Glutaredoxin And Thioredoxin Systems In The Ocular Lens And Their Relation To Cataract

Marjorie F. Lou
University of Nebraska-Lincoln
USA
Cataract: A degenerative disease

- Cataract is the leading cause of blindness in the world
- 45% of all blindness, 20 million/yr and growing (WHO)
- 50% of the population over 65 will develop cataract
- Cataract surgery is the only effective way to restore vision, but with some degree of complications (PCO)
- Huge expense in the US healthcare ($3.5 billion/year)
- A major cause of blindness in the 3rd world countries
Structure of Human Eye

- Cornea
- Lens
- Retina
- Optic Nerve
Figure 3: Comparison of Normal Human eyes vs Cataract eyes.

Normal: Clear lens, no opacity.

Cataract: Opaque lens, cloudiness.

Left images: Appearance of the eye (front view).
Right images: Appearance of the lens (side view).
Figure 3: Vision from a clear and an opaque lens

Normal vision

Cataract vision
Schematic Diagram of a Mammalian Lens

- A non-neuronal, non-vascular and transparent tissue
- No organnelle in differentiated fiber cells
- Highest GSH in the body (4 – 8 mM)
- High Protein content----30% of its wet weight

Excellent Research Model for studying Aging and Protein modifications
Association of oxidative stress with eye diseases--cataract

The evidences in the literature
Oxidation-related Biochemical Changes Found in Human Cataracts

- Decrease in protein –SH
- Increase in protein –SS-
- Protein high molecular weight (HMW) aggregates
- Methionine sulfoxide and cysteic acid in proteins
- Increase in PSSG and PSSC
- Loss of GSH
- DNA damage
- lipid peroxidation
- Elevation of $\text{H}_2\text{O}_2$ in Aqueous
- Epidemiological studies
Oxidation-related Biochemical Changes Found in Human Cataracts

- Decrease in protein –SH
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Oxidation Of Protein Thiols

Protein $\text{-SH}$

- $\text{-S-SX}$ Mixed Disulfide
- $\text{-SO}_2\text{H}$ or $\text{-SO}_3\text{H}$
- $\text{-S-S}$
- $\text{PSSP}$

$X$: Glutathione, Cysteine
GSH Pool Decreases with Aging in Normal Human Lenses

Age (years)

GSH (µmol/g wet wt)
Oxidation of Protein Thiols Increases With Aging in Normal Human Lenses
Cataractous Human Lens:

*Low in GSH and

*High in PSSP

*High Molecular Weight Aggregates
Protein-thiol mixed disulfides:
PSSG and PSSC may be an intermediate step in protein aggregation and cataract formation
Dynamics of GSH in the eye lens

- Cysteine
- Glycine
- Glutamic acid
- GSH
- PS
- SG
- GSSG
- PSSP
- NADP⁺
- NADPH
- GR
- [O]
- X

Processes:
- GSH to GSSG
- GSSG to GSH
- GSH to PSSP
- PSSP to GSH
Mechanism for Oxidative Cataract
The Possible Role of Protein-thiol Mixed Disulfide (PSSG)

Native Protein → GSH → [O] → GSSG

Modified Protein

Changed Protein Conformation

Insolubility
Opacification
Pigmentation

PSSP and Other Forms of Protein conjugates
Quantification of Protein-thiol Mixed Disulfides

\[
\begin{align*}
\text{S} & \text{S-Cysteamine} \quad \text{[O]} \\
\text{S} & \text{S-Cysteine} \\
\text{S} & \text{S-Glutathione}
\end{align*}
\]

\[
\begin{align*}
\text{SO}_3\text{H} & \\
\text{SO}_3\text{H} & \\
\text{SO}_3\text{H}
\end{align*}
\]

+ Taurine
Cysteic acid
Glutathione sulfonic acid (GSO_3H)

Released in solution:
Taurine
Cysteic acid
GSO_3H

10% TCA

Protein PPT (discarded)

Anion Exchange
Amino acid analyzer
In a normal lens, only < 1% of GSH is bound to protein as PSSG

