RB biochemistry

Redox Biology: Biochemistry

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Biochemistry Summary

Biochemical Lecture

I. Sources of the instigators
   A. Exogenous sources of radicals
   B. Endogenous
      NADPH oxidase
      NADPH P450 oxidoreductases
      Xanthine oxidase
      Mitochondria
      Nitric oxide Synthase
      Heme oxygenase

II. Detoxification:
   A. Enzymatic
      SOD, CAT, GPx
      DT-Diaphorase (2-e transfer)
   B. Scavenging
      GSH
      Ascorbate
      Tochopherol
Oxygen Reduction

Reduction of oxygen to $\text{O}_2^-$ and $\text{H}_2\text{O}_2$

Outer-sphere electron transfer

Thus, the determining factor is $E$ and access

$\text{O}_2 + \text{e}^- \rightarrow \text{O}_2^-$  - 0.33 V  \hspace{1cm} \text{O}_2 + \text{e}^- + \text{H}^+ \rightarrow \text{HO}_2^-  - 0.46$ V

$\text{O}_2^- + \text{e}^- \rightarrow \text{H}_2\text{O}_2$  +0.95 V

$pK_a = 4.7$

Substance with more negative reduction potential than -0.33 V can spontaneously reduce oxygen to superoxide.

Paraquat/methyl viologen  \hspace{1cm} \text{MV}^+ + \text{O}_2 \rightarrow \text{MV}^{2+} + \text{O}_2^-$

$\Delta E = (+0.45) + (-0.33) = +0.12$
Redox Cycling

Redox Cycling to form Reactive Oxygen Species

Types of Compounds

Ubiquione (CoQ)
Menidione
Adraimycin (Doxorubicin)
Tochoperol

Quinone

Semi-Quinone

GSH
GS-Q
Prot-SQ

Diphenol

Dopamine derivatives, Catecholic Estrogens

Adrenalin

Quinone

Semi-Quinone

Diphenol

Para

Ortho
Menadione and Dopamine

Menadione (Vitamin $K_3$)

Menadione $\xrightarrow{\text{HbO}_2}$ Met Hb + $SQ^-$ $\xrightarrow{}$ Thiol $O_2^-$

dopamine $\xrightarrow{\text{hydroxylate}}$ 6-hydroxydopamine
Pro-Oxidant vs Antioxidant

Pro-oxidant versus Antioxidant

Quinone $\Leftrightarrow$ SQ$^*$ $\Leftrightarrow$ QH$_2$

O$_2^-$ \quad Fe(III) reduction \quad Pro-oxidant

Pro-oxidant: SQ to Quinone

Anti-oxidant: reduce the SQ to QH$_2$.

Location is essential and pharmacokinetic stability water versus fat soluble
Vitamins Working in Concert

Vitamin E = TcOH

TcOH + OH; LOO or NO₂ → TcO• → CoQH₂ → SQ•

DHA and Asc ↙ 2 Asc ↩ Cytosol

Carotenoid

R

Car + OH; LOO → CarOH or CarOOL

NO₂ → Asc → Car•
Xenobiotic Metabolism

Activation of Quinones, Paraquat etc.

\[ X \xrightarrow{\text{NADPH Cytochrome P450 Oxidoreductases}} X^- + 1 \text{ electron} \]

Detoxification of Quinones, Paraquat etc.

\[ X \xrightarrow{\text{DT-Diaphorase or Quinone Reductase (NQ1, P450 reductase super family)}} XH_2 + 2 \text{ electrons} \]

Broccoli extracts induce DT-Diaphorase (NQ1) expression
Mitochondrial Sources of ROS

Mitochondrial Sources of ROS

Site I  →  Site II  →  Site III  →  Site IV

NADH

O₂  →  O₂⁻

Ubiquinone (Q)  ↔  Ubasemiquione (SQ)

Ubiquinone (Q)  ↔  Ubisemiquione (SQ)

O₂⁻  ↔  H₂O₂

H₂O₂  ↔  Ubiphenol (H₂Q)

pKₐ = 4.7

O₂⁻  →  O₂

O₂  →  H₂O

Cytochrome c oxidase

Polarization/depolarization of the mitochondria can effect reduction where the proton gradient can be important to reduction of O₂.
Energetics of ROS Formation

