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Thioredoxin: An Unauthorized Biography

by

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Abbreviations

Ala, alanine
Cys, cysteine
Gly, glycine
GRX, glutareductase
Pro, proline
TRX, trx, thioredoxin

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Abstract

Thioredoxins are small, ubiquitous dithiol proteins that play an important role in maintaining the redox balance in cells. Thioredoxins are also important in a variety of cellular tasks, such as DNA synthesis, redox signaling, and growth of tumor cells. Additionally, thioredoxins can repair disulfides in proteins that have been damaged by hydrogen peroxide as well as directly scavenge free radicals. For these reasons, thioredoxins have been investigated by researchers as promising drug targets. This review focuses on the biochemistry of the thioredoxins.

Introduction

Thioredoxins are ubiquitous, highly conserved, 12 kDa, dithiol proteins [1] with many important roles, including DNA synthesis (it provides the hydrogen for the reduction of ribonucleotides) [2], regulation of gene expression [J, 91], protein folding and enzyme function [1], and cancer cell growth [4]. Although thioredoxins are highly reducing ($E^{0'}$ = -240 mV), there are only 20 to 30 specific targets of thioredoxins—mostly catalytic and regulatory [5].

Background

Thioredoxins may either be cytosolic, as in the case of yeast thioredoxins 1 and 2 [12]. However, there is also an important function for them in the mitochondria, as with yeast thioredoxin 3 (TRX3) [12]. This makes sense, because many reactive oxygen species are created in the mitochondria during oxidative metabolism. Thus TRX3 is in the right place at the right time to prevent significant oxidative damage. Only a few groups have quantified cellular thioredoxins. In *E. coli*, the intracellular concentration is estimated at 15 µM. Pea chloroplasts, another source of ROS, contain 100 µM thioredoxin [5]. The thioredoxin content of mitochondria has not yet been examined, but one would expect concentrations similar to that found in chloroplasts.

Thioredoxin Structure

Thioredoxins are soluble, dithiol proteins of about 12 kDa, and 105 to 110 amino acids long [5]. They are highly conserved proteins. For example, the yeast thioredoxin proteins Trx1p and Trx2p share 78% sequence identity [7]. Compared to *E. coli* thioredoxin, the thioredoxins of other species range have between 27 and 69 % sequence identity [6]. Despite any sequence

differences, the three-dimensional structures are highly conserved across species [6]. At the active site, there is a characteristic CXXC motif. Typically, the sequence is –Cys-Gly-Pro-Cys- , but occasionally it is –Cys-Ala-Pro-Cys- [5].

In the best-characterized thioredoxin, *E. coli* thioredoxin (Figure 1), β-sheets are arranged in a twist at the hydrophobic center of the molecule, while 4 α-helices form the surface [5]. Residues 29 to 37 form the "thioredoxin fold," the active site that protrudes into the molecule. (See Figure 1.) This so-called thioredoxin fold has also been found in 4 protein classes.

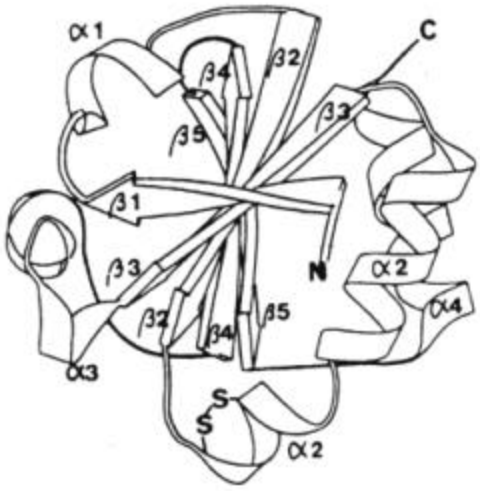


Figure 1: *E. coli* thioredoxin. β-sheets are arranged in a twist at the hydrophobic center of the molecule, while 4 α-helices form the surface. The disulfide bond which forms the thioredoxin fold, as well as the C- and N-termini of the molecule are indicated. Adapted from [6].

