„Oxidant stress“ - Basic Concepts

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Basic Concepts: recommended resource

http://www.sfrbm.org/sections/education/frs-presentations
Defining oxidant stress

“Redox imbalance leading to over-abundance of oxidants”

- is this too simplistic?

no universally agreed definition

issues with semantics

Is term “stress” always appropriate?
Oxidant stress

Damage
dysfunction and
disease

Signalling
regulatory or
adaptive response
Common illustrations of oxidant stress

Easy but inadequate concept?

Not all redox components are in equilibrium
• chemically
• spatially
• Temporally

Require some consideration of chemistry

*Genome Biology* 2002, 3(7):reviews1019.1–1019.6
increased oxidant $\rightarrow$ global biomolecule oxidation $\rightarrow$ injury $\rightarrow$ cell death

antioxidant

increased oxidant $\rightarrow$ biosensor oxidation $\rightarrow$ adaptive signaling $\rightarrow$ regulation / survival
Why do many people think oxidants are simply “bad” and antioxidants “good”?

In 1991 the Linus Pauling Institute recommended daily doses of

- 6g to 18g vitamin C
- 400 to 1600 IU vitamin E
- 25000 IU of vitamin A,

plus other supplements

Evidence?
Re-evaluated 19 vitamin E antioxidant trials between 1994 and 2004

136,000 patients in North America, Europe and China

Death rate increased: 1 in 20 chance of dying earlier if 200IU Vitamin E

25% Americans use vitamin E supplements, with >60% taking >400IU day

How many die early?
Vitamin supplementation trials - smoking

The **Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study**

29,000 male smokers randomly assigned to beta carotene, vitamin E, both, or placebo.

After 6 years lung cancer incidence was 16 percent higher in supplement group.

The all-cause death rate was also 8% higher.

*Trial stopped early*

The **Beta-Carotene and Retinol Efficacy Trial (CARET)**

18,000 smokers, former smokers, or workers exposed to asbestos.

Randomly assigned to beta-carotene and vitamin A (or placebo).

Four years follow up lung cancer incidence was 28 percent higher in supplement group.

All-cause death rate was also 17% higher.

*Trial stopped early*
Vitamin trials in other diseases – pre-eclampsia

Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial

L Poston, A L Briley, P T Seed, F J Kelly, A H Shennan, for the Vitamins in Pre-eclampsia (VIP) Trial Consortium*

Vitamins C and E and the Risks of Preeclampsia and Perinatal Complications


No benefit from supplementation

- indications of harm?
Molecular basis of oxidant stress (damage)

oxidant + target biomolecule

↓

target biomolecule oxidation = damage
Molecular basis of oxidant stress (signalling)

oxidant + target biomolecule

\[ \downarrow \]

target biomolecule oxidation = signalling

How can we differentiate damage from signalling?
Defining oxidation and reduction

These are reactions where electrons are transferred from one species to another.

Oxidation is the loss of electrons from a species
- the agent causing the loss of electrons is an oxidant

Reduction is the gain of electrons
- the agent donating the electrons is a reductant

Oxidation and reduction in terms of hydrogen transfer
- oxidation is loss of hydrogen
- reduction is gain of hydrogen

\[ 2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{G-S-S-G} + 2\text{H}_2\text{O} \]
Major biological oxidants
Reactions of oxidants with biological targets

Oxidants can target virtually all biological molecules

• DNA, RNA, cholesterol, lipids, carbohydrates, proteins and antioxidants

Extent of target oxidation depends on many factors

• concentration of oxidant and target
• rate constant for reaction of oxidant with target
• location of target versus oxidant
• occurrence of secondary damaging events
• occurrence of transfer reactions
• repair and scavenging reactions

target oxidation = signalling or damage?
## Biological oxidant species – reaction rates

