Antioxidant Enzymes

A. Superoxide Dismutase (SOD) – 1969, Fridovich and McCord

1. Function

a. Enzymatic

$$O_2^{\bullet -} + O_2^{\bullet -} + 2\,H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Only enzyme known to act on a radical. Revolutionary idea: the presence of SOD implies $O_2^{\bullet -}$ produced in cell during normal metabolism.

b. Biological

Superoxide theory of oxygen toxicity:

1. $O_2^{\bullet -}$ produced in normal cell metabolism
2. $O_2^{\bullet -}$ is reactive & toxic
   (false, but Fe-catalyzed Haber-Weiss produces $^{\bullet}O$)
3. SOD protects cell by scavenging $O_2^{\bullet -}$
   (but produces H_2O_2, toxic)

*Note – SOD is a primary antioxidant enzyme – acts on a ROS

2. Forms

Differences:
1. a.a. sequence

2. active metal site

3. cellular location

Table 1: Procaryotic Cells - SOD

	MW/Da	Subunits
FeSOD	40,000	2 dimer
MnSOD	40,000	2 dimer
	80,000	4 tetramer

Table 2: Eucaryotic Cells - SOD

	MW/Da	Subunits
MnSOD	88,000	4
CuZnSOD	32,000	2
ECSOD	135,000	4
ECMnSOD	150,000	2,4

3. Intracellular Location of SOD's

a. Procaryotes:
MnSOD – matrix (dimer)
Fe SOD – outer membrane

b. Eucaryotes:
CuZnSOD – cytoplasm, nucleus, lysosomes
MnSOD – mitochondrial matrix
EC(CuZn)SOD – plasma membrane, extracellular
ECMnSOD – plasma membrane

B. Catalase (CAT)

History

- Thernard, discoverer of H_2O_2, first noted in 1818 that animal tissues could decompose H_2O_2.
- 2.Loew in 1901 introduced the name catalase for the natural compound that decomposes H_2O_2.
- Wolft and de Stoecklin achieved first hemoglobin-free purification in 1910.

1. Functions

a. Enzymatic Functions

1. Catalytic

$$2\ H_2O_2 \xrightarrow{CAT} H_2O + O_2$$ * note oxygenates

2. Peroxidative first substrate is H_2O_2

$$ROOH + HQOH \longrightarrow QO + ROH + H_2O$$

A. $$H_2O_2 + AH_2 \longrightarrow A + 2\ H_2O$$

B. $$ROOH + AH_2 \longrightarrow H_2O + ROH + A$$

b. Biological Functions

1. Removes H_2O_2, adds O_2
2. Protects against lipid peroxidation
3. May participate in alcohol metabolism
4. In bacteria, low CAT mutants are hypersensitive to H_2O_2

In Drosophilia, null mutants age faster

Acatalasemia – Deficiency in human blood CAT. Oral ulcerations develop gangerene. No other long term effects. Neutrophil function is depressed.

2. Location & Forms

There are many forms of CAT. Most contain Fe – heme, but some contain Mn.
Most anaerobic bacteria do not contain CAT, most aerobic bacteria contain CAT.

a. E. Coli 2 CAT
1. HIP – periplasmic membrane
   - tetramer MW 337,000
   - 2 molecules of protoheme IX per tetramer
   - bifunctional: catalytic or peroxidative
   - inducible by H_2O_2 or ascorbate
   - increased during log growth