The pK_a values of active site cysteines play an important role in the reduction potential of the thioredoxin. Normally, a cysteine in an ordinary thiol will have a pK_a of approximately 9.5. The N-terminal cysteine is usually a strong nucleophile and has an unusually low pK_a, while the C-terminal cysteine is buried and higher. For example, using *E. coli* thioredoxin, one group found that the pK_a of the N-terminal cysteine was 7.6, whereas that of the buried, C-terminal cysteine was 11.1. *E. coli* thioredoxin is the most reducing member of the thioredoxin family

($E^{0'}$ = -270 mV). By contrast, the most oxidizing thioredoxin, *E. coli* DsbA ($E^{0'}$ = -122 mV) has a pK_a of only 3.5 at its N-terminal active site cysteine [8]. Several explanations for this phenomenon of the lowered pKa have been offered. In the case of DsbA, there is an electrostatic interaction with the side chain of a histidine that is between the two cysteine residues [8].

Thioredoxin/Thioredoxin Reductase System

Thioredoxin plays an important role in the transfer of electrons from NADPH to ribonucleotide reductase [9]. With its redox potential of –240 mV [5], thioredoxin transfers electrons to ribonucleotide reductase. Thioredoxin is regenerated through reduction by thioredoxin reductase. NADPH supplies electrons for further reductions in the pathway (Figure 2).

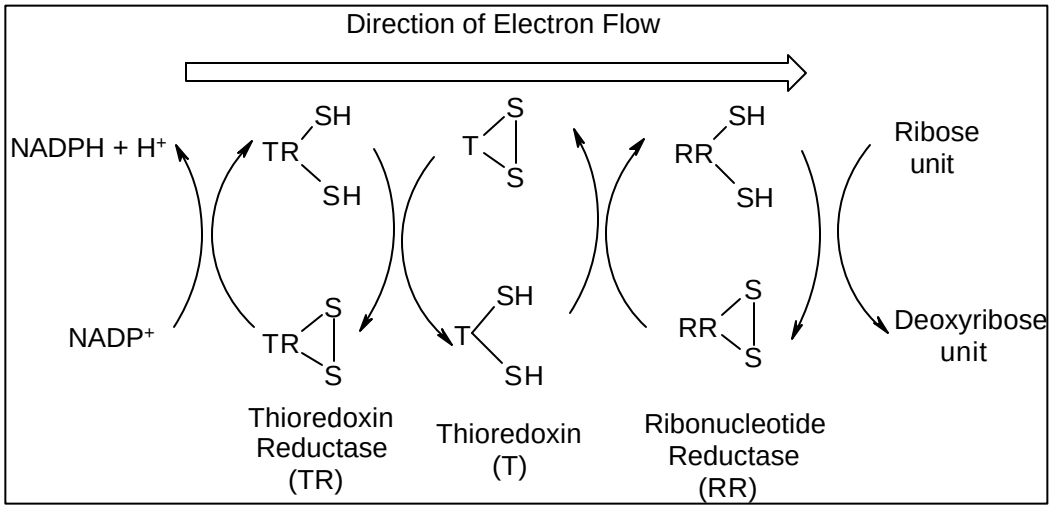


Figure 2: The Thioredoxin/Thioredoxin Reductase System. Electrons flow from NADPH to deoxyribonucleotides. Adapted from [9].

Other Reactions of Thioredoxin

Bacterially-produced thioredoxin and thioredoxin reductase from *Mycobacterium tuberculosis* reduces peroxides and dinitrobenzenes in a reaction that is optimal under anaerobic