<table>
<thead>
<tr>
<th>Species</th>
<th>Time Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl radical (•OH)</td>
<td>$10^{-9}$</td>
</tr>
<tr>
<td>Alcoyl radical (RO•)</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>Singlet oxygen (1O2)</td>
<td>$10^{-5}$</td>
</tr>
<tr>
<td>Peroxynitrite anion (ONOO-)</td>
<td>0.05 – 1.0</td>
</tr>
<tr>
<td>Peroxyl radical (ROO•)</td>
<td>7</td>
</tr>
<tr>
<td>Nitric oxide (•NO)</td>
<td>1 - 10</td>
</tr>
<tr>
<td>Semiquinone radical</td>
<td>minutes/hours</td>
</tr>
<tr>
<td>Hydrogen peroxide (H$_2$O$_2$)</td>
<td>Spontaneous / hours / days (accelerated by enzymes)</td>
</tr>
<tr>
<td>Superoxide anion (O2•-)</td>
<td>Spontaneous / hours / days (by SOD accelerated to $10^{-6}$)</td>
</tr>
<tr>
<td>Hypochlorous acid (HOCl)</td>
<td>depends on substrate</td>
</tr>
</tbody>
</table>
Lipid peroxidation

Fatty acid with three double bonds

Hydrogen abstraction by Hydroxyl radical

Unstable carbon radical

Molecular Rearrangement

Conjugated diene

Oxygen uptake

Peroxyl radical

Hydrogen abstraction ↔ Chain reaction

Lipid hydroperoxide

malondialdehyde

4-hydroxynonenal

ethane/pentane

e tc.

damaged membranes
= compromised cell

Secondary Oxidants
protein adducts
(injury, signaling)

DNA adduct
Other reactive oxidized lipids that adduct proteins

Martin et al, Vasc Pharm (2005)
IB: anti protein-HNE

Supinski et al, Crit Care (2010)

DNA Oxidation

hundreds of different DNA oxidation states
/sites of oxidative attack shown (→)

bases and sugars susceptible

strand-breaking oxidations

mutagenic
DNA Oxidation – examples

8-Oxo-deoxyguanosine (oxo8dG)
8-Oxo-deoxyadenosine
5-Hydroxy-2-deoxycytidine (5-HMdU)
Thymidine glycol

Monitored using HPLC methods
ELISA kits also available
## Protein Oxidation – susceptible residues

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Modifications</th>
</tr>
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<tbody>
<tr>
<td>Cys</td>
<td>many modifications – see slides to follow</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine sulfoxide</td>
</tr>
<tr>
<td>Tyr</td>
<td>Dityrosine, nitrotyrosine, chlorotyrosines, dopa</td>
</tr>
<tr>
<td>Trp</td>
<td>Hydroxy-and nitro-tryptophans, kynurenines</td>
</tr>
<tr>
<td>Phe</td>
<td>Hydroxyphenylalanines</td>
</tr>
<tr>
<td>Val, Leu</td>
<td>Hydroperoxides</td>
</tr>
<tr>
<td>His</td>
<td>2-Oxohistidines, asasparagine, aspartate, HNE-His</td>
</tr>
<tr>
<td>Glu</td>
<td>Oxalic acid, pyruvic acid</td>
</tr>
<tr>
<td>Pro</td>
<td>Hydroxyproline, pyrrolidone, glutamic semialdehyde</td>
</tr>
<tr>
<td>Thr</td>
<td>2-Amino-3-ketobutyric acid</td>
</tr>
<tr>
<td>Arg</td>
<td>Glutamic semialdehyde, chloramines</td>
</tr>
<tr>
<td>Lys</td>
<td>MDA-Lys, HNE-Lys, acrolein-Lys, CML</td>
</tr>
</tbody>
</table>
Peroxynitrite (OONO\(^-\)) and nitrotyrosine formation

\[
\text{O}_2 \xrightleftharpoons{\text{e}^-} \text{O}_2^- \xrightarrow{2\text{H}^+} \text{H}_2\text{O}_2 \xrightarrow{2\text{H}^+} \text{OH}^- \xrightarrow{2\text{H}^+} \text{H}_2\text{O}
\]

