PRO-OXIDANTS OR ANTIOXIDANTS

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Pro-oxidants and Antioxidants

What Characterizes an Antioxidant

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What is an Antioxidant?

Meaning can depend on context

Definition (Halliwell & Gutteridge 2007)

A substance that, when present at a low concentration compared with that of an oxidizable substrate, inhibits oxidation of the substrate.

Pro-oxidant

A species that causes or promotes oxidation.

Depending on the circumstances, a compound may exhibit pro- or antioxidant activity.

Examples include:

polyphenols, thiols, α-tocopherol

Antioxidant Classification

Function

- Free radical scavengers
- Scavengers of non-radical oxidants
- Compounds that inhibit generation of oxidants
- Compounds that induce the production of antioxidants

Antioxidant Classification

Function

- **Free radical scavengers**

  Ascorbic acid

- **Scavengers of non-radical oxidants**

  Catalase (H_2O_2); thiols (HOCl)

- **Compounds that inhibit generation of oxidants**

  Metal chelators

- **Compounds that induce the production of antioxidants**

  Isothiocyanates (sulforaphane)

Antioxidant Classification

Structure

- Enzymatic
- Small molecule

Locality

- Water soluble
- Lipophilic

Antioxidants act in different ways

- What is effective against one oxidant may be ineffective against another
- Localization is important
- Compounds may enhance the antioxidant capacity of cells but be ineffective in test tube assays

You need to know your oxidant

ROS is not an entity

Reactive oxygen species are individuals

One electron	Two electron (Non-radical)
Free radicals	Hydrogen peroxide Peroxynitrite Hypochlorous Acid

Interconversion

$$H_2O_2 + Fe^{2+}(\text{chelate}) \longrightarrow Fe^{3+}(\text{chelate}) + OH^- + OH^{\bullet}$$

$$ONOO^- + CO_2 \longrightarrow NO_2^{\bullet} + CO_2^{\bullet -}$$

$$H_2O_2 + \text{tyrosine} \xrightarrow{\text{peroxidase}} H_2O + \text{tyrosyl radical}^{\bullet}$$

Requirements for a Reaction

- Thermodynamics
  - Free energy change must be negative
- Kinetics
  - Activation energy must be overcome

Qinghai-Tibet Railway, highest point 5,072 m above sea level

But in between, the Himalayas (Mt. Everest 8,848 m)

ΔG is -ve

Lucknow, India 128 m

Thanks to Henry Forman

Thermodynamics v Kinetics

Hydrogen Peroxide (H_2O_2) v Hypochlorous Acid (HOCl)

- H_2O_2 is the stronger oxidant (higher reduction potential)
- Activation energy for most 2e oxidation reactions of H_2O_2 is high
- Activation energy is much lower for reactions of HOCl
- HOCl is a much more reactive oxidant

But for most radical reactions -

Activation energy low

Pecking order based on reduction potential

Radical		Reduction potential (V) *
Hydroxyl	$OH^{\bullet},H^{+}/H_2O$	2.31
Carbonate	$CO_3^{\bullet -},H^{+}/CO_3^{2-}$	1.78
Nitrogen dioxide	$NO_2^{\bullet}$	1.04
Superoxide	$O_2^{\bullet -}/2H^{+}/H_2O_2$	0.94
Phenoxyl	$PhO^{\bullet},H^{+}/PhOH$	0.90
Cysteinyl	$Cys^{\bullet}/CysS^{-}$	0.92
Ascorbyl	$Asc^{\bullet -},H^{+}/Asc^{2-}$	0.28

*pH 7 (Relative to NHE) Buettner 1993

Identifying Oxidant Targets

- Reactions occur in competition.
- Determined by rate constants and concentrations.