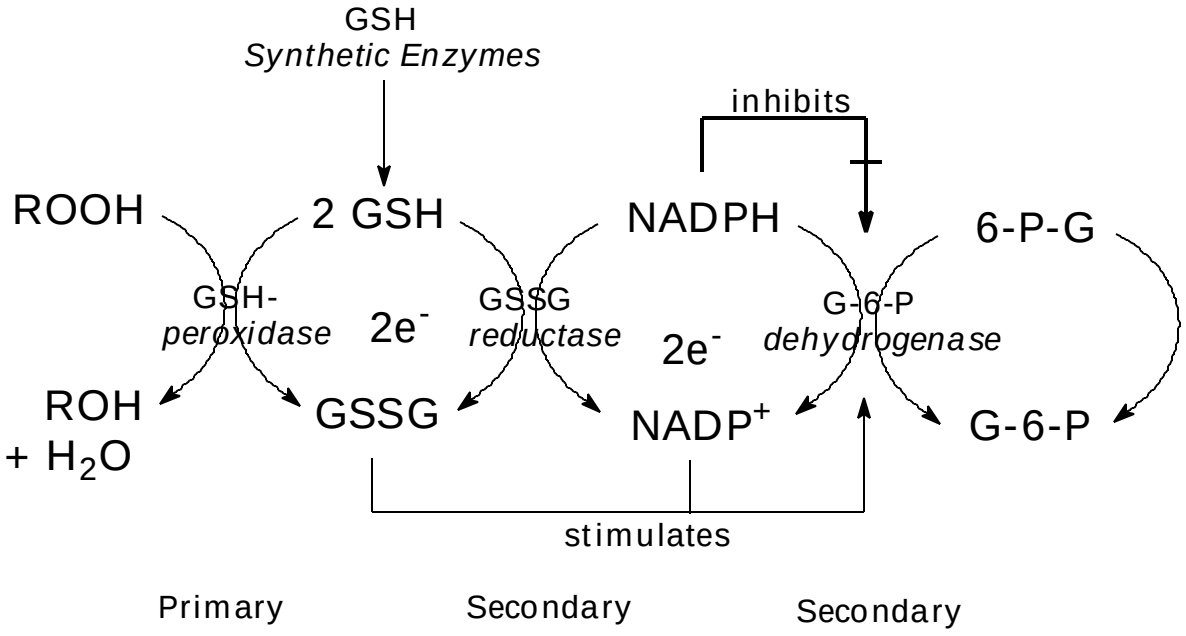
2. HPII – cytoplasmic
   - tetramer
   - 2 molecules of protoheme IX per tetramer
   - monofunctional, peroxidatic activity only
   - not inducible by H_2O_2 or ascorbate
   - increased during stationary phase of growth

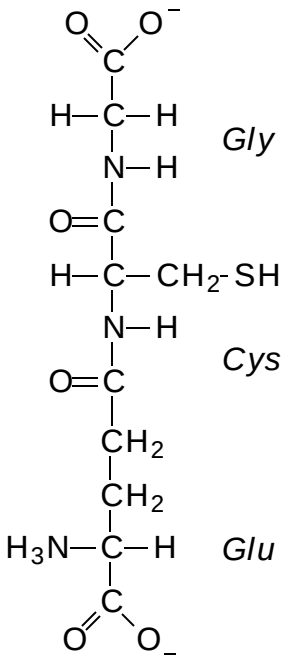
b. Maize – 3 CAT found in different cells and expressed differentially during development. All are tetramers of MW 240,000. Each has one or two amino acids different.

c. Human – At least 2 forms. Found in cytoplasm and peroxisomes. One report found CAT in cytoplasmic granules of eosinophils. *J Histochem Cytochem* **30**: 697, (1982). Tissues – Most in liver (hepatocyte, peroxisomes) and erythrocyte (cytoplasm). Some found in brain, heart, skeletal muscle, and kidney. Heart CAT found in mitochondria. B. Freeman, 1991.

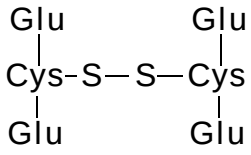
C. Glutathione – rids of H_2O_2 or ROOH (hydroperoxide)

1. General Scheme





Glutathione



Glutathione disulfide

1. Glutathione Peroxidase (GPx)

Discovered by Mills in 1957

A. Function

1. Enzymatic

$$ROOH + 2\,GSH \xrightarrow{GPx} ROH + H_2O + GSSG$$

- ❖ Unspecific for hydroperoxides. Can be about anything from H_2O_2 to peroxidized membranes and DNA.
- ❖ Specific for GSH. Similar compounds have little reactivity.
- ❖ It yields a single oxidation product, in contrast to heme peroxidases.