Energetics of ROS formation from Site III

\[ E^o = -0.33 \]
\[ E^o = 0.04 \]
\[ \Delta E = -0.29 \]
\[ \Delta G \sim 6 \text{ kcal} \]
\[ K = 0.01 \]

\[ SQ^+ + O_2 \rightarrow e^- \]
\[ Q + O_2^- + NO \rightarrow ONOO^- \rightarrow NO_3^- \]
\[ H_2O_2 \rightarrow \text{Spin trap} \]
\[ \text{Water reduction at Site IV} \]

Aconitase

MnSOD

Over expression of MnSOD leads to increase DCHF oxidation

DHR
DCHF
Inner-Sphere Reduction Examples

Examples of Inner-sphere reduction of Oxygen

**Bleomycin**

BLMFe(III) → BLMFe(II) → Fe(II)O₂ or Fe(III)O₂⁻

Substrate oxidation → OH and Fe(IV) → 1 electron oxidants

BLMFe(II) + e⁻ → Fe(II)O₂ or Fe(III)O₂⁻

O₂⁻ + H₂O₂ → Fe(V) or Fe(III)O₂H⁺

2 electron, oxygen transfer hydroxylation

**Prolyl Hydroxylase**

Fe(III) → Fe(II) + e⁻ → Fe(O₂⁻)

Hydroxylation of proline → Fe(III)O₂⁻ + e⁻ → Fe(II)O₂

O₂ → Fe(II)O₂⁻
P450 System

Different reduction mechanism of O₂ by P450 system

Cytochrome P450

- Fe³⁺ + O₂ → Fe²⁺O₂ + e⁻
- Fe³⁺ - O₂²⁻
- Fe⁵⁺=O
- 1.4 -1.6 V

Peroxide shunt

- +H₂O₂

Substrate oxidation

- H₂O

P450 reductase

- NADPH → FAD → FMN → P450
- Q → SQ⁺ → O₂
Enzymatic Generation Purpose

Enzymatic generation of Reactive Oxygen Species

Purpose:

- As an antimicrobial agent
- as a by product of metabolism
- Part of signal transduction mechanisms
Hydrogen Peroxide Formation Example

Examples of Hydrogen peroxide formation

Glucose Oxidase (Glc oxidase: Bacterial)
\[ O_2 + \text{Glucose} \rightarrow \text{gluconolactone} + H_2O_2 \]

Polyamine oxidase (PAO)
\[ O_2 + \text{spermine} \rightarrow \text{acrolein} + \text{putrascine} + H_2O_2 \]

Xanthine Oxidase (XO)
\[ O_2 + \text{Xanthine} \rightarrow \text{Urate and } O_2-/H_2O_2 \]
\[ O_2 + \text{Hypoxanthine} \rightarrow \text{Xanthine and } O_2-/H_2O_2 \]

NADPH oxidase (NOX)
\[ \text{NADPH} + 2O_2 \rightarrow 2O_2^- + \text{NADP}^+ \]
Xanthine Oxidase

Purpose:
- Purine metabolism
- Secretion of milk drops
- Detoxification of aldehydes
- Generation of ROS

Xanthine → XD → Uric acid + NADH

Xanthine Oxidase (XO)

HX → X + ROS

Uric acid + O$_2^-$/H$_2$O$_2$

Disease and injury (XO)

XD to XO by proteolysis or thiol oxidation
Xanthine Oxidase

Homodimer
HX or X
Urate

Inhibited by allopurinol (abundant in goats milk)

O₂
FAD → FADH₂

O₂⁻/H₂O₂

Mouse utilizes XO more than humans

Moco

Mo

Fe₂S₂

Xanthine Oxidase
NADPH Oxidase

NADPH oxidase (NOX)

**Purpose:**
- Antimicrobial
- Regulation of cell-surface signaling
- Regulation of physiological function

**Phagocytic (Phox)**

Membrane bound
- \( gp91^{phox} \)
- \( p21^{phox} \)

Cytosolic regulators
- \( p47^{phox} \)
- \( p67^{phox} \)
- \( p40^{phox} \)

Small GTPase/rac1/Rac2

**Vascular NOX**

- NOX-1 colon VSMC, prostate
- NOX-2 innate immune system
- NOX-3 inner ear
- NOX-4 kidney
- NOX-5 spleen (human only)
Nitric Oxide Synthase