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>Age</th>
<th>GSH</th>
<th>Mixed disulfides†</th>
<th>GSO$_3$H</th>
<th>CSO$_3$H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey (3)</td>
<td>1 yr</td>
<td>3.33±0.15</td>
<td>0.013±0.001</td>
<td>0.010±0.001</td>
<td></td>
</tr>
<tr>
<td>Bird (emu)(3)</td>
<td>3 yr</td>
<td>1.48±0.08</td>
<td>0.023±0.003</td>
<td>0.020±0.001</td>
<td></td>
</tr>
<tr>
<td>Rat (6)</td>
<td>1.5 m</td>
<td>4.48±0.43</td>
<td>0.014±0.010</td>
<td>0.320±0.100</td>
<td></td>
</tr>
<tr>
<td>Squirrel (4)</td>
<td>Adult</td>
<td>6.75±1.05</td>
<td>0.140±0.020</td>
<td>0.060±0.030</td>
<td></td>
</tr>
<tr>
<td>Dog (3)</td>
<td>1 yr</td>
<td>8.00±0.55</td>
<td>0.260±0.050</td>
<td>0.060±0.010</td>
<td></td>
</tr>
<tr>
<td>Pig (4)</td>
<td>6 m</td>
<td>6.94±0.67</td>
<td>0.041±0.010</td>
<td>0.035±0.020</td>
<td></td>
</tr>
<tr>
<td>Guinea pig (3)</td>
<td>2 yr</td>
<td>9.59±0.50</td>
<td>0.406±0.040</td>
<td>0.024±0.002</td>
<td></td>
</tr>
<tr>
<td>Bovine (2)</td>
<td>6 m</td>
<td>7.54±0.59</td>
<td>0.180±0.070</td>
<td>0.110±0.020</td>
<td></td>
</tr>
<tr>
<td>Rabbit (6)</td>
<td>3 m</td>
<td>11.41±0.50</td>
<td>0.191±0.050</td>
<td>0.025±0.003</td>
<td></td>
</tr>
<tr>
<td>Human (6)</td>
<td>19-20 yr</td>
<td>2.62±1.30</td>
<td>0.220±0.150</td>
<td>0.040±0.013</td>
<td></td>
</tr>
</tbody>
</table>

* Data is expressed as μmole/g wet weight, mean±S.D.; n, number of samples.
†GSO$_3$H represents PSSG and CSO$_3$H represents PSSC.
PSSG only forms under oxidative stress

<table>
<thead>
<tr>
<th>Rat lens</th>
<th>Free GSH*</th>
<th>Mixed disulfides*†</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GSO₃H</td>
<td>CSO₃H</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3.766 ± 0.193</td>
<td>0.0142</td>
<td>0.0990</td>
<td></td>
</tr>
<tr>
<td>Glucose (30 mM)</td>
<td>2.582 ± 0.419</td>
<td>0.0200</td>
<td>0.1197</td>
<td></td>
</tr>
<tr>
<td>H₂O₂ (0.5 mM)</td>
<td>1.891 ± 0.444</td>
<td>0.2700</td>
<td>0.1213</td>
<td></td>
</tr>
<tr>
<td>H₂O₂ + Glucose</td>
<td>1.316 ± 0.448</td>
<td>0.2714</td>
<td>0.1219</td>
<td></td>
</tr>
</tbody>
</table>

*Data is expressed as μmole/g wet weight of lens; mean ± S.D. (n= 4)
†Analysis data obtained from four pooled lenses.
GSO₃H : PSSG; CSO₃H: cysteic acid
PSSG forms in all oxidant-induced Cataracts

<table>
<thead>
<tr>
<th>Cataract models</th>
<th>PSSG</th>
<th>PSSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{O}_2$</td>
<td>↑↑↑</td>
<td>No change*</td>
</tr>
<tr>
<td>Hyperbaric oxygen</td>
<td>↑↑↑</td>
<td>↑↑↑↑↑</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>↑↑↑↑</td>
<td>No change*</td>
</tr>
<tr>
<td>Photo-oxidation</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>UVA</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>X-ray</td>
<td>↑</td>
<td>↑↑↑↑</td>
</tr>
<tr>
<td>Selenite (mature)</td>
<td>−</td>
<td>↑</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