For two substrates, the ratio of the amounts of oxidant reacting with each is given by

$$\frac{k_1\,[\text{substrate 1}]}{k_2\,[\text{substrate 2}]}$$

Radical Scavengers as Antioxidants

One radical begets another radical

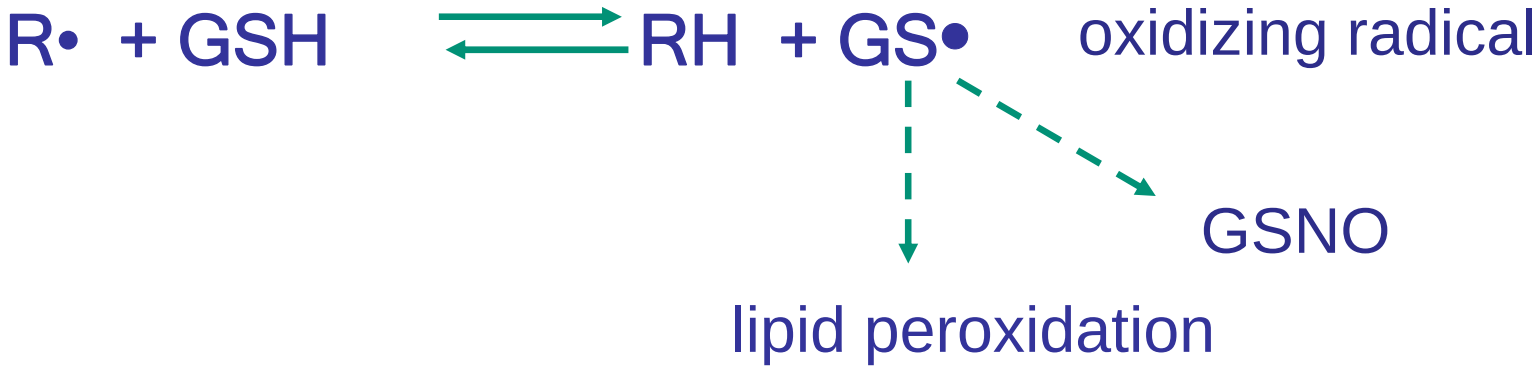
Radicals propagate chain reactions

Whether a scavenger is an antioxidant depends on the fate of the secondary radical

