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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Thioredoxin

A Tale of Two Cysteines

By

Patricia A. Rose

125 B Chemistry Building
Department of Chemical and Biochemical Engineering
University of Iowa
Iowa City, IA 52242

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Abbreviations:

AP1 = activator protein 1
NF-kB = nuclear factor kB
NADPH = reduced nictotinamide diphosphate
H_2O_2 = hydrogen peroxide
DTT = dithiothreitol
DETA-NO = diethylene triamine-nitric oxide
MTT= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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Abstract:

Thioredoxin is a protein found in most species. It has a conserved active site, Trp-Cys-Gly-Pro-Cys-Lys, where the Cys residues are able to cycle through oxidized and reduced states. This redox ability of thioredoxin is used to activate essential enzymes and proteins. It is assisted by thioredoxin reductase and NADPH. Thioredoxin can form a noncovalent dimer that cannot be reduced by thioredoxin reductase. Thioredoxin can work in the cytoplasm and extracellularly. It can both stimulate and inhibit cell growth depending on the microenvironment. It can reduce the number of free radicals in a system. Thioredoxin can also help cells survive in the presence of nitric oxide. Thioredoxin works in the best of times and the worst of times, keeping proteins reduced.

Introduction

During the worse of times and the best of times, thioredoxin works to keep sulfhydryl proteins reduced. It was originally isolated in the 1960's from *Eschericha coli* B as a low molecular weight, heat stable protein [1]. Thioredoxin is a 12-kDa protein that is found in many organisms, including bacteria, plants and humans. Each specie thioredoxin contains a conserved sequence of Trp-Cys-Gly-Pro-Cys-Lys in the active site [2]. The cysteine residues, working in concert, alternate between –S-S- (oxidized) and SH (reduced) states. This redox ability is used to activate essential enzymes and proteins [3]. Thioredoxin reduces ribonucleotides to deoxyribonucleotides [3]. Transcription factors, such as AP-1 [2], TFIIIC [2] and NK-?B [4], can be activated by thioredoxin. Thioredoxin can also help cells recover from exposure to hydrogen peroxide [5]. This versatile protein does not work alone. In most systems, thioredoxin (Trx) needs thioredoxin reductase (TrxR) and NADPH. From Figure 1, when thioredoxin reduces an oxidized protein, it becomes oxidized. TrxR and NADPH reduce the oxidized thioredoxin and then the cycle can be repeated [6].

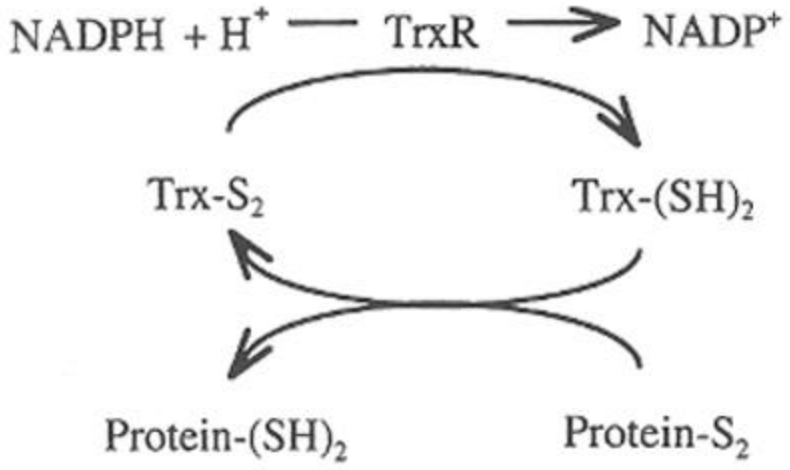

Figure 1: Scheme of oxidoreductase activities of the thioredoxin system. The figure shows the reduction of the active site disulfide in oxidized thioredoxin, Trx-S_2, to a dithiol in reduced thioredoxin, Trx-$(SH)_2$, by thioredoxin reductase (TrxR) and NADPH generating Trx-S_2.

This paper will briefly explain the physical characteristics of thioredoxin, localization, its role in oxidative stress, radical removal, impact on cell growth and reaction with NO.