2. Biological
   Removal of H_2O_2:

   Genetic or alimentary deficiency in GPx suffer hemolytic episodes if exposed to drugs generating $O_2^{\bullet-}$, H_2O_2, or lipid peroxides.

   Removal of other hydroperoxides:
   - ❖ Protection against lipid peroxidation
   - ❖ Protection against DNA hydroperoxides

   Arachidonic acid cascade
   - ❖ Catalyzes formation of prostaglandins

B. Location and Forms
   GPx is not found in bacteria or higher plants, but found in all eukaryotes.

   Amounts: high (liver); moderate (heart, lung, brain); low (muscle).

   Four known forms:
   1. Cytosolic GPx (GPX-1)
      Bovine erythrocytes are usually studied; soluble.
      Tetrameric protein of MW = 85,000 Da
      Rat liver MW = 75,000 Da
      Human erythrocyte = 95,000 Da
      Human placenta = 85,500 Da
      Equal subunits of MW = 21,000 Da
      Each subunit contains a Se; no other metal.
      Active site contains a selenocysteine.

2. Human Plasma GPx
   Tetramer 21.5 to 22.5 kDa per subunit. One Se per subunit.
   1529 bp, 226 a.a. Synthesized and secreted by kidney.
   Distinct from cytosolic (49% homology) and phospholipid GPx.

3. "Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, $GSHP_x$ –GI." *J Biol Chem* **268:** 2571-2576, 1993.

   Tetrameric protein localized in cytosol.

   Monomer MW = 22,000, 190 amino acids.

   Similar substrate specificities as cytosolic GPx ($GSHP_x$ – 1).

   Both reduce H_2O_2, tert-butylhydroperoxide, amino hydroperoxide, and linoleic acid hydroperoxide, but not phosphatidylcholine hydroperoxide.

4. Phospholipid hydroperoxide glutathione peroxidase (PH-GPx).

   First isolated from pig heart in 1982. Active toward hydroperoxides of phospholipids. The rest of GPx's require phospholipase to clip hydroperoxides. Rat liver PH-GP-x needs detergent for activity, pig heart does not.

   Rat liver – monomer, MW = 22,000 Da
   Pig heart – monomer, MW = 20,000 Da

   Contains Se. Active site is conserved, but the rest of the protein is quite different. Homology is 25% for plasma EC-GPx and 35% for GPx (with PH-GPx) in terms of amino acids.

PH-GP-x occurs in two forms. Long form (23 kDa) is mitochondrial GPx and includes a signal peptide for transport to mitochondria. Short form (20 kDa) found in cytoplasm.

Long form, but not short form, protected strongly against apoptosis induced by glucose deprivation, or exposure to 2-deoxyglucose, etoposide, staurosporine, UV radiation, cycloheximide, and actinomycin D *J Biol Chem* **274:** 29294-29302, 1999. Results suggested that "hydroperoxide, produced in mitochondria, is a major factor in apoptosis and that mitochondrial PH-GPx might play a critical role as an anti-apoptotic agent in mitochondrial death pathways."

5. Mitochondrial matrix GPx – never isolated.

   MnSOD produces hydrogen peroxide in mitochondrial matrix and there is no CAT there, so it seems likely there is a peroxide-removing enzyme in the mitochondrial matrix. PH-GPx is in the mitochondrial membrane, not in the matrix. Recently, Shijun Li in my lab has transfected GPx1 and shown it goes into mitochondria. Unusual, since GPx1 has no mitochondrial signal sequence.

2. Glutathione-S-transferases (GSTs)

Non-Se containing GPx found in 1976 by Lawrence and Burk.

