Thioredoxin and glutaredoxin systems

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ROLE OF THIOREDOXIN AND GLUTAREDOXIN SYSTEMS IN DNA SYNTHESIS

**THE THIOREDOXIN SYSTEM**

\[ \text{NADPH} + \text{H}^+ \xrightarrow{\text{THIOREDOXIN REDUCTASE}} \text{NADP}^+ \]

\[ \text{Trx-S}_2 \xrightarrow{\text{RIBONUCLEOTIDE REDUCTASE}} \text{Trx(SH)}_2 \]

\[ \text{rNDP} \xrightarrow{\text{RIBONUCLEOTIDE REDUCTASE}} \text{dNDP} \rightarrow \rightarrow \text{dNTP} \rightarrow \text{DNA} \]

**THE GLUTATHIONE REDUCTASE**

\[ \text{GSSG} \xrightarrow{\text{GLUTATHIONE REDUCTASE}} 2\text{GSH} \]

\[ \text{NADPH} + \text{H}^+ \xrightarrow{} \text{NADP}^+ \]
RNR Subunits

- **R1 Subunit**
  - Substrate (NDPs) binding site
  - Allosteric effector (ATP/dNTPs) binding sites
  - Redox-active —SH groups

- **R2 Subunit**
  - A diferric-tyrosyl radical cofactor

**The expression and activity of RNR subunits are highly regulated to maintain an optimal dNTP pool, which is required to maintain genetic fidelity**
In an S-phase T cell > 100 000 disulfides are formed per second!

Mammalian RNR is different from the *E.coli* enzyme: Zahedi Avval & Holmgren  JBC, 284, 8233-8240, 2009
Recombinant RNR subunits were used to evaluate/characterize the kinetics of the electron donors. Further characterization of the kinetics should contribute insights into the mechanism of mammalian RNR reaction.
RNR Activity & Cell Cycle - Ribonucleotide Reductases are critical for cell cycle progression

Proliferating S-phase cell

- Nucleus
- Mitochondrion
- Circular DNA
- Replicating DNA
- R1/R2 RNR
- dNTPs for DNA replication
- R1/p53R2 RNR
- dNTPs for basal DNA repair

Nonproliferating G0/G1 cell
The recently identified p53R2 regulatory subunit of RNR is directly induced by p53 and functions as a catalytic partner of the regulatory subunit of R1

A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage

Hiroshi Tanaka, Hirofumi Arakawa, Tatsuya Yamaguchi, Kenji Shiraishi, Seisuke Fukuda, Kuniko Matsui, Yoshiki Takei & Yusuke Nakamura

NATURE | VOL 404 | 2 MARCH 2000
Reductants for R1-p53R2 complex

R1 + p53R2 + DTT/Trx/Grx1

+ 10 mM GSH

+ 5 mM GSH

[\text{[dCDP]} \text{ nmol}]

DTT
Trx
Grx1
Grx1
Electron transport in RNR

Mammalian cells!
**Conclusions**

The mechanism of human ribonucleotide reductase is different from that of E.coli and glutaredoxin functions via a monothiol mechanism and in principle any glutaredoxin (dithiol) can act together with GSH.

Since GSH is high (4-10 mM) in cells this may act to ensure the supply of dNTPs for DNA repair (BER with APE-1/Ref1) in nondividing cells with very low RNR levels.

Thioredoxin system is high capacity for S-phase or when GSH is low or oxidized. Tumors have often very upregulated RNR. Unbalanced dNTP pools favour genetic instability?

But if Se is low? TrxR not active- use GSH system plus high RNR? p53R2 is constitutive for DNA repair and mitochondrial DNA synthesis in nondividing cells.
Thioredoxin System

Glucose → 100 µM NADPH+H+ (Pentose cycle)

1 µM TR-S₂ FAD → 1-20 µM Trx-(SH)₂

many µM Protein-(SH)₂

external Trx
Asp26 and Lys 57 in E. coli Trx aid in catalysis
Mechanism of thioredoxin catalysis
Rate Constants

(1) Trx-(SH)$_2$ + H$_2$O$_2$ $\overset{\text{slow}}{\longrightarrow}$ Trx-S$_2$ + 2H$_2$O

(2) Trx-(SH)$_2$ + insulin-S$_2$ $\overset{k_2=5\times10^4 \text{ M}^{-1}\text{s}^{-1}}{\longrightarrow}$ Trx-S$_2$ + insulin-(SH)$_2$

(3) Trx-S$_2$ + DTT $\overset{k_2=1500 \text{ M}^{-1}\text{s}^{-1}}{\longrightarrow}$ Trx-(SH)$_2$ + DTT

(4) DTT + insulin-S$_2$ $\overset{k_2=3 \text{ M}^{-1}\text{s}^{-1}}{\longrightarrow}$ DTT + insulin-(SH)$_2$