Physical Characteristics of Thioredoxin

Thioredoxins from different species have approximately 27 to 69 % sequence homology to *Escherichia coli* thioredoxin [6]. The variation between the thioredoxin forms can be seen with extra cysteines or import sequences. Two forms of human thioredoxin have been cloned, thioredoxin 1 (Trx – 1) and thioredoxin 2 (Trx – 2). Trx – 1 contains the conserved catalytic site with the two cysteine residues and three additional cysteines at positions 62, 69 and 73 [2]. Human Trx – 1 has been located on chromosome 9 at band 9q32 [7]. Trx – 2 contains the conserved site and a mitochondria import sequence on the 5' end [2]. *E. coli* produced thioredoxin only contains the conserved catalytic site thioredoxin [2]. All of the different thioredoxin forms, including the forms mentioned above, have the same overall structure containing five beta sheets forming a hydrophobic core that is surrounded by 4 alpha helices [2]. The active site, denoted by the sulfhydryl bridge (S-S bridge) is located on the second beta sheet to the second alpha helix (please see figure 2) [8]. The active site contains the Trp-Cys-Gly-Pro-Cys-Lys sequence and is called the thioredoxin fold [8].

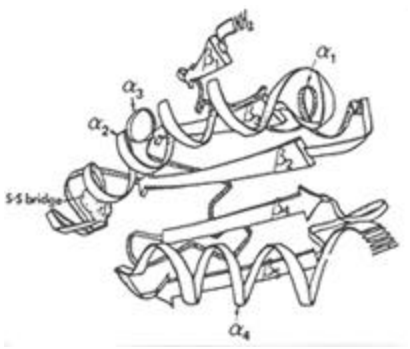

Figure 2: Tertiary structure of thioredoxin. A drawing of the three dimensional structure of thioredoxin-S_2 with the four α-helices and the five strands of β-pleated sheet. Note the disulfide bond in the protrusion on the left, which is the conserved active site.

Human thioredoxin, Trx-1, can from a homodimer that is held together noncovalently. Dimer formation is concentration and pH dependent [9]. The cysteine 73, which is not in the catalytic site, can align with a second Cys 73 from an adjacent molecule. The asparagine at location 60 also assists in the dimer formation. If the Cys 73 is mutated to another amino acid then dimers are not able to form. Activity of the mutated thioredoxin was shown to be higher than the native form [10]. One explanation for this result is that the test conditions allowed the native thioredoxin to form dimers, lowering the overall capacity for reduction.

The dimer cannot be reduced by thioredoxin reductase even though the active sites are still theoretically available for reduction (please see figure 3, a2 position) [13]. When DTT was used, the dimer thioredoxin active sites could be reduced [13].

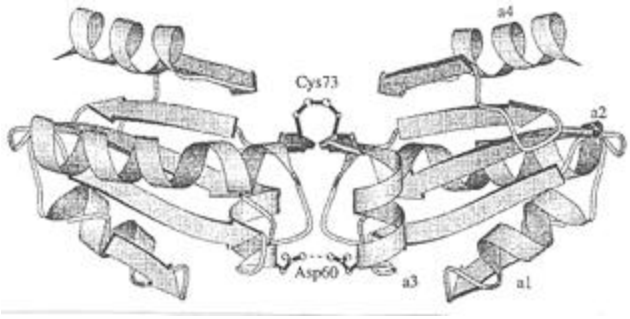

Figure 3: Thioredoxin noncolvalent dimer formation.

The noncovalent dimer has an apparent dissociation constant (K_{app}) that is pH dependent. The K_{app} increased by 27 fold when the pH was changed from 3.8 to 8.0 changing the constant from 6 to 166 µM, respectively [9].