* PSSC forms after longer period of stress
PSSG and PSSC increase with age in the human lenses

Dickerson and Lou, 1992
Reversal of H$_2$O$_2$-induced Protein thiolation in Rat Lens Organ Culture

- Media containing 0.5 mM H$_2$O$_2$
- Media-free H$_2$O$_2$-treated

Graph A:
- Incubation Time (hours)
- GSH (μmole/g dry wt.)

Graph B:
- Incubation Time (hours)
- GSSG (μmole/mg protein)

- ○ Control
- ● H$_2$O$_2$-treated
- Recovery
The Thiol Oxidation Damage Repair Systems

- **GSH-dependent Glutaredoxin system**

\[ \text{GSH} \xrightarrow{\text{Grx}} \text{PSH} \]
\[ \text{GSSG} \xrightarrow{\text{Grx}} \text{PSSP} \]

- **NADPH-dependent Thioredoxin system**

\[ \text{PSH} \xrightarrow{\text{TR}} \text{TRx-(SH)_2} \]
\[ \text{PSSP} \xrightarrow{\text{TR}} \text{TRx-S}_2 \]

Grx: Glutaredoxin
TRx: Thioredoxin
TR: Thioredoxin Reductase
Lens Grx

- Isolated and purified from bovine lens
- Purified recombinant human Grx
- Identical gene sequence and enzymatic properties same as Grx from other human tissues
- Prefer PSSG over PSSC as its substrate (dethiolation)
- Has dehydroascorbate reductase activity (reduces oxidized ascorbate)
- Has cytosolic Grx1 and mitochondrial Grx2
Functions of Grx in the lens:

* Dethiolate PSSG, PSSC in structural proteins
* Re-activate oxidation damaged key metabolic enzymes
* Control redox signaling
Function of Grx in the lens:

* Dethiolate PSSG, PSSC in structural proteins
  - $\alpha$, $\beta$ and $\gamma$ crystallin proteins
  - $\alpha$-crystallin is a chaperone protein; it protects other proteins from precipitation.
  - PSSG in alpha can inhibit its chaperone function
Grx can reduce the oxidized lens structural proteins (crystallins)

Function of Grx in the lens:

* Re-activate oxidation damaged key metabolic enzymes

Glyceraldehyde 3 phosphate dehydrogenase (G3PD) in cytosol
Complex 1 in mitochondria
The Glycolysis Pathway in the Lens

Under Oxidative Stress

GSH/GSSG ↓↓
PSSG ↑
PSSC ↑

Glycolysis ↓
Gluconeogenesis ↑
**In vitro Model System**---Human Lens Epithelial (B3) Cells (HLE B3) Treated by a Bolus of \( \text{H}_2\text{O}_2 \) (0.1 mM)

![Graph showing decreasing oxidative stress followed by recovery over time with \( \text{H}_2\text{O}_2 \) levels in the medium (mM)]
Reversal of PSSG and PSSC formed in HLE B3 cells after Exposing to a Bolus of H$_2$O$_2$ (0.1 mM)

G3PD loses activity under oxidative stress

G3PD: glyceraldehyde 3-phosphodehydrogenase

Min

Lactate
G3PD
H₂O₂

Xing and Lou, 2003
**Reactivation of Oxidatively-Damaged (inactivated) G3PD by Grx1 only**

<table>
<thead>
<tr>
<th>Condition</th>
<th>G3PD Activity (mU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
</tr>
<tr>
<td>None: 15 min in 0.1 mM H$_2$O$_2$</td>
<td></td>
</tr>
<tr>
<td>+ GR</td>
<td>~7</td>
</tr>
<tr>
<td>+GSH</td>
<td>~8</td>
</tr>
<tr>
<td>+GST</td>
<td>~12</td>
</tr>
<tr>
<td>+Grx 1</td>
<td>~20</td>
</tr>
<tr>
<td>+DTT</td>
<td>~25</td>
</tr>
</tbody>
</table>

