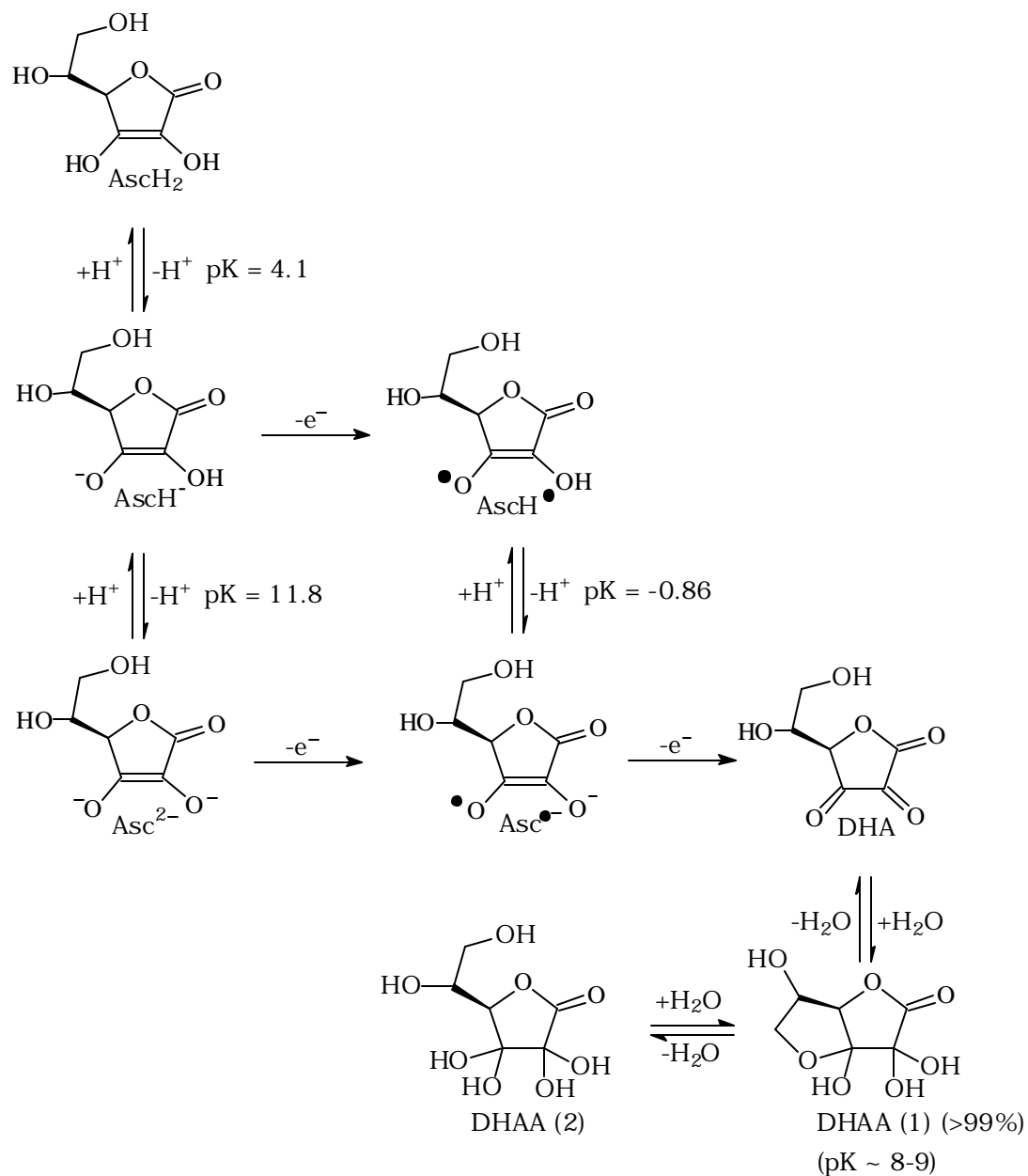
(AscH₂)

