Thioredoxin and glutaredoxin systems

Arne Holmgren

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden
arine.holmgren@ki.se
ROLE OF THIOREDOXIN AND GLUTAREDOXIN SYSTEMS IN DNA SYNTHESIS

THE THIOREDOXIN SYSTEM

NADPH + H+  \[\text{THIOREDOXIN REDUCTASE}\] \[\rightarrow\] Trx-S₂  \[\rightarrow\] Trx(SH)₂

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

THE GLUTAREDOXIN SYSTEM

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

NADPH + H+  \[\rightarrow\] 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
Molecular Mechanisms of Thioredoxin and Glutaredoxin as Hydrogen Donors for Mammalian S Phase Ribonucleotide Reductase

Farnaz Zahedi Avval¹ and Arne Holmgren²

From the Medical Nobel Institute for Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, SE-17177 Stockholm, Sweden

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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
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, Hirotumi Arakawa, Tatsuya Yamaguchi, Kenji Shiraiishi, Seisuke Fukuda, Kuniko Matsui, Yoshiki Takei & Yusuke Nakamura

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

R1 + p53R2 + DTT/Trx/Grx1

- DTT
- Trx
- Grx1

+ 5 mM GSH
+ 10 mM GSH

[dCDP] nmol

0 1 2 3

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

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

(2) \( \text{Trx-(SH)}_2 + \text{insulin-S}_2 \rightarrow \text{Trx-S}_2 + \text{insulin-(SH)}_2 \) \( k_2 = 5 \times 10^4 \text{ M}^1\text{s}^{-1} \)

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

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

(5) \( \text{DTT} + \text{insulin-S}_2 \) catalyzes \( \text{Trx} \)

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

- Thiol redox control
- Phage fl, T7
- T7 DNA pol
- Chloroplast photosynthesis regulation
- NO
- Nitrogen fixation regulation
- Cytochrome biogenesis
- S-S in proteins and folding
- Protein disulfide isomerase (PDI) family
- 24 genes in man

Chemotactic factor

Growth factor

Trx80 (monocytes)

Reductive enzymes

rNDP $\rightarrow$ dNTP $\rightarrow$ DNA

-MetSO-$\rightarrow$ -Met-

SO$_4^{2-}$ $\rightarrow$ SO$_3^{2-}$ $\rightarrow$ Cys

Peroxiredoxins (Prx 1-6)

Trx fold proteins
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 co-cytokine involved in cell growth
- Trx is controlled by TXNIP (TBP2)
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
Trx1 Redox Western Blot after IAA alkylation in 8 M urea
Redox state of Trx1 in HeLa cells treated by ATG or Auranofin

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-0 SH -1 SH -2 SH -3 SH -4 SH -5 SH

Du, Y et al unpublished results
Redox state of Trx2 in HeLa cells treated by ATG or Auranofin

Control  5  10  100  2  5  10 (μM)
ATG
Auranofin

-0 SH
-1 SH
-2 SH
HeLa cells viability of combined treatment by ATG with Ebselen
Redox shift of Trx1 and Trx2 in HeLa cells treated by Aurothioglucose with Ebselen
GSH reduces TrxS$_2$

![Graph showing the effect of GSH on TrxS$_2$ reduction](image)

**Insulin assay**
Trx1 redox signaling

Trx(SH)$_5$ → TrxS$_2$ → TrxS$_4$ (Inactive)

Sub(ox) → Sub(re) → H$_2$O$_2$ → H$_2$O

GSH system

Cell membrane

Oxidative stress
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 65 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.

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.
b  cDNA encoding selenium-containing rat TrxR

Secis | SECIS
---+---

TGA

Open reading frame  3'-UTR

TGA

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.
Glutaredoxin System

- Concentration: 100 µM NADPH+H^+ to NADP^+
- 1 µM GR-S2 to GR-(SH)_2
- 1-10 mM 2GSH to GSSG
- 1-20 µM Grx-S2 to Grx-(SH)_2
- Many µM Protein-(SH)_2 to 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

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<th>LD50</th>
<th>Grx2+</th>
<th>50 µM</th>
<th>Grx2-</th>
<th>0.5 µM</th>
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![Graph showing viability of HeLa (+/- Grx2) after adriamycin/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

Fe\textsuperscript{II} Cys GSH

iron-sulfur assembly machinery

oxidative stress

GSSG, oxidation

active apo-Grx2 (stressed cells)

inactive holo-Grx2 (normal cells)

free GSH
A NOVEL BIOLOGICALLY ACTIVE SELENO-ORGANIC COMPOUND—I

GLUTATHIONE PEROXIDASE-LIKE ACTIVITY IN VITRO AND ANTIOXIDANT CAPACITY OF PZ 51 (EBSELEN)

ARMIN MULLER, ENRIQUE CADENAS, PETER GRAF and HELMUT SIES
Institut für Physiologische Chemie I, Universität Düsseldorf, Moorenstrasse 5, D-4000 Düsseldorf 1
Federal Republic of Germany

A NOVEL BIOLOGICALLY ACTIVE SELENO-ORGANIC COMPOUND—II

ACTIVITY OF PZ 51 IN RELATION TO GLUTATHIONE PEROXIDASE

ALbrecht WENDEL,* Martina FAUZEL, Hasan SAFAYHI, Gisa TIEGS and Rainer Otter
Physiologisch-chemisches Institut der Universität, Hoppe-Seyler Str. 1, 7400 Tübingen, Federal Republic of Germany
Novel antibiotic principle for GSH negative bacteria targeting TrxR

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 catalyse 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 S-nitrosylation and S-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.