**Homodimer**

NOS-1 nNOS or neuronal NOS
NOS-3 eNOS or endothelial NOS

NOS-2 iNOS or inducible NOS

Constitutive and calcium sensitive
Induced and calcium insensitive

**P450 reductase domain**

NADPH
FMN
FAD

Calmodulin binding

**Heme oxidase domain**

O₂
Arginine/N-hydroxyarginine (NOHA)
Nitric Oxide Synthase

130kd

Arginine

Citrulline + NO

Heme oxygenase

Reductase domain

Tetrahydrobiopterin (BH₄)
Calmodulin (Cm)

FMN

FAD

NOS_{ox}

NOS_{red}

NADPH-dependent P450 reductase

In cells can exist as monomers which then are converted to dimers
NOS Biochemistry

Threshold of NO

\[ \text{Fe(NO)} \rightleftharpoons \text{Fe(II)} + \text{NO} \]

\[ K_{i}^{\text{NO}} \]

iNOS >> nNOS or eNOS

\[ K_{m}^{\text{O}_2} \]

eNOS, 25 \( \mu \text{M} \); iNOS, 120 \( \mu \text{M} \); nNOS, 400 \( \mu \text{M} \)
Heme Oxygenase

P450 superfamily
Numerous factors induce HO-2

HO-1
HO-2

Heme

\[
\text{M} \quad \text{V} \quad \text{M} \quad \text{V} \quad \text{M} \quad \text{V} \quad \text{M} \\
\text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N}
\]

\[\text{HO} \quad + \quad \text{CO} \quad \longrightarrow \quad ?\]

Biliverdin

\[
\text{NADPH} \quad \text{NADP}^+ \\
\text{M} \quad \text{V} \quad \text{M} \quad \text{V} \quad \text{M} \quad \text{V} \quad \text{M} \\
\text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N}
\]

Guanylyl cyclase
p38 activation

Antioxidant
Biochemistry of Prevention of Oxidative or Nitrosative stress

Different classes

Preventive
Inhibition of production of instigators

Interception and Quenching
Scavenging of reactive intermediates formed

1) Want the product to be innocuous
2) The product could be biologically recycled
3) Prevent chain reactions from occurring (chain breaking)
Biochemistry Reactive Oxygen Species

Biochemistry Reactive Oxygen Species

O_2^- → H_2O_2

XO

NOX

NOS_{unc}

Peroxidases

Cl^- → Br^- → NO_2^- → HOCl → HOBr → NO_2

Antimicrobial

MAPK

NF\kappa B

p53

Aconitase inhibition

COX

LipOX

Fenton

LOOH
Preventive Mechanisms

\[ \text{Preventive Mechanisms} \]

\[ \text{O}_2^- \downarrow \text{SOD} \]

Catalase
Heme protein peroxisome

\[ \text{H}_2\text{O}_2 \]

Glutathione peroxidase (GPX)
Selenium containing protein
Cytosol and mitochondria

LOOH

Peroxiredoxin (Prx)
Isoforms: Prx 1-6
Contains reactive thiol
1-4 two cys
5 one cys
6 one cys

Location
Cytosol
Mitochondria
Peroxisome
Plasma
Chemical Biology of Nitric Oxide

Direct

Inhibition of Respiration

Guanylyl cyclase

NOS

Radical scavenging
Antioxidant properties

Inhibition of P450

Indirect

O$_2^-$

ONOO$^-$ + NO

NO$_2$

N$_2$O$_3$

Antitumor
Antipathogen

Oxidation
Nitration

Nitrosation
Scavenging of NO

Scavenging of NO (Prevention of RNOS formation)

\[
\begin{align*}
\text{NOS} & \xrightarrow{\text{HbO}_2} \text{NO} \\
\text{NO}_3^- & \xrightarrow{\text{MbO}_2} \text{NO} \\
\text{Fe}=\text{O} & \xrightarrow{} \text{NO}_2^- \\
\text{k} & > 10^7 \text{ M}^{-1} \text{ s}^{-1}
\end{align*}
\]

General Cellular Consumption
Mechanism to be determined

Mitochondria?