**Legend**

- **Control**: original activity
- **None**: 15 min in 0.1 mM H$_2$O$_2$
- **GR**: glutathione reductase
- **GSH**: glutathione S-transferase
- **GST**: glutathione S-transferase
- **Grx**: glutaredoxin
- **DTT**: dithiothreitol

* p < 0.001

**15 min after H$_2$O$_2$ stress**
Transient up-regulation of Grx (TTase) under oxidative stress*

*Human lens epithelial cells

Raghavachari et al, 2001
Re-activation of G3PD depends on the amount of Grx activity in cells
Grx, but not TRx, can reactivation of G3PD activity in clear human lens.

Why?---likely G3PD is only being oxidized to PSSG but not to PSSP.
Regeneration of G3PD Activity in Human Cataractous Tissue by Grx (TTase)

Function of Grx2 in mitochondria

---Complex I protection

Grx2 protects cells from oxidative stress-induced apoptosis by---

• Protects complex I activity
• Preserves ATP generation in ETS
Mitochondria complex I function

• Complex I (NADH dehydrogenase) is the first complex in the mitochondrial respiratory chain.

• Complex I not only generates ATP but also plays a critical role in cell apoptosis.
Complex I activity in WT and Grx2 KO mouse lens epithelial cells

Under oxidative stress, the lens epithelial cells from Grx2 KO mouse lose more complex I activity and easily become apoptotic.
The lens epithelial cells from Grx2 KO mouse have lower ATP level, and decrease more under oxidative stress comparing with the cells from WT mouse.
Function of Grx in the lens:

* Control redox signaling

Growth factor, cytokine stimulate cell signaling by
- Generating ROS to targeting the oxidation sensitive PTPs
- Allowing Kinase to phosphorylate the Receptor
- Cell signaling begins
- PTP reactivated by Grx or Trx or both
- Phosphate is removed from the receptor
- Cell signaling is terminated
ROS controls Cell Signaling through thiol oxidation

PTP: protein tyrosine phosphatase
E: regulating enzyme—Grx or Trx or both

GF, cytokine
NOX 4

PTP
Active
SH
SSG (or SOH)

PTP Inactive

Receptor

Target

P-

X

P

+ P

ROS

Signalizing
The target of ROS in PDGF stimulation: Low molecular weight protein tyrosine phosphatase (LMW-PTP) and PDGFR

ROS signaling

active

inactive

Y857
Inactivation of Human Recombinant LMW-PTP by \( \text{H}_2\text{O}_2 \)

![Graph showing the inactivation of LMW-PTP activity with increasing \( \text{H}_2\text{O}_2 \) concentrations.](Image)
Re-activation of LMW-PTP by Grx (TTase) and TRx systems

![Graph showing the re-activation of LMW-PTP activity with different concentrations of GSH and different treatments. The graph compares DTT, GSH only, GSH+TTase, and GSH+TTase+TRx systems. The y-axis represents LMW-PTP activity in mU/μg protein, and the x-axis shows GSH concentrations in mM (0, 1, and 10 mM).]
LMW-PTP activity in PDGF–stimulated wild type (WT) and Grx1 knockout (KO) cells

Xing et al. BBA 1774:545 (2007)
PDGF-stimulated cell signaling

Xing et al. BBA 1774:545 (2007)
Thioredoxin system in the lens

1) Thioredoxin (cytosolic Trx1 and mitochondrial Trx2) and thioredoxin reductase are present in the lens

2) Trx and TR are transiently upregulated under oxidative stress

3) Trx system re-activates many essential proteins/ enzymes via PSSP dethiolation

4) Trx participates in redox signaling (reduce AP-1 bound Ref1 to activate AP-1 binding to DNA)

5) Anti-apoptosis by binding to ASK

6) TBP-2 is present to negatively regulate its bioavailability
Trx System Can Re-activate G3PD From Human Cataractous Lenses:
Why? --- Likely G3PD has been oxidized to both PSSG and PSSP

Yan et al., Mol Vis (2006)
Under oxidative stress, cell increases its reducing power by suppressing TBP-2 expression and upregulating Trx.