AscH₂ is a Di-acid

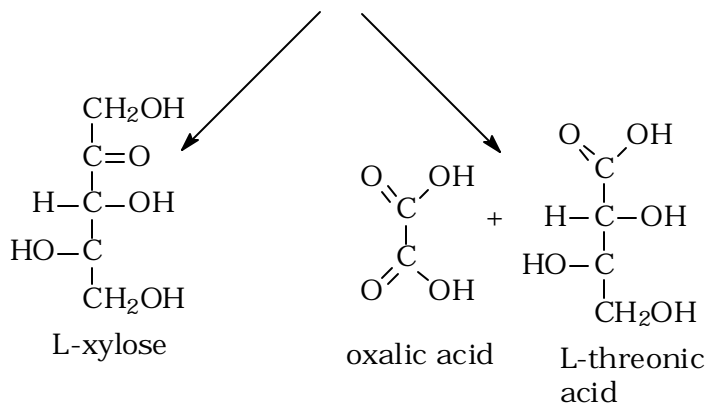
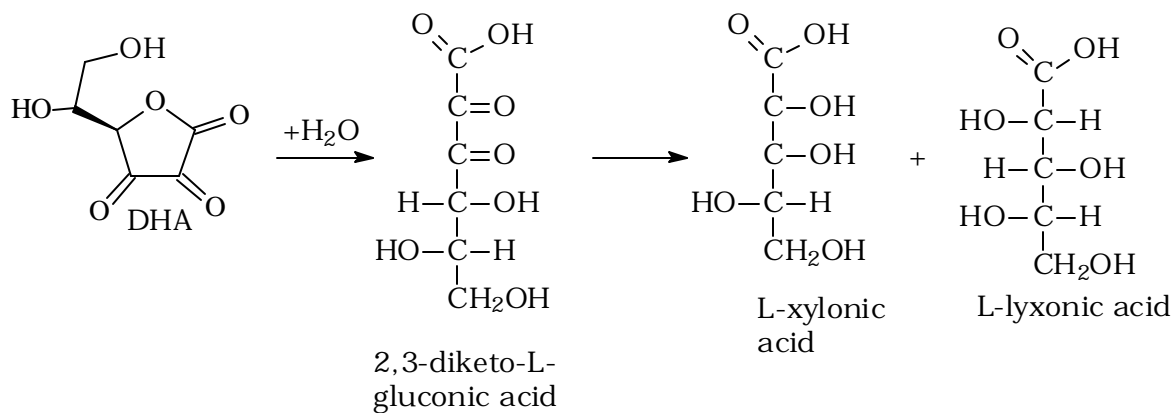
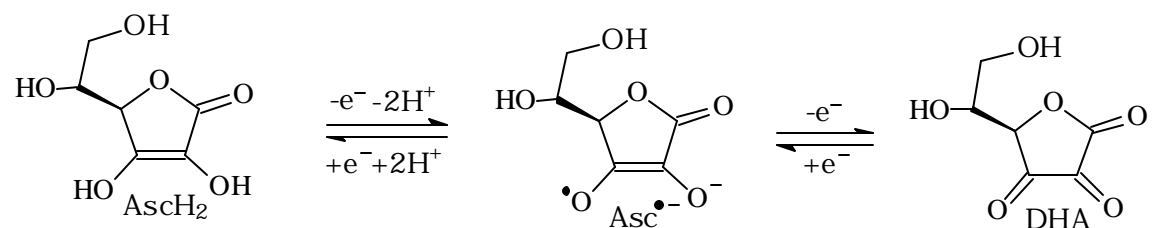


At pH 7.4, 99.95% of vitamin C will be present as AscH⁻; 0.05% as AscH₂ and 0.004% as Asc²⁻. Thus, the antioxidant chemistry of vitamin C is the chemistry of AscH⁻.

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.