Critical to compartmentalizing NO effects
Glutathione Peroxidase Biochemistry

NADPH

Glutathione reductase (GR)

GSSG

GSH

Prot-SeSG + H₂O

GPX

Prot-Se⁻

H₂O₂

LOOH

ONOO⁻

Prot-Se-OH + LOH

NO₂⁻

Selenium compound Ebselen
Peroxiredoxin

Over expressed in cancer cells
Breast carcinoma
Hepatocellular carcinoma
Prostate
Oral
Lung

Peroxiredoxin (Prx)

Prx (1-4)

H$_2$O$_2$

NADPH $\rightarrow$ Trx$_{\text{red}}$

Trx(S-S) $\rightarrow$ Trx(SH)$_2$

Trx = Thioredoxin (ER)
Trx$_{\text{red}}$ = thioredoxin reductase (FAD)

NADPH $\rightarrow$ FADH$_2$ $\rightarrow$ Reduced S-S

Both GR and Trx$_{\text{red}}$ use hydride transfer to reduce the disulfides to sulfides
Seleno-Cysteine Reduction

Reduction of a seleno-cysteine

A selenylsulfide in a protein is reduced by a cysteine-exchange reaction and the resulting disulfide is then reduced by electron transfer. This example shows the reduction of thioredoxin (Trx) by thioredoxin reductase (TrxR).

Glutathione Metabolism

NADPH

GR

GSH ---\rightarrow GS

Oxidant

OH/Fe=O

NO₂

GSH

GSSG

O₂

SOD

O₂⁻ \rightarrow H₂O₂

GPX

H₂O

O₂

GSSG

GSH

GSH

ONOO⁻

GSOH

GSOO

SOD

O₂⁻ \rightarrow H₂O₂

GPX

H₂O
Abatement of Nitrosative Stress

NO → \[ \text{O}_2 \] → \[ \text{N}_2\text{O}_3 \] → GSH → Cu(I)ZnSOD → GSH + NO

Both NO and HNO toxicity is abated by GSH

Protein SNO + GSH → Protein S-SG

GSSG + \[ \text{HNO} \] → GSSG + GSH → GSH

GS(O)NH\(_2\) ← GSNOH → GSH

GSH → \[ \text{NH}_2\text{OH} \] → GSH

\[ \text{Cu}(\text{II})\text{ZnSOD} \] → Cu(I)ZnSOD → GSH + NO
Glutathione and Ascorbate Pools

Glutathione and Ascorbate Pools Communicate

GSSG \rightarrow 2 \text{ GSH}

Protein Disulfide Isomerase (PDI)

Glutaredoxin (cytosol)

\text{Asc}^{-} \quad \overset{Y^*}{\longrightarrow} \quad \text{Asc}^* \quad \overset{+0.17}{\longrightarrow} \quad \text{DHA}

\text{AscH}^- \quad \overset{-0.28}{\longrightarrow} \quad \text{Reduce metals}

\text{Increase Metal-mediated Oxidation}

\text{Trx}_{\text{red}} \quad \overset{\text{NADPH}}{\downarrow}
Urate and Uric Acid

Human Plasma 0.2-0.4 mM

Xanthine

XD or XO

\[
\begin{align*}
\text{UH}_3 & \rightleftharpoons \text{UH}_2^- & \text{UH}_2^- & \rightleftharpoons \text{UH}^{2-} \\
pK_{a1} = 5.4 & & pK_{a2} = 9.8
\end{align*}
\]

Uric acid

OH  NO₂

\[
\begin{align*}
>10^9 & \\
10^7
\end{align*}
\]

Urea and HOC-CHO

Glyoxylic acid

Oxalic acid
Interaction of NO and ROS

Interaction of NO and ROS

HO-1 → CO and Bilirubin

Heme

NOX → Lipid Peroxidation

NO → SOD

RNOS → H₂O₂

O₂⁻ → LOOH

Ferrochelatase

NOS → NO

Ox

GR → GSSG

GSH

Heme and iron regulation is critical to the redox balance
Oxygen and NAPDH Essential

All enzymes require Oxygen and NADPH

O₂ → XO → NOX

NADPH versus NADH

Metabolism of iron, oxygen and glucose will be critical factors in the cellular redox state

Now the adventure begins the biochemistry/chemistry in biology