**tyrosine**

\[
\text{HO} - \text{NH}_2 - \text{OH}
\]

\[
\text{e}^- + 2\text{H}^+ + \text{O}_2^- \xrightarrow{2\text{H}^+} \text{H}_2\text{O}_2 \xrightarrow{2\text{H}^+} \text{OH}^- \xrightarrow{2\text{H}^+} \text{H}_2\text{O}
\]

\[
\text{NO}^- \xrightarrow{\text{OONO}^-} \text{denitrase?}
\]

**3-nitrotyrosine**

\[
\text{HO} - \text{NH}_2 - \text{OH}
\]

**phosphotyrosine**

\[
\text{HO}_3\text{PO} \xrightarrow{\text{mimic?}} \text{NH}_2
\]

Nitro-Tyrosine Polyclonal

Peroxynitrite Degraded Peroxynitrite Pervanadate

\[
\text{Peroxynitrite} \quad \text{Degraded Peroxynitrite} \quad \text{Pervanadate}
\]
Methionine oxidation

NADPH $\rightarrow$ thioredoxin reductase $\rightarrow$ thioredoxin $\rightarrow$ thioredoxin reductase $\rightarrow$ NADP$^+$

$\rightarrow$ cellular redox system

$\rightarrow$ cofactors

MSRA$_{ox}$ $\rightarrow$ MSRA $\rightarrow$ biologically irreversible

mild oxidants $\rightarrow$ strong oxidants

methionine (Met) $\rightarrow$ methionine sulfoxide (MetO) $\rightarrow$ methionine sulfone (MetO$_2$)
Methionine oxidation in cardiac CaMKII

Methionine oxidation induces constitutive activation

Cardiac dysfunction
Cysteine thiol – potential oxidant sensor

$$\text{PSH} \rightarrow \text{PS}^-$$

(thiol) (thiolate anion)
remember this for later..
... peroxiredoxin proteins and „floodgate”
How can selective / specific oxidant-signalling occur when there are so many thiols in the cell?

1-5mM glutathione plus other small thiols intracellular

1000s different protein thiols

Doesn’t the oxidant just react with all these thiol targets?

not all thiols are „equal“

some are more nucleophilic than other

nucleophiles react with electrophiles (oxidants)

this provides a molecular basis for selective oxidant signalling
Thiol pKa determines its reactivity

\[ \text{RSH} \quad \text{↔} \quad \text{RS}^- + H^+ \]

*pKa is the pH at which } [\text{RSH}] = [\text{RS}^-]*

*or pH when the thiol is 50% ionised*

Oxidants (e.g. H\(_2\)O\(_2\)) will react very, very slowly with \( \text{RSH} \).

... but very, very fast with \( \text{RS}^- \).
Thiol pKa determines its reactivity

\[ RSH \quad \text{unreactive} \quad \text{acid (H}^+\text{)} \quad RS^- + H^+ \quad \text{reactive} \]

\[ \text{base (OH}^-\text{)} \]

pKa = acid dissociation constant (pH at which the molecule ionises)
Thiol pKa determines its reactivity

\[ RSH \quad \text{↔} \quad RS^- + H^+ \]

*\( pKa \) is the pH at which [RSH] = [RS-]*

*or pH when the thiol is 50% ionised*

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<th>Thiol molecule</th>
<th>p( Ka )</th>
<th>%RS(^-) at pH 7.4</th>
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<tr>
<td>Cysteine</td>
<td>( \sim 8.5 )</td>
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Protein S-nitrosylation (-SNO formation)
covalent adduction of nitric oxide (NO)
aka S-nitrosation
aka nitrosothiol
Protein S-nitrosylation

Table 1 | Enzymatic activities that are involved in S-nitrosylation/de-nitrosylation