Location of Thioredoxin

Thioredoxin can exert its effect from inside and outside the cell (please see figure 5) [7]. When proteins become oxidized they can form aggregates. These aggregates can cause the protein to become inactivated. Thioredoxin helps to keep the cell in a nonaggregated, reduced state. Thioredoxin may influence gene transcription by reducing NF-kB. NF-kB is a nuclear binding that has been implicated in transcriptional control of many genes. When it is in the cytoplasm, it is bound to an inhibitor. Thioredoxin can remove the inhibitor by reduction, allowing NF-?B to be transported into the nucleus [4]. Thioredoxin can have an effect by interacting with the cell surface. It has been shown that thioredoxin in the presence of interferon-? can cause an antiproliferative effect on Hela cells [11]. The exact method for this growth inhibition is unknown but it may be due to dimer formation, depletion of NADPH and the production of free radicals [11].

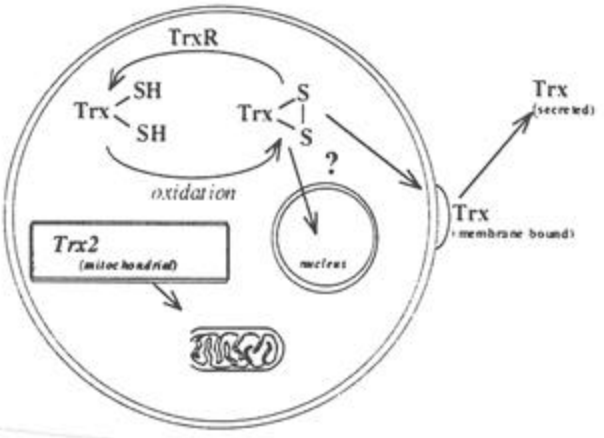

Figure 5: The compartmentalization of the thioredoxin system in a mammalian cell (modified from 8)

Thioredoxin in Oxidative Stress

When proteins become oxidized they can loose their activity. Glyceraldehyde 3-phosphate dehydogenase (GPD) is an enzyme that works in the process of glycolysis and it is sensitive to hydrogen peroxide oxidation. To determine if thioredoxin could repair the oxidative damage, GPD was subjected to hydrogen peroxide for five minutes followed by treatment with catalase to remove the H_2O_2 [5]. GPD activity was determined using a reaction that leads to a change in absorbance at 340 nm [5]. After the treatment with H_2O_2, the activity of GPD was reduced by 50% [5]. Thioredoxin with thioredoxin reductase and NADPH were able to restore the enzyme activity (please see figure 5) [5]. A significantly higher concentration of DTT, compared to the thioredoxin system, could also restore the GDP enzyme activity [5].

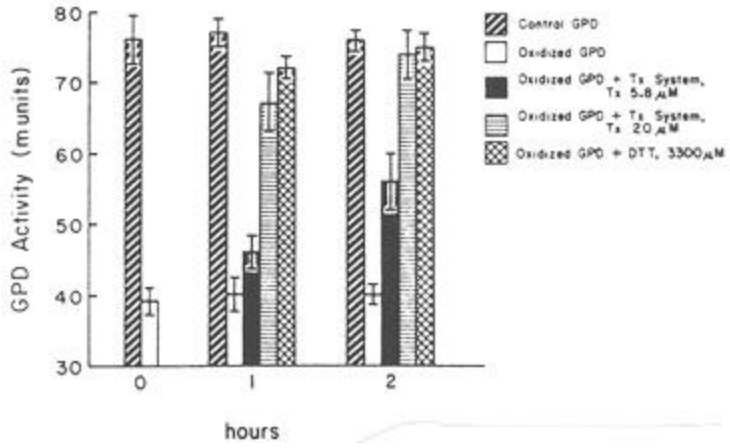

Figure 6: Recovery of the activity of H_2O_2 – treated glyceraldehydes-3-phosphate dehydrogenase after treatment with thioredoxin. Solutions of GPD at a concentration of 1 mg/mL were used in each condition and tested at 37 degrees with 5.8 or 20 µM thioredoxin, 1.5 ug of thioredoxin reductase, 0.25 mM NADPH, or 3300 µM DTT in 50 mM Tris, pH 7.4, 2.5 mM EDTA.