Dose of oxidation: 0.1 mM H$_2$O$_2$ HLE-B3 cells

Liyanage et al. EER (2007)
The relationship of Grx and TRx systems in aging and cataractous human lenses

* Age-dependent loss in activity
* Very low activity in cataract lens
Glutathione Reductase

Glutathione Reductase

P<0.02

P<0.03
Thioredoxin

Thioredoxin Reductase

P<0.0001

P<0.02

Trx (mU/g wet wt)

<= 40 yr  40 - 60 yr  60 - 70 yr  >70 yr
(3)  (9)  (8)  (5)

<= 40 yr  40 - 60 yr  60 - 70 yr  >70 yr
(3)  (9)  (8)  (5)
Grx and Trx systems are mostly inactive in cataract tissues

<table>
<thead>
<tr>
<th>Age</th>
<th>TTase</th>
<th>GR</th>
<th>TR</th>
<th>TRx</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>25.88</td>
<td>60.25</td>
<td>4.74</td>
<td>14.43</td>
</tr>
<tr>
<td>69</td>
<td>35.98</td>
<td>239.87</td>
<td>62.68</td>
<td>25.59</td>
</tr>
<tr>
<td>71</td>
<td>53.72</td>
<td>86.22</td>
<td>7.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Cataract</td>
<td></td>
<td></td>
<td></td>
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</table>

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<thead>
<tr>
<th>Age</th>
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<th>GR</th>
<th>TR</th>
<th>TRx</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>83.26</td>
<td>1021.48</td>
<td>146.12</td>
<td>36.89</td>
</tr>
<tr>
<td>69</td>
<td>90.66</td>
<td>893.79</td>
<td>122.69</td>
<td>44.95</td>
</tr>
<tr>
<td>71</td>
<td>107.91</td>
<td>1017.49</td>
<td>137.72</td>
<td>28.42</td>
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</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>TTase</th>
<th>GR</th>
<th>TR</th>
<th>TRx</th>
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<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What is the effect of Grx 1 or Grx 2 gene knockout in mouse?

Phenotype—-Faster cataract formation

- $\text{H}_2\text{O}_2$-induced cataract in vitro
- UV radiation-induced cataract in vivo
- Age-associated cataract in vivo
Grx2 knockout mice have high prevalence of cataract during aging

<table>
<thead>
<tr>
<th>Age (m)</th>
<th>Grx2 KO</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 m</td>
<td>0/12</td>
<td>0/12</td>
</tr>
<tr>
<td>5 m</td>
<td>1/10</td>
<td>0/12</td>
</tr>
<tr>
<td>7 m</td>
<td>2/8</td>
<td>0/8</td>
</tr>
<tr>
<td>8 m</td>
<td>2/6</td>
<td>2/10</td>
</tr>
<tr>
<td>9 m</td>
<td>6/12</td>
<td>2/10</td>
</tr>
<tr>
<td>11 m</td>
<td>11/12</td>
<td>3/10</td>
</tr>
<tr>
<td>13 m</td>
<td>10/10</td>
<td>8/8</td>
</tr>
<tr>
<td>16 m</td>
<td>10/10</td>
<td>8/8</td>
</tr>
</tbody>
</table>
Opacity score in wild type and Grx2 knockout mice

<table>
<thead>
<tr>
<th>Age</th>
<th>WT</th>
<th>Grx2 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 m</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 m</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>7 m</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>8 m</td>
<td>0.2</td>
<td>0.33</td>
</tr>
<tr>
<td>9 m</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>11 m</td>
<td>0.4</td>
<td>0.92</td>
</tr>
<tr>
<td>13 m</td>
<td>1.38</td>
<td>*</td>
</tr>
<tr>
<td>16 m</td>
<td>1.63</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* indicates significance.
Slit lamp images in wild type and Grx2 knockout mice
In Summary
Thiol repair is essential to prevent cataract formation