A. Mechanism:
   The enzymatic function is the same as glutathione peroxidase, *i.e.* rids cells of hydroperoxides.

   ❖ GST does not act on H_2O_2!

$$ROOH + GSH \xrightarrow{GST} GSOH + ROH$$

$$GSOH + GSH \xrightarrow{\text{nonenzymatic ?}} GSSG + H_2O$$

B. Function:
   1. Biological
      a. GST may function as GPx when Se is low.
      b. Detoxification of foreign compounds
         - conjugation with GSH (catalytic)
         - binding with ligands which are not substrates
         - covalent bond formation with very reactive compounds leading to inactivation and destruction of GST
      c. Conjugation reactions involving endogenous comounds, *i.e.* make steroids, prostaglandins, etc.

C. Location of GST:
   Eukaryotic cells: cytoplasm, nucleus, cell surface, not mitochondria

   Tissue: liver, red cell, intestine

   Accounts for 10% of soluble protein in liver – wow!

D. Structure of GST
Liver – dimer with 4 possible subunits

Ya(22,000 Da); Yb (23,500 Da); Yb'(23,500 Da); Yc (25,000 Da).

Subunits combine to form 6 isozymes:
YaYa, YaYc, YcYc, YbYb, YbYb', Yb'b'

Only proteins with Ya or Yc exhibit high GPx activity.
In other organs there are other subunits, *i.e.* placental Yp – correlates with liver cancer.

Yb GST is a major glucocorticoid binding protein.

3. Glutathione Reductase (GR)

Function

A. Enzymatic

$$GSSG + NADPH + H^+ \xrightarrow{GR} NADP^+ + 2\ GSH$$

Other substrates besides GSSG: only mixed disulfides between GSH and γ-glutamylcysteine or CoA.

B. Biological

- Removes GSSG, which is toxic.
- Keeps GSH in reduced form so it can be used.
- There are families with low levels of GR in red cells. OK under normal circumstances, but under oxidative stress, red cells hemolyze.
- Location in eukayrotic cell: cytoplasm, mitochondria
- Where is the signal sequence?

Thioredoxin Peroxidases

Thioredoxin (TRX) – is a low molecular weight protein found in both prokaryotic and eukaryotic cells. MW of human protein is 11,500.

The cysteine residues at conserved Cys32-Gly-Pro-Cys35-Lys undergo reversible oxidation reduction catalyzed by NADPH-dependent selenium-containing flavoprotein TRX reductase.

TRX can stimulate growth and inhibit apoptosis. Can be translocated into the nucleus to activate transcription factors.

Protein serves as hydrogen donor for ribonucleotide reductase, protein sulfoxide methionine reductase, TRX peroxidase, and protein tyrosine phosphatase.

TRX1 is located in cytosol, nucleus and also secreted. TRX2 only in mitochondria.

1. TRX reductase – TRXR or TR

Converts oxidized TRX to reduced form using electrons from NADPH.

$$TRX{-}S{-}S{-}TRX + 2H^{+} \xrightarrow{TRXR} 2\ TRX{-}SH$$

- Homodiomeric protein with a redox active disulfide and one FAD per subunit.
- Belongs to superfamily of flavoprotein disulfide reductases that includes GR, dihydrolipoamide reductase, mercuric reductase, and alkylhydroperoxide reductase.
- Mammalian TRXR has sequence Gly-Cys-Secys-Gly.
- TRSR1 in cytoplasm, TRXR2 in mitochondria. *J Biol Chem* **274:** 4722-4734,1999.

2. TRX peroxidase – TRXP or TPX

Can remove hydrogen peroxide from cells. Analagous to GPX.