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate(s)</th>
<th>Action/Product</th>
<th>Mechanism</th>
</tr>
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<tr>
<td>Ceruloplasmin (other multi-Cu²⁺ proteins including laccase)</td>
<td>Glutathione</td>
<td>GSNO</td>
<td>1) CPCu(II) + NO → CPCu(II)-NO + GSH → CPCu(II) + GSNO + H⁺ + 1e⁻ 2) CP[Cu cluster]ox + 4e⁻ → CP[Cu cluster]red 3) CP[Cu cluster]red + 4H⁺ + O₂ → CP[Cu cluster]ox + 2H₂O Net: 4NO + 4GSH + O₂ → 4GSNO + 2H₂O</td>
</tr>
<tr>
<td>Glycophan-1</td>
<td></td>
<td>SNO-glycophan-1</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Haemoglobin</td>
<td>Hb([ι-Cys93]-NO)</td>
<td></td>
</tr>
<tr>
<td>Hæmoglobin-FeNO</td>
<td>Self (auto- S-nitrosylation)</td>
<td>Hb([ι-Cys93]-NO)</td>
<td>1) Hb([Fe(II)]NO)[Fe(II)]Cys + 4O₂ ↔ (Hb([Fe(II)]O₂)CysNO + 1e⁻ 2) Hb([Fe(II)]NO)[Fe(II)]Cys + 4O₂ ↔ Hb([Fe(II)]O₂)CysNO</td>
</tr>
<tr>
<td>Thioredoxin/ thioredoxin reductase</td>
<td>SNO-protein (NO-synthase, PKC?) GSNO</td>
<td>De-nitrosylation</td>
<td>De-nitrosylation</td>
</tr>
<tr>
<td>GSNO-reductase</td>
<td>GSNO</td>
<td>GSNO metabolism (GSNO ↔ SNO-protein equilibrium)</td>
<td>1) Protein-SNO + GS⁻ ↔ protein-S⁻ + GSNO 2) GSNO + NADH + H⁺ → GSNH₀H + NAD⁺</td>
</tr>
<tr>
<td>NOS</td>
<td>NOS (auto-S-nitrosylation)</td>
<td>Inhibition of NO production</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-nitrosylation of multiple substrates</td>
<td>Regulation of substrate function, location and protein-protein interactions</td>
<td></td>
</tr>
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</table>


S-nitrosylation has the similar enzymatic control mechanisms as phosphorylation.
More complex biomolecules can also react with thiolate

\[ \text{15-deoxy-} \Delta^{12,14-}\text{PGJ}_2 \]

alpha beta unsaturated carbonyls

* = electrophilic carbons

more selective oxidant-signalling

Compared to \( \text{H}_2\text{O}_2 \), HNO, HOCl etc?
electronegative oxygen atom pulls electrons from the double bond
Resulting "electrophilic carbon" is very reactive with protein thiolate.
Michael reaction or Michael addition

Conjugation (adduction of lipid to protein cysteine)
Reaction cycle of Peroxiredoxins (decomposition of $\text{H}_2\text{O}_2$)

$S_p$ = peroxidatic thiol ($\text{pKa} \sim 4.5 = \text{very reactive} = \text{very fast reaction}$)

$S_R$ = resolving thiol

$\text{disulfide reductase}$

$\text{Oxidase}$

$p$-$\text{H}$

$\text{S}_p$ = peroxidatic thiol ($\text{pKa} \sim 4.5 = \text{very reactive} = \text{very fast reaction}$)

$\text{S}_R$ = resolving thiol
The aldehyde of glyceraldehyde-3-phosphate reacts with the cysteine thiol to form a thiohemiacetal.

Oxidation to a carboxylic acid (in a ~ thioester) occurs, as NAD$^+$ is reduced to NADH.

The “high energy” acyl thioester is attacked by $P_i$ to yield the acyl phosphate ($\sim$P) product.