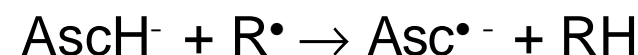


Ascorbate Falling Apart

Kinetics of AsCH⁻ Reactions

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

These rate constants are for the reaction:



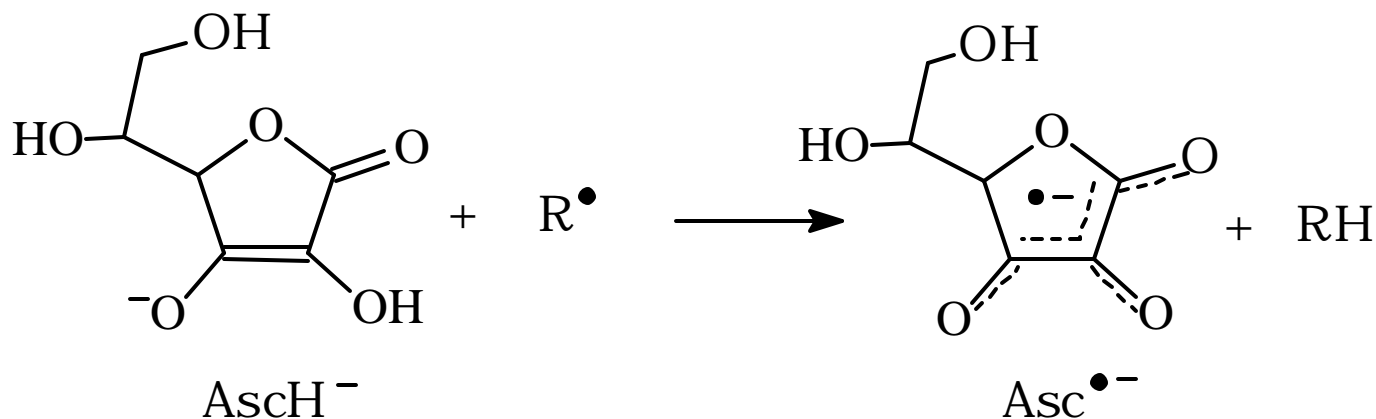
AsCH⁻ reacts rapidly with these and similar oxidants making it an outstanding donor antioxidant.

^a Estimated k_{obs} for TO• when in a biological membrane.

^b k is pH dependent, thus this is k_{obs} at pH 7.4.

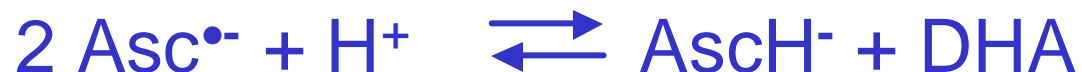
Adapted from: Buettner GR, Jurkiewicz BA. (1996) Catalytic metals, ascorbate, and free radicals: combinations to avoid. *Rad Research* **145**:532-541.