(5) DTT + insulin-S$_2$ catalyzes Trx $\downarrow$ DTT + insulin-(SH)$_2$ free A and B chain which precipitates

*from A. Holmgren, J. Biol. Chem., 254, 9627-9632, 1979*
Thioredoxin

- thiol redox control
- phage f1, T7
- T7 DNA pol
- chloroplast photosynthesis regulation
- NO
- nitrogen fixation regulation
- cytochrome biogenesis
- Trx

- Chemotactic factor
- growth factor
- Trx80 (monocytes)
- reductive enzymes
  - rNDP -> dNTP -> DNA
  - MetSO -> Met-
  - SO4^2- -> SO3^2- -> Cys

- Peroxiredoxins (Prx 1-6)
- Trx fold proteins

- S-S in proteins and folding
  - Protein disulfide isomerase (PDI) family
  - 24 genes in man
Thioredoxin (Trx)

- Trx is upregulated by oxidative stress
- Trx in oxidized form is present in plasma
- Trx added to medium is taken up into cells
- High Trx protects from inflammatory cells
- Trx stimulates GSH synthesis
- Trx stimulates cystine transport
- Trx is a growth factor for monamine neurons
- Trx is a cocytokine involved in cell growth
- Trx is controlled by TXNIP (TBP2)
Human Trx1
Regulation of hTrx activity via Cys-62, Cys-69 and Cys-73

- Redox signaling by hydrogen peroxide via induction of NADPH oxidases may reversibly inactivate Trx in an autoregulatory process via two disulfides.
- GSNO will S-nitrosylate Cys residues and inactivate catalytic activity; but reversibly. Either Cys-62 and Cys-69 in fully reduced protein, or Cys-73 in the two-disulfide oxidized protein.
- Thioredoxin system controls S-nitrosylation of proteins. Trx acts as a donor of NO by transnitrosylation or in denitrosylation reactions.
Oxidative stress + Nitrosative stress

Human Trx

Reduced Trx

Protein-disulfide reductase activity in the presence of NADPH and TrxR

Human Trx

Nitrosylation of other proteins, e.g. caspase 3

Secretion?

Generation of Trx80?

Cell membrane
Thioredoxin System

Glucose

Conc: 100 \text{ \( \mu \)M} NADPH+H^+

Pentose cycle

NADP^+

1 \text{ \( \mu \)M} \text{TR-S}_2\text{FAD}

1-20 \text{ \( \mu \)M} \text{Trx-(SH)}_2

many \text{ \( \mu \)M} \text{Protein-(SH)}_2

external Trx
The Swedish scientist, JÖNS JACOB BERZELIUS (1779–1848), is regarded by many as the 'Father of Modern Chemistry'. Although trained as a physician, his natural interest was in chemistry. Through his studies he discovered the elements Silicon, Cerium, Selenium, and Thorium. He was the first to apply a standard symbol and name to the elements. In 1808 he wrote a chemistry book that was the standard text for 30 years. He also published a yearly review of chemical studies from 1831 to 1848. He was the recipient of many awards during his lifetime for his contributions to the advancement of chemistry.

DIMITRY MENDELEYEV (1834–1907), was born in Tobolsk, Siberia, and studied science at the University of St. Petersburg. In 1866 he devised the first logical arrangement of the 63 known elements, which he called 'The Periodic Table of the Elements.' Because of the precise organization of this table he was able to predict the existence of many elements that were as yet unknown, including Gallium and Germanium. Element number 101, Mendelevium, is named in his honor.

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The periodic table of the elements.
a H₂N- FAD NADP(H) Central Interface COOH

- Gln - Ala - Gly - Cys - SeCys - Gly - Ter (human TrxR)

--- CAG GCT GGC TGC TGA GGT TAA ---

... ... ... ... ...

--- CAG TCT GGC TGC TGA GGT TAA ---

- Gln - Ser - Gly - Cys - SeCys - Gly - Ter (rat TrxR)

b cDNA encoding selenium-containing rat TrxR

TGA

--- Open reading frame --- 3'-UTR ---

SECIS

c cDNA encoding selenium-deficient rat TrxR mutant enzymes

TGC (Cys)
TCA (Ser)
TAA (Stop)

--- Open reading frame ---
Fig. 6. Model of the complex of rat TrxR with human Trx. Color coding for TrxR is as in Fig. 3, Trx is shown in green. Residues at the Trx–TrxR interface are shown as stick models. The positions of the sulfur atoms of the catalytic disulfide (Cys-59–Cys-64) and the cysteine residues Cys-32, Cys-35, Cys-497, and Cys-498 are indicated by yellow spheres.
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THE THIOREDOXIN SYSTEM

NADPH + H+ \rightarrow \text{Trx-S}_2 \xrightarrow{\text{TRX REDUCTASE}} \text{Trx(SH)}_2 \rightarrow \text{Trx-S}_2 \xrightarrow{\text{TRX REDUCTASE}} \text{NADP}^+ \\