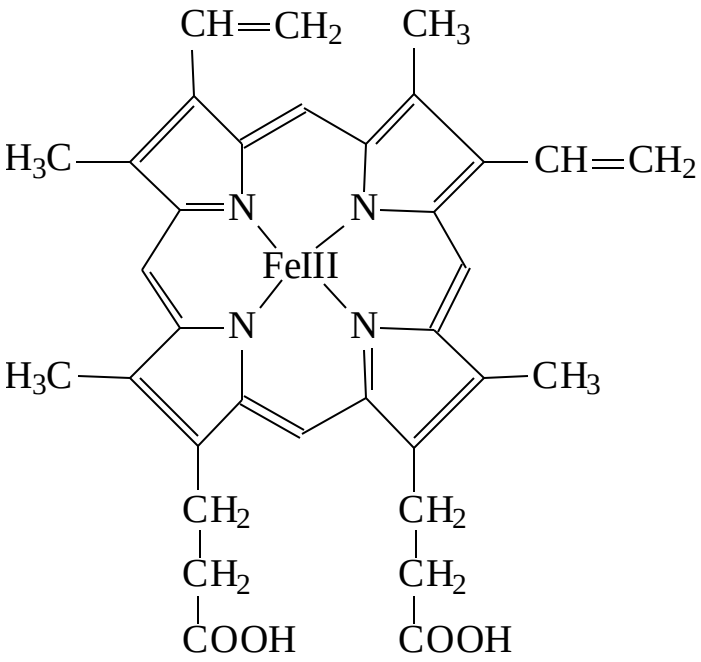
$$2\ TRX{-}SH + H_2O_2 \longrightarrow TRX{-}S{-}S{-}TRX + 2\ H_2O$$

- Some forms can remove phospholipid hydroperoxides.
- Prevents apoptosis.
- Different forms found in cytoplasm and mitochondria.
- Also called peroxiredoxins.
    - Exhibit hydrogen peroxide and alkyl hydroperoxide reductase activities.
    - There are six subfamilies based on amino acid structure.
    - For many of these proteins, endogenous sulfhydryl cofactor still not known.

Other Antioxidant Enzymes

Nonspecific peroxidases

- Non specific means there are lots of substrates. Substrates *in vivo* are often unknown.
- Usually assayed with artificial substrates (guaicol, benzidine, o-dianisidine, etc.)
- Found exclusively in plants and bacteria. Found less often in animal systems.
- Usually are heme containing.



Examples in Animal Tissue

A. Myeloperoxidase (MPO)

Found in mature neutrophils. Must abundant protein in neutrophils (10% protein).

Function - Enzymatic

$$H_2O_2 + X^- + H^+ \xrightarrow{MPO} HOX + 2\ H_2O$$

Usually, X = Cl^-, so the product is HOCl $\rightarrow$ $^{\bullet}OH$, $^1O_2$
HOCl = hyopchlorous acid

Biological – kills bacteria, etc.

Location – cytoplasmic granules and membrane

Structure – MW 130,000 – 160,000 Da

B. Eosinophil peroxidase (EPO)

Found in eosinophils

Function – same as MPO, but uses Br^- predominantly (can also use SCN^-).

Location – cytoplasmic granules

Structure – Human EPO
715 a.a., MW 81,036 Da from cDNA
20-25 a.a. are for signalling

- Purified EPO is 70-72 kDA
one heavy chain 50-55 kDa
one light chain 10-15 kDa

- Homology between human EPO and MPO
72% cDNA
70% a.a.

C. Lactoperoxidase

Found in milk & saliva

Function – Enzymatic

$$SCN^- + H_2O_2 \longrightarrow H_2O + OSCN^-$$

(thiocyanate) (hypothionate, toxic)

- Biological
  Kills bacteria, H_2O_2 comes from bacteria

D. Ovoperoxidase

Found in egg granules; hardening of egg.

Polyspermy inhibited by respiratory burst, production of H_2O_2, and crosslinking of tyrosines in egg membrane to toughen it.

E. Thyroid peroxidase

Function – Iodination of phenolic ring of tyrosine in thyroglobulin.
- Coupling the iodinated tyrosines from iodothyronines. The H_2O_2 needed is supplied by membrane NADPH-oxidase.

Location – Membrane bound hemeprotein

Medical importance – Makes thyroid hormone
- Target Ag in autoimmune destruction of thyroid in Hashimoto's thyroiditis. Ab attacking thyroid peroxidase.

Repair Systems

A. Radical Repair – vitamins C & E, but rely on enzymes for recycling.

B. DNA Repair – specific enzymes

C. Protein Repair *via* degradation of abnormal proteins. Not really repair, proteolysis.

D. Lipid Repair – PLA_2 and FA-CoA.