Ascorbate

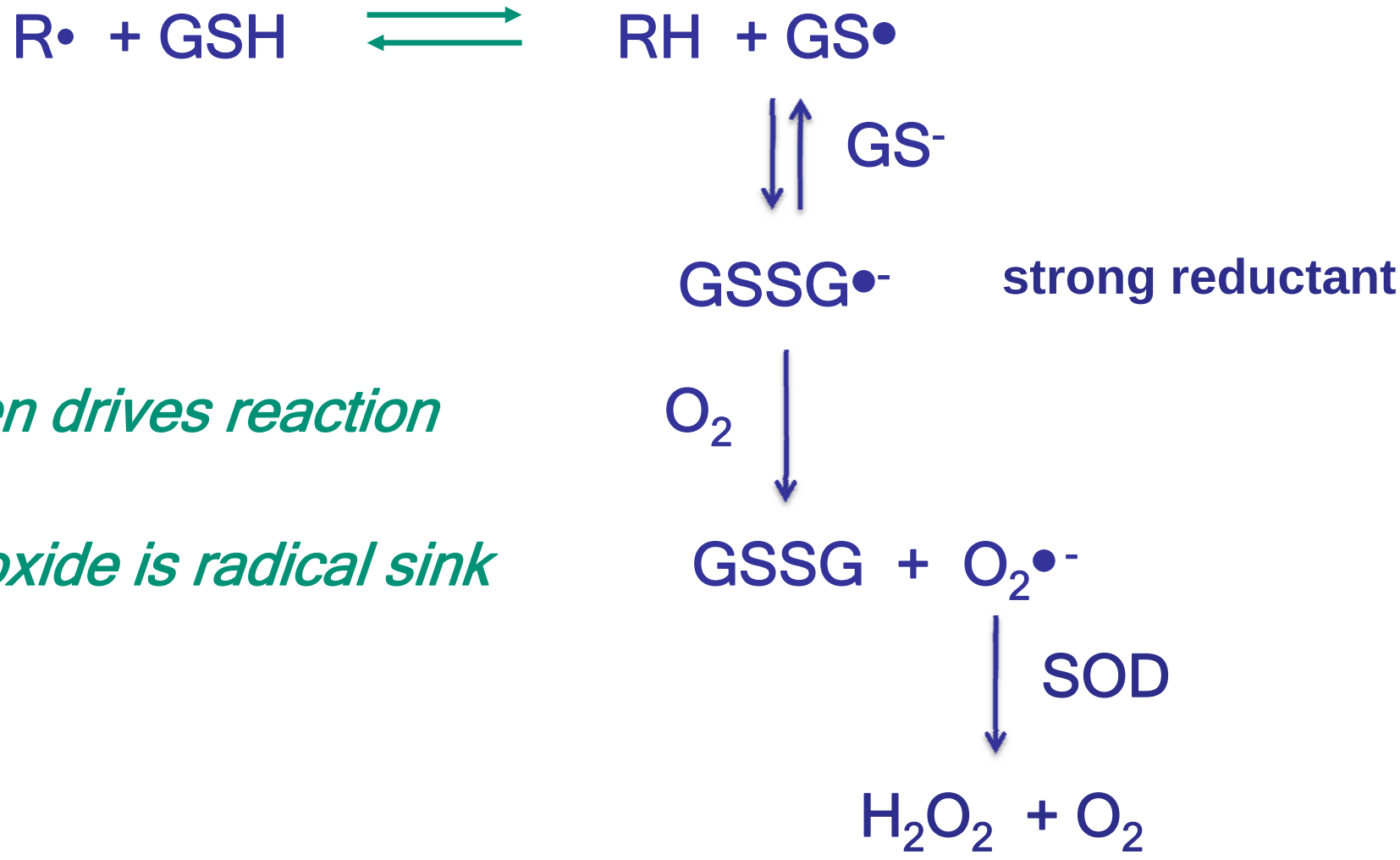
Antioxidant

Reduced Glutathione (GSH)



Pro-oxidant?

GSH as an Antioxidant



Winterbourn 1993

Radical Sinks

Superoxide

Ascorbyl radical

Interplay with 2e Chemistry

$2\ Asc^{\bullet -} \longrightarrow$ ascorbate + dehydroascorbate

dehydroascorbate + GSSG $\longrightarrow$ ascorbate + GSH

Vitamin E - Pro- or Antioxidant?

Antioxidant: Lipid peroxidation

$LOO^{\bullet} + Toc \longrightarrow LOOH + Toc^{\bullet}$

$Toc^{\bullet} + LOO^{\bullet} \longrightarrow$ chain termination

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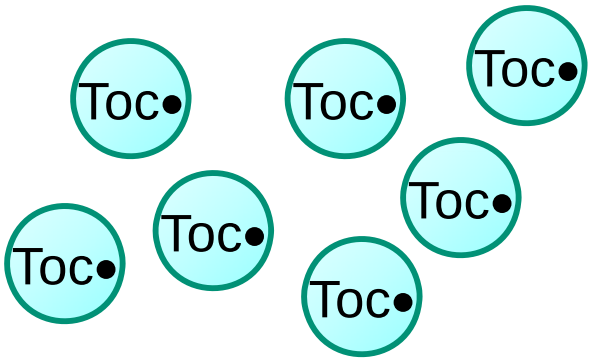
Pro-oxidant: In LDL particles



$Toc^{\bullet} + \text{Lipid-H} \longrightarrow \text{Lipid}^{\bullet}$

UNLESS…. $Toc^{\bullet} + \text{Lipid-H} \longrightarrow \text{Lipid}^{\bullet}$

Thomas & Stocker 2000

Hydrogen Peroxide

Common assumptions

- Oxidative damage

  Acts via 1e (radical) mechanism

  Metal centres

- Redox signalling

  Undergoes 2e oxidations

  Thiol targets

Antioxidant Protection against H_2O_2

Ie mechanisms

- Chelators
- Radical scavengers
- Enzymatic breakdown

2e mechanisms

- Low molecular weight “antioxidants” react slowly with H_2O_2
- Radical scavengers are ineffective
- Enzymatic breakdown