AscH⁻ is a Donor Antioxidant



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

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

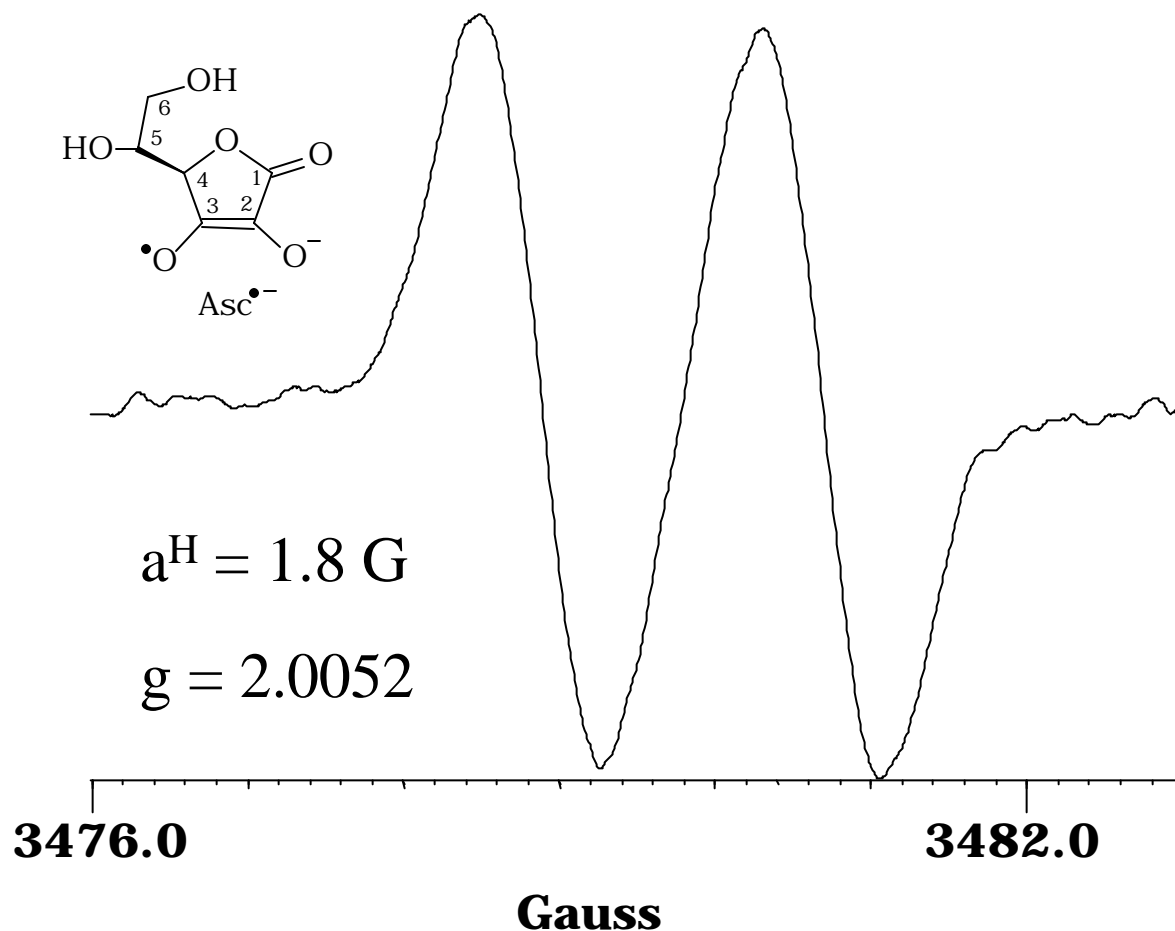


$$K = \frac{[\text{DHA}] [\text{AscH}_2]_{\text{total}}}{[\text{Asc}^{\bullet-}]^2 [\text{H}^+] [1 + \text{H}^+ / K_{\text{AscH}_2}]} = 5 \times 10^{14} \text{ M}^2$$

Because of this equilibrium reaction, $\text{Asc}^{\bullet-}$ can be formed if both, DHA and 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_{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^{•-}