$H_2O_2$ inactivates GAPDH by thiol oxidation.
Examples of proteins that are modified / regulated by H$_2$O$_2$

Protein tyrosine phosphatase 1B (PTP1B)

## Comparing thiol targets of $\text{H}_2\text{O}_2$

<table>
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<th>Protein tyrosine phosphatases</th>
<th>$k_{\text{H}_2\text{O}_2}$ M$^{-1}$s$^{-1}$</th>
<th>Conc.</th>
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<td>PTP1B ($pK_a \sim 5.6$)</td>
<td>20</td>
<td>1 µM</td>
<td>0.0004</td>
</tr>
<tr>
<td>GAPDH ($pK_a \sim 5.3$)</td>
<td>$\sim 500$</td>
<td>100 µM</td>
<td>1</td>
</tr>
<tr>
<td>Peroxiredoxins ($pK_a \sim 4.5$)</td>
<td>$&gt;100,000$</td>
<td>50 µM</td>
<td>99</td>
</tr>
</tbody>
</table>

How can PTP1B or GAPDH ever be stoichiometrically modified by $\text{H}_2\text{O}_2$ when peroxiredoxin is so reactive and also abundant? ????

adapted from work of Christine Winterbourn
### Comparing thiol targets of H$_2$O$_2$

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Some proteins that are easily oxidized by H$_2$O$_2$ in isolation are unlikely to be directly oxidized in a cell - until more favourable targets (peroxiredoxins) are oxidized.

adapted from work of Christine Winterbourn
Peroxiredoxins should prevent oxidation of less abundant / lower pKa thiols

Oxidase $\rightarrow$ H$_2$O$_2$ $\rightarrow$ PTP1B

$\sim$100% $\sim$0%

How can the phosphatase ever be stoichiometrically oxidised?
peroxiredoxin peroxidase catalytic cycle

\[
\text{[H}_2\text{O}_2\text{]} \quad 0 \quad 10 \quad 100 \quad (\mu\text{M})
\]

\[
\frac{\text{[H}_2\text{O}_2\text{]}}{} \quad 2 \quad 4 \quad 6
\]

detects only this oxidation state

hyperoxidation and inactivation

Jönsson et al., 2005, Biochemistry
signalling → non-Prx target thiol → \( \text{H}_2\text{O}_2 \)

Science 2003 (Perspective: An Overoxidation Journey with a Return Ticket)
Vol. 300. no. 5619, pp. 592 - 594

“floodgate” opens

hyperoxidation and inactivation

Jönsson et al., 2005, Biochemistry
"repair" of hyperoxidised peroxiredoxin by sulfiredoxin enzyme - "floodgate" closes

signalling ↔ non-Prx target thiol ↔ H$_2$O$_2$

"floodgate" opens

peroxiredoxin peroxidase catalytic cycle

hyperoxidation and inactivation
Alternative to floodgate

![Diagram showing the process of Prx catalyzed disulfide exchange and its effect on target S\(^{-}\)NH\(^+_3\) groups.]

- Prx catalyzes the exchange of disulfide bonds (Cys-S\(_p\), Cys-S\(_R\)) with target S\(^{-}\)NH\(^+_3\) groups.
- The reaction sequence involves the oxidation of Prx with \(\text{H}_2\text{O}_2\), converting Cys-S\(_p\)H to Cys-S\(_p\)OH and Cys-S\(_R\)H to Cys-S\(_R\)H.
- The final result is no signal for the target S\(^{-}\)NH\(^+_3\) groups.
Alternative to floodgate

Prx \text{Cys-S}_p
\downarrow
\text{Cys-S}_R

\text{disulfide reductase}

Prx \text{Cys-S}_pH \xrightarrow{\text{H}_2\text{O}_2} \text{Cys-S}_R\text{H}

Prx \text{Cys-S}_pOH \xrightarrow{\text{Cys-S}_R\text{H}} \text{target}

\text{signal}
Alternative to floodgate

disulfide reductase

Prx Cys-S\_p

Cys-S\_R

target

Prx Cys-S\_p

NH\_3^+

Prx Cys-S\_p\_H

H\_2O\_2

Cys-S\_R\_H

target

S

NH\_3^+

Prx Cys-S\_p\_OH

Cys-S\_R\_H

target

S

NH\_3^+