Defences against hydrogen peroxide

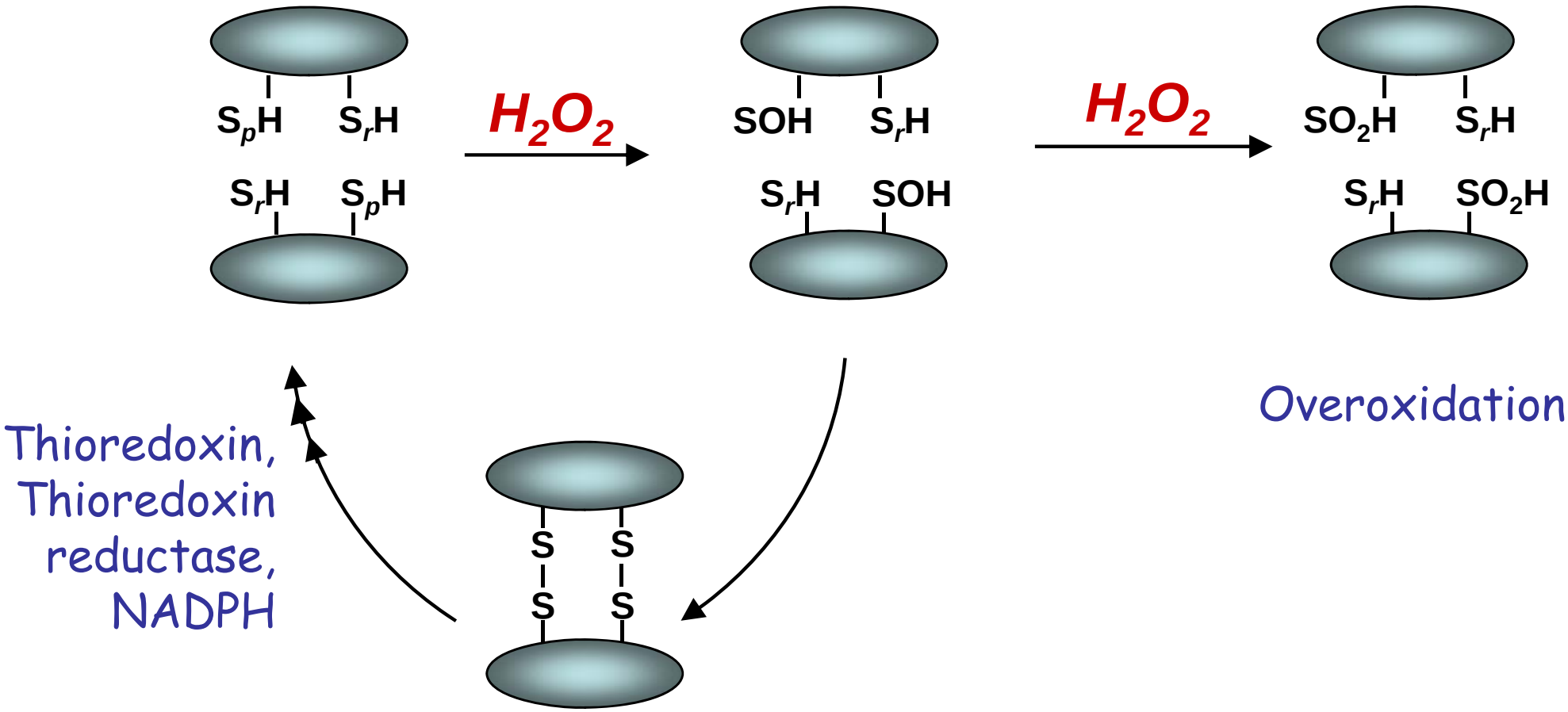
- Catalase
- Glutathione peroxidase / GSH cycle
- Peroxiredoxin / thioredoxin cycle