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

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

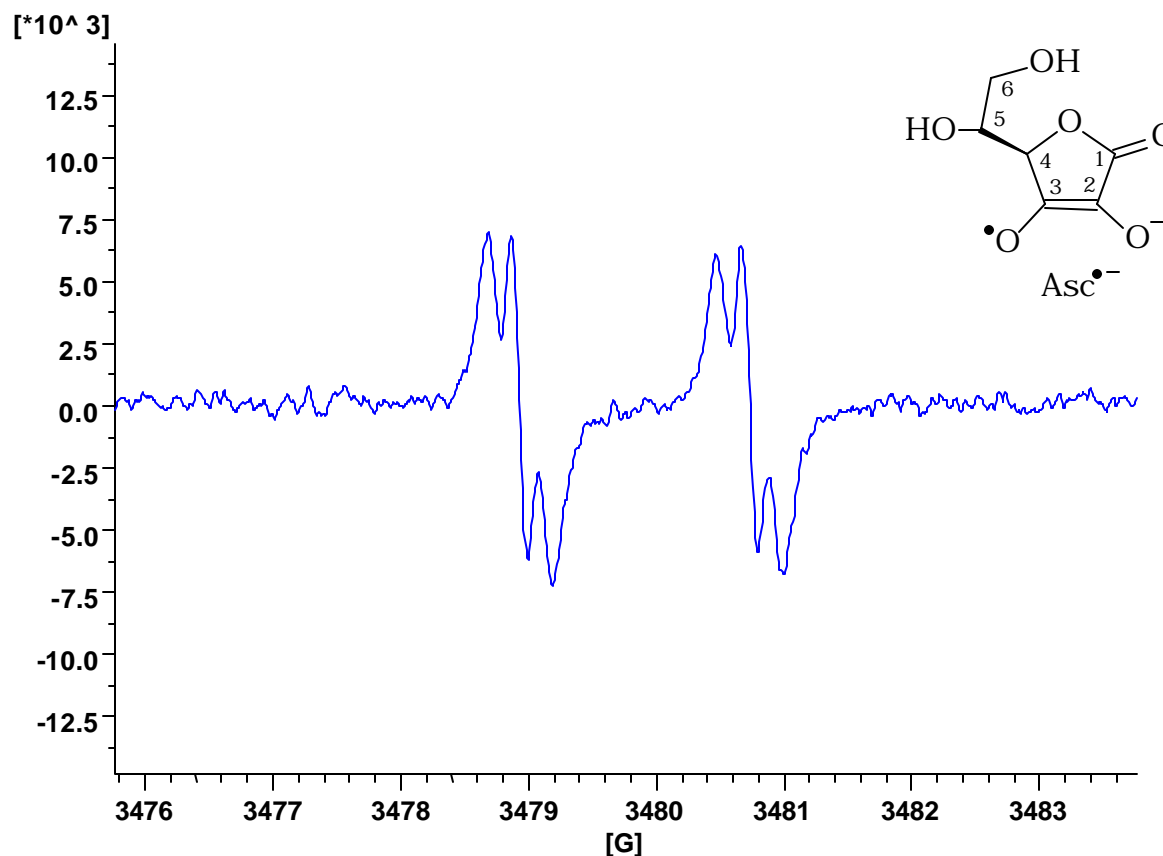
Higher Resolution EPR

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

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

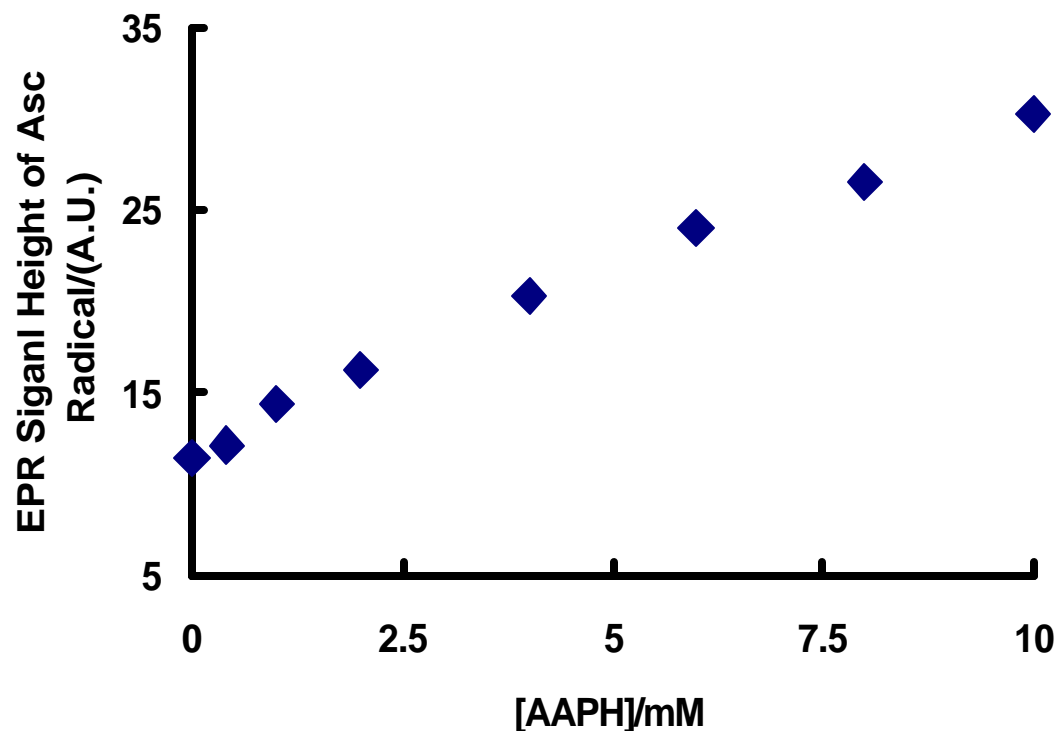
$$a^H_5 (1) = 0.07 \text{ G}$$

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



Asc•⁻, Real Time Marker of Oxidative Stress

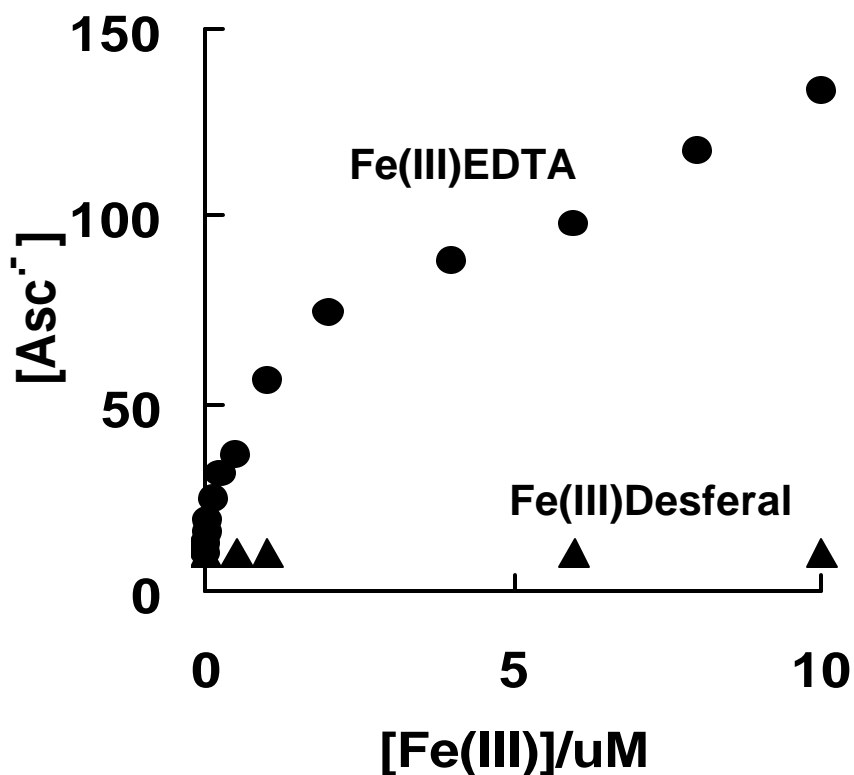
Ascorbate Radical in Plasma



$[\text{Asc}\bullet^-]_{\text{ss}}$ 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 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^{•-}, 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⁺ 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 ($\epsilon_{265} = 14,500 \text{ M}^{-1} \text{ cm}^{-1}$ 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 $\text{Asc}^{\bullet-}$ 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 $\text{Asc}^{\bullet-}$ 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, α -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, α -tocopherol, and ascorbate. *Arch Biochem Biophys.* **300**:535-543.