Thioredoxin and Free Radical Reduction

Ascorbic acid and dimethoxybenzoquinone can be used to produce free radicals. These free radicals can be seen in electronic spin residence. Pigiet *et al*. showed that the thioredoxin system could rapidly reduce the free radical concentration [5]. These results indicate that the thioredoxin system may be able to reduce toxic oxidants.

Thioredoxin in Cell Growth and Cancer

Thioredoxin is produced in many cell types including lymphocytes, fibroblasts and cancer cells [12]. The data indicates that cell produced thioredoxin it is able to stimulate a higher production of cytokines, including TNF, IFN-? and IL-2 [13]. Thioredoxin with IFN- ? can exert an antiproliferative effect on Hela cells [11]. The mechanism for growth inhibition has not been elucidated but it may be due to depletion of NADPH and tryptophan.

A variety of human primary tumors have elevated levels of thioredoxin compared to normal tissue and that once the tumor was removed the levels of thioredoxin reduced [2]. This might indicate that thioredoxin is a key to the progression of cancer. Where thioredoxin does not produce cancer directly but by keeping the oxidative stress to a minimum, allows the cell to live. This is especially problematic to the efficacy of cancer chemotherapy treatments. These drugs induce cellular oxidative stress in an attempt to kill the cell and thioredoxin is repairing the damage [3]. However, this interference is not universal. Thioredoxin from *E. coli* or catalytic site mutants are unable to stimulate growth even if the concentrations are 50 times what is found in the plasma [14].

Thioredoxin and NO

Nitric oxide (NO) is produced by the oxidation of L-arginine by NO synthetase [15]. When it is generated in macrophages it can contribute to antimicrobial and antitumoral activities *in vitro* and *in vivo* [15]. These activities are not generated by the NO directly but are from reactions leading to the production of reactive oxygen species or other nitrogen species [16].

Experiments with THP-1 cells, an immortalized line of monocytes, showed that TRX could increase the cell survival in the presence of NO. THP-1 cells were manipulated to contain the TRX gene or an empty cassette. When the cells were challenged with a NO donor, DETA-NO, 70% of the cells with the TRX gene could survive for 48 hours [16]. Only 20% of the cells without the elevated expression of thioredoxin could survive the 1 mM NO treatment (please see figure 7) [16].

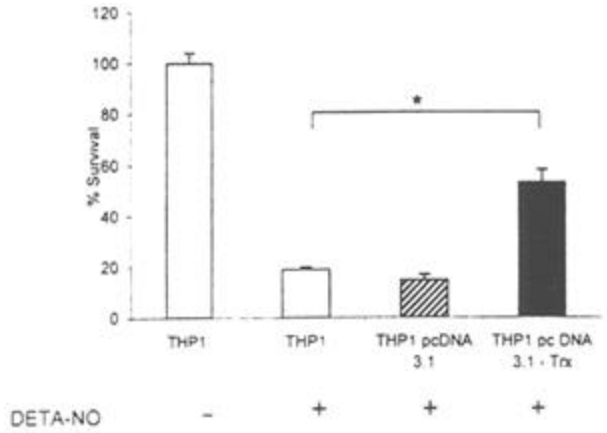

Figure 7. Susceptibility of Trx-transfected THP-1 cells to NO damaging effects. Susceptibility of both THP1 transfected cell line to exogenous NO cytotoxicity was evaluated using the MTT assay. Percentage cell survival was calculated as the recovery of viable cells exposed to 1 mM DETA-NO compared with the cell culture recovery in medium alone. The experiments were performed in triplicate and the results presented are representative of four separate experiments. Statisical analysis was performed using Student's t test in comparison with untreated cells: *$P < 0.05$.

Conclusion:

Thioredoxin is a ubiquitous protein that keeps the inside of the cell reduced. It works in signal transduction and DNA synthesis. Thioredoxin can form a dimer that can no longer be reduced by thioredoxin reductase. During oxidative stress thioredoxin can keep enzymes activated. Cell growth can be modulated by thioredoxin, which has been shown to have both stimulatory and inhibitory characteristics. It can help cells survive in the presence of nitric oxide. Thioredoxin can mediate the activities of a cell during the best of times and the worst of times.