THE GLUTAREDOXIN SYSTEM

NADPH + H+ \rightarrow \text{Grx-(SH)}_2 \xrightarrow{\text{GRX REDUCTASE}} \text{Grx-S}_2 \rightarrow \text{Grx-(SH)}_2 \rightarrow \text{GSSG} \rightarrow 2 \text{GSH} \rightarrow \text{NADP}^+ \\

\text{rNDP} \xrightarrow{\text{RIBONUCLEOTIDE REDUCTASE}} \text{dNDP} \rightarrow \text{dNTP} \rightarrow \text{DNA}
Glutaredoxin System

Concentration:
- 100 μM NADPH+H⁺
- 1 μM GR-S₂
- 1-10 mM 2GSH
- 1-20 μM Grx-S₂
- many μM Protein-(SH)₂

Reactions:
- NADP⁺ → GR-S₂ → FAD → GSSG → Grx-(SH)₂ → Protein-S₂
Solution structure of E.coli Grx1 in complex with GSH
Mixed disulfide mechanism

Dithiol mechanism
Human cells have two Grx genes. Grx1 is the classical enzyme in the cytosol/nucleus or secreted and extracellular in plasma. Grx2 has splice forms with Grx2a targeted to mitochondria. Grx2 is unusual........
Mitochondrial Grx2 can be reduced both by GSH and TrxR. Mixed disulfides with GSH are important substrates and Grx2 controls glutathionylation of proteins in e.g. complex 1.
Gene silencing of hGrx2

siRNA-mediated gene silencing of hGrx2 in HeLa cells

Kinetic of the knock down using siRNAT28
Gene silencing of hGrx2

adriamycin/doxorubicin

✗ anthracycline antibiotic
✗ anti-cancer drug
✗ induction of mitochondrial apoptosis via formation of ROS
✗ formation of complexes with iron
✗ toxicity is iron-dependent
✗ highest toxicity in heart muscle cells
Gene silencing of hGrx2

Viability of HeLa (+/- Grx2) after Adriamycin/doxorubicin treatment

**LD50**
- Grx2^+: 50 µM
- Grx2^-: 0.5 µM

![Graph showing viability of HeLa cells with and without Grx2 expression after doxorubicin treatment](image)
hGrx2 binds a [2Fe2S]-cluster
Stability of Grx2 iron-sulfur complex: Stabilized by GSH but destroyed by oxidation
crystal structure of hGrx2 (2fls)
protein de-/glutathionylation

anti-apoptotic

reduction of low molecular weight disulfides

**active apo-Grx2** (stressed cells)

**inactive holo-Grx2** (normal cells)

Fe\(^{2+}\) Cys GSH

iron-sulfur assembly machinery

oxidative stress

GSSG, oxidation

free GSH
Model for the disulfide pairing and nitrosylation of human Grx1 and Grx2 cysteine residues
Summary I

• Trx, TrxR and NADPH exist in all cells and is a 4 billion years old system as a general protein disulfide reductase.
• Trx has a CXXC active site where the N-terminal C residue is the nucleophilic thiolate operating via a mixed disulfide mechanism.
• Mammalian cells have Trx1 in cytosol with 3 structural Cys residues which can regulate activity by making two disulfides. In contrast mammalian Trx2 in mitochondria is more similar to bacterial Trx.
• TrxRs in bacteria, yeast and plants are smaller specific enzymes with a CXXC active site.
• TrxR in mammalian cells are larger and selenoenzymes with a broad substrate specificity and have a C-terminal GCUG active site where U is a selenocysteine residue.
• TrxR1 is cytosolic, TrxR2 is mitochondrial and TGR is thioredoxin-glutathione reductase with a Grx domain particularly in testes and the only enzyme in some parasitic worms.
Summary II

- Glutathione (GSH) is present in high concentrations in almost all cells (grampositive bacteria have no GSH like Staph. aureus). Made by two enzymes from Glu, Cys and Gly. GSH/GSSG (>100:1) is very high in the cytosol and particularly during DNA replication when GSH is accumulated in the nucleus.
- Grx catalysis GSH-disulfide-oxidoreductions and is a thioredoxin fold protein with a GSH binding site recognizing GSH for its reduction of the active site and uses GSH-mixed disulfides as substrates. Large number of proteins are regulated by glutathionylation and Grx.
- In contrast nitrosylation and denitrosylation of proteins is catalyzed by thioredoxins if not directly by GSNO and GSH for nonenzymatic nitrosylation and denitrosylation.
- Grx1 and Grx2 are dithiol glutaredoxins in mammalian cells where Grx2 has splice forms with Grx2a in the mitochondria. Grx2 is also present as an inactive dimeric 2 Fe-2S iron sulfur protein. The monothiol glutaredoxin Grx5 is also a 2Fe2S protein involved in heme synthesis.
- Trx and Grx function in a large number of reactions in cells with overlapping functions in some reactions like electron transport to ribonucleotide reductase.