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

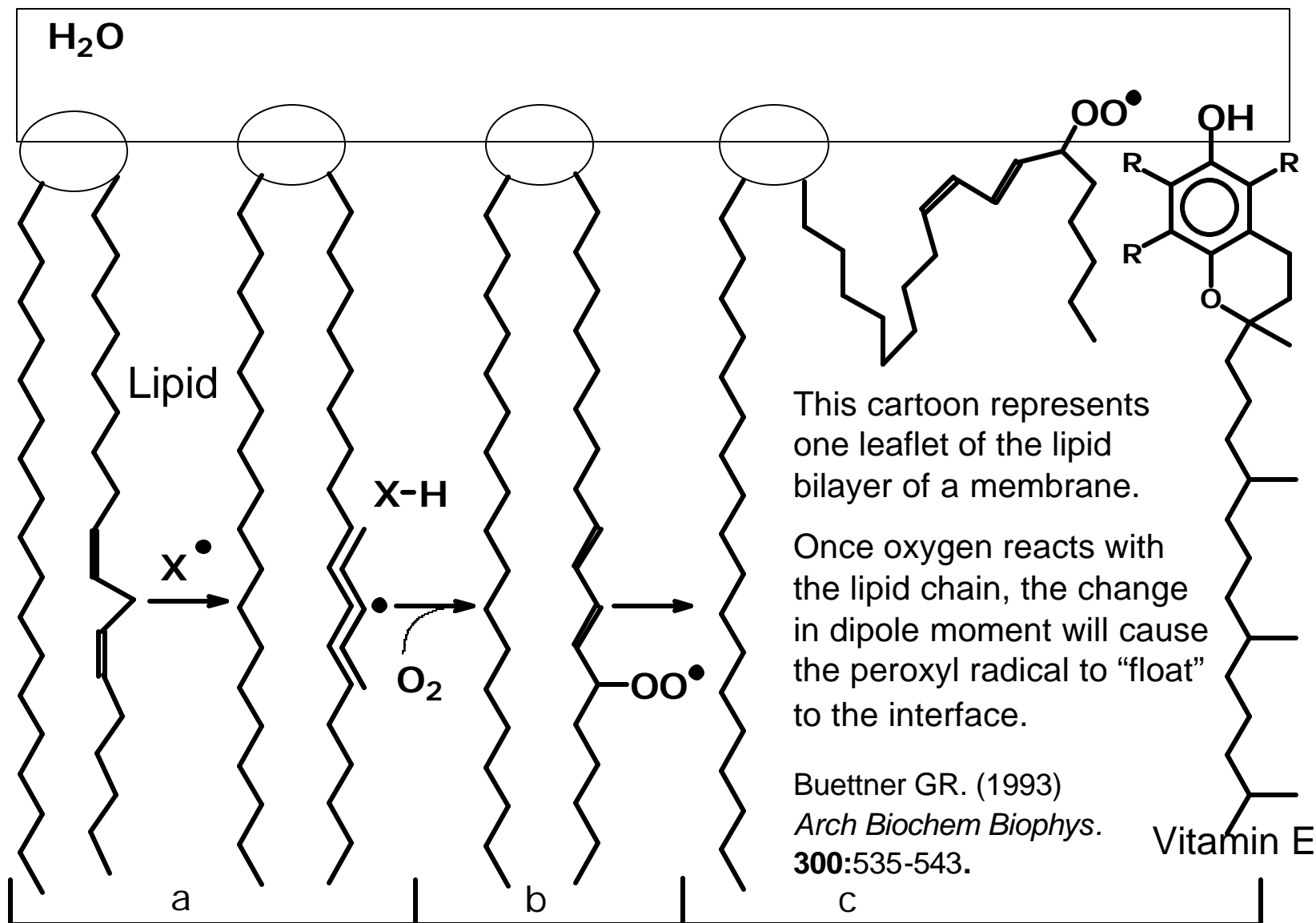
C and E as Co-antioxidants

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

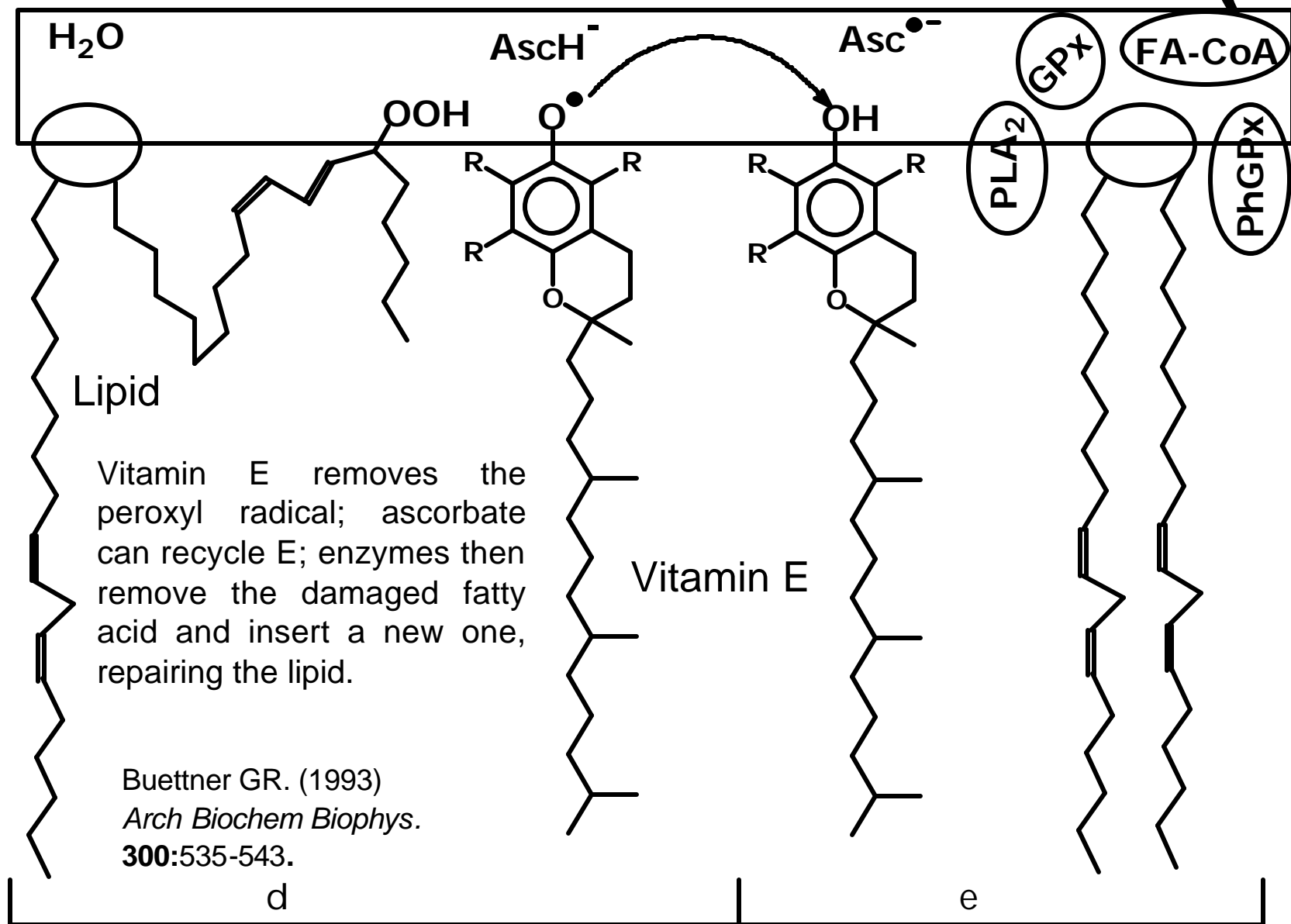


This mechanism is clearly important in protecting LDL