References:

1. Laurent, T.C., Moore, E.C., Reichard, P. (1964) Enzymatic synthesis of deoxyribonucleotides IV. Isolation and characterization of thioredoxin, the hydrogen donor from *Escherichia coli* B. J. Biol. Chem., 239, 3436-3444.
2. Powis, G., Mustacich, D., Coon, A. (2000) The role of the redox protein thioredoxin in cell growth and cancer. Free Radical Biology and Medicine. 29, 312-322.
3. Davis Jr., W., Ronai, Z., Tew. K.D. (2001) Cellular thiols and reactive oxygen species in drug-induced apoptosis. J. of Pharm. and Exp. Ther., 296, 1-6.
4. Toledano, M.B. and Leonard, W.J. (1991) Modulation of transcription factor NF-?B binding activity by oxidation-reduction in vitro, Proc. Natl. Acad. Sci. USA, 88, 4328-4332.
5. Spector, A., Yan, G., Huang, R.C., McDermott, M.J., Gascoyne, P.R.C., Piglet, V. (1988) The Effect of H2O2 upon Thioredoxin-enriched Lens epithelial cells, J. Biol. Chem., 4984-4990.
6. Arner, E.S.J. and Holmgren, A. (2000) Physiological functions of thioredoxin and thioredoxin reductase, Eur. J. Biochem., 267, 6102-6109.
7. Heppell-Parton, A., Cahn, A., Bench, A., Lowe, N., Lehrach, H., Zehetner, G., Rabbitts, P. (1995) Thioredoxin, a mediator of growth inhibition, maps to 9q31. Genomics, 26, 379-381.
8. Reutimann, H., Straub, B., Luisi, P.L., Holmgren, A. (1981) A conformational study of thioredoxin and its trypic fragments, J. Biol. Chem., 256, 6796-6803.
9. Andersen, J.F., Sanders, D.A.R., Gasdaska, J.R., Weichsel, A., Powis, G., Montfort, W.R. (1997) Human thioredoxin homodimers: Regulation by pH, role of aspartate 60, and crystal structure of the aspartate 60 ? asparagine mutant, Biochemistry, 36, 13979-13988.
10. Ren, X., Bjornstedt, M., Shen, B., Ericson, M.L., Holmgren, A. (1993) Mutagenesis of structural half-cystine (sic) residues in human thioredoxin and effects on the regulation of activity by selenodiglucathione, Biochemistry, 32, 9701-9708.
11. Deiss, L.P. and Kimchi, A. (1991) A Genetic tool used to identify thioredoxin as a mediator of growth inhibitory signal, Science, 252, 117-120.
12. Rubartelli, A., Bajetto, A., Allavena, G., Wollman, E., Sitia, R. (1992) Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway, J. Biol. Chem., 267, 24161-24164.
13. Schenk, H., Vogt, M., Droge, W., Schulze-Osthoff, K. (1996) Thioredoxin as a potent costimulus of cytokine expression, J. Immuno., 765-771.
14. Oblong, J.E., Berggren, M., Gasdaska, P.Y., Powis, G. (1994) Site-directed mutagenesis of active site cysteines in human thioredoxin produces competitive inhibitors of human thioredoxin reductase and elimination of mitogenic properties of thioredoxin, J. Biol. Chem., 269, 11714-11720.
15. Evans, T.J., Buttery, L.D.K., Carpenter, A., Springall, D.R., Polak, J.M., Cohen, J. (1996) Cytokine-treated human neutrophils contain inducible nitric oxide synthase that produces nitration of ingested bacteria, Proc. Natl. Acad. Sci. USA, 93, 9553-9558.
16. Ferret, P.J., Soum, E., Negre, O., Wollman, E.E., Fradelizi, D. (2000) Protective effect of thioredoxin upon NO-mediated cell injury in THP-1 monocytic human cells, Biochem. J., 346, 759-765.
17. Holmgren, A. (1979) Thioredoxin catalyzes the reduction of insulin disulfides by dithiothreitiol and dihydrolipoamide, J. Biol. Chem., 254, 9627-9632.