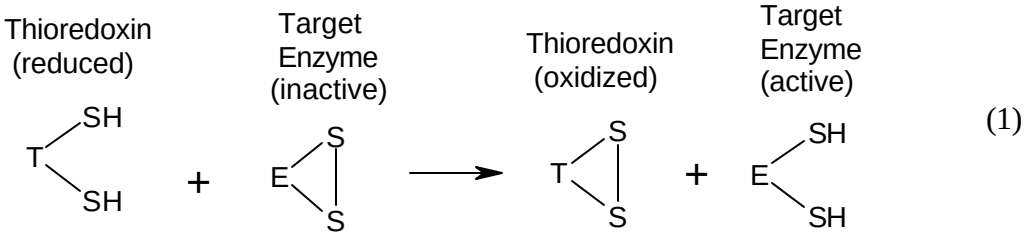
conditions and pH = 8.0 [10]. This study also found that reduction of cumene hydroperoxide and *tert*-butyl hydroperoxide were not catalyzed by thioredoxin. Only thioredoxin reductase and NADPH were needed to reduce these bulky, hydrophobic hydroperoxides. Kinetic data is summarized in Table 1 [10].

Table 1: Kinetic Parameters for the Reductions of Hydroperoxides by Thioredoxin and Thioredoxin Reductase. Adapted from [10].

	Enzymes Present	K_m (mM)	V_{max} ($\mu M^{-1}min^{-1}$)	k_{cat} (min^{-1})	k_{cat}/K_m ($M^{-1}min^{-1}$)
H_2O_2	TR (60 µM) Trx (1 µM)	2.7 ± 0.5	175 ± 9	2.9	1100
Cumene Hydroperoxide	TR (60 µM)	15 ± 6	24 ± 5	0.4	26
tert-Butyl Hydroperoxide	TR (60 µM)	23 ± 8	13 ± 2	0.2	9

Kirkpatrick *et al.* studied a series of alkyl 2-imidazolyl disulfides in the thioredoxin/ thioredoxin reductase system. Short-chain alkyl analogues such as 1-methylpropyl 2-imidazolyl disulfide are substrates for thioredoxin reductase. Analogues with branched alkyl chains or benzyl groups competitively inhibit thioredoxin reductase. Cys 73 of human thioredoxin appears to be the site of this inhibition, as a cysteine to serine mutation abolishes the inhibition [4].

Thioredoxin can modulate enzyme function by reducing a disulfide bond in the enzyme according to reaction 1 [1].



(1)

Thioredoxins are important in modulating function of the transcription factor Yap1p in the yeast *Saccharomyces cerevisiae* [3]. Deletion of thioredoxin genes TRX1 and TRX2 resulted in constitutive Yap1p activation. Deletion of the glutaredoxin genes (GRX1 and GRX2) did not activate Yap1p. Carmel-Harel *et al.* propose two mechanisms to explain the role of thioredoxins in the response to oxidative stress [3]. In the first, the lack of reduced thioredoxin prevents the thioredoxin-dependent peroxidases from removing oxidants such as hydrogen peroxide. Since more oxidants are present, the transcription factor Yap1p becomes oxidized, modulating its activity. In the second model, Yap1p is inefficiently reduced in the absence of a large oxidative stress. Oxidized, activated Yap1p accumulates. Microarray data supports both models. It is likely that a combination of these models describes the actual situation [3].

Thioredoxin is also important in relieving a cell of oxidative injury. For example, *E. coli* thioredoxin can reduce the H_2O_2-generated protein disulfides. It can reverse the H_2O_2 inactivation of glyceraldehyde-3-phosphate dehydrogenase. Most important, however, is its ability to directly scavenge free radicals generated in an ascorbic acid/2,6-dimethoxy-p-benzoquinone system [11].

Detection of Thioredoxin

Although thioredoxin has several possible substrates, the classical method for detecting it is by its ability to reduce insulin. In this assay, the amount of reduced insulin is determined by absorbance at 405 nm [12]. This assay provides us with a reliable, standard way of measuring thioredoxin levels, which has been in use for many years.

Summary

The thioredoxins represent a very important family of proteins. These small dithiols maintain the redox balance of the cell, repair oxidant-damaged cellular proteins, aid DNA synthesis, and perform signaling functions in the cell. When thioredoxins are dysregulated, they can play a role in tumorigenesis. Thus, it will continue to be important to study the thioredoxins, as they may well prove to be important drug targets.

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