from unwanted oxidations, because LDL lacks enzymes that could recycle TO^\bullet . But its importance in cells and tissues is still being debated.

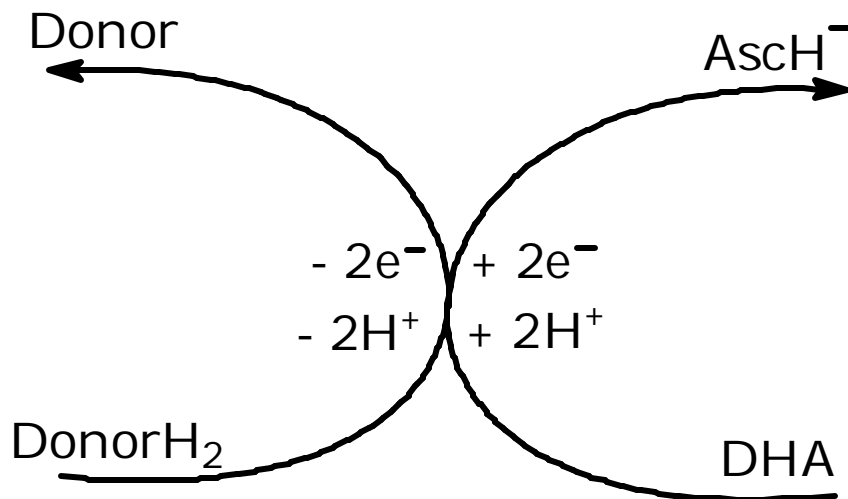
C and E as Co-Antioxidants (1)



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.