Peroxiredoxins

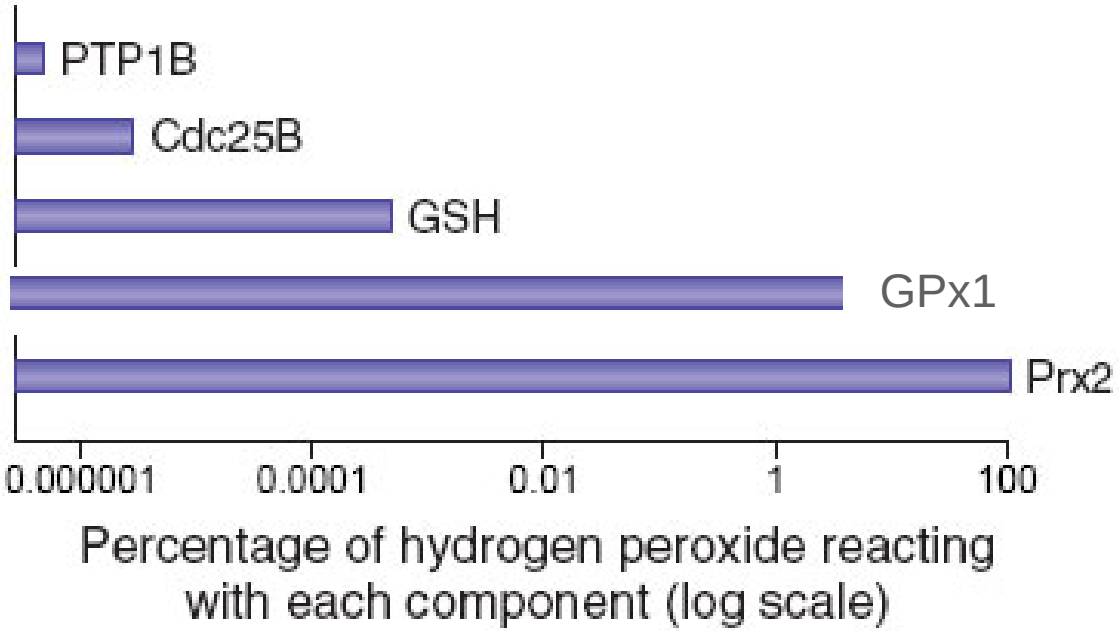
- Ubiquitous class of antioxidant or signaling proteins
- Present in cells at high copy numbers
- Highly reactive with H_2O_2 ($k>10^7\ M^{-1}s^{-1}$)
- 2-cys and 1-cys forms

2-Cys Peroxiredoxins

Peroxiredoxin / thioredoxin cycle



Simulation of cellular targets for H_2O_2



Based on rate constants and estimated cellular concentrations, and assuming homogeneous system (Winterbourn 2008)

Dietary “Antioxidants”

Approximate plasma concentrations of antioxidants

ascorbic acid	20 - 80 μM
α-tocopherol	50 μM
carotenoids	0.1 - 0.4 μM
polyphenols	0.01 - 0.1 μM
isothiocyanates	0.1 - 1 μM

To be effective physiologically

- Must be able to compete with endogenous scavengers
- Must interact with endogenous pathways
- May have alternative effect

Many dietary antioxidants act as chemoprotectants

Activate NRF2/KEAP pathway to induce Phase II response

Includes induction of antioxidant and repair enzymes

Activity may reflect radical scavenging properties but is not due to antioxidant action

Paracelsus (1493-1541)

All substances are poisons . . .

The right dose differentiates a poison from a remedy

Are the best antioxidants poisons?

Is a little of something bad good for you?

Final Comments

- Antioxidant efficacy depends on the oxidant

- Different strategies are needed for 1 electron and 2 electron processes

- Radical scavengers can be pro-oxidant unless linked to a radical sink

- Effective scavenging of H_2O_2 is generally enzymatic

- Selectivity of H_2O_2 for cell signaling proteins is likely to require site co-localization

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