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Thioredoxin: a family conserved

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Abbreviations
α - alpha
β - beta
Cys – cysteine
IR – ionizing radiation
Trx – thioredoxin
TrxR – thioredoxin reductase

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Abstract

Thioredoxins (Trx) comprise a family of small globular redox proteins that consist of a five stranded β (beta) sheet structure that comprises the hydrophobic core, and four α (alpha) helices that surround the core and form the external surface of the protein. Each member of this family shares a highly conserved active domain of five amino acids that includes two specific cysteine (Cys) residues that are involved in redox reactions. This sequence has been conserved throughout evolution across different species. The primary function of Trx is to break protein disulfide bonds by reducing their Cys residues. Through this activity Trx is involved in a variety of different biological activities ranging from antioxidant capabilities to transcriptional regulation. Trx is critical to a host of functions in cells, tissues, and organisms from primitive bacteria to humans.

Introduction

Thioredoxin was first discovered in 1964 in *E. coli* [1]. It was first characterized as a small redox protein in bacteria and mammals in 1967. After Trx was first purified in mammals from rat Novikoff hepatoma, it was rediscovered numerous times under different names such as interleukin-1, interleukin-2, and early pregnancy factor [1]. When the correct amino acid sequence of Trx was published, all of these proteins were determined to be identical and are know known as Trx [2]. Trx family members are present in a wide variety of eukaryotic and prokaryotic species. While family members may vary slightly, each member shares a conserved catalytic site. Trx activity has been observed predominately in the cytoplasm, but significant activity has also been observed in the nucleus, mitochondria and outside the cell [1]. It has been reported that oxidative stress can induce the expression of Trx [3]. The Trx induction increases the amount of reduced intracellular proteins, which protects against oxidative damage [3]. The primary function of Trx is to reduce oxidized Cys groups on proteins. Trx can act as many different biological factors including antioxidant, growth factor, cofactor, transcription factor regulator, and apoptosis inhibitor [2]. One example of its role as a biological factor is it can protect the lens of the eye from oxidative damage and cataracts [3]. This paper will provide an overview of the structure, biochemistry and activities of Trx.

Structure

Thioredoxin is a 12 kDa protein that exists with a compact globular structure. It consists of a five stranded beta sheet that comprises the hydrophobic core that is surrounded by four alpha helices on the external surface of the protein [1]. Despite the vast functions and activities of Trx, all family members share a common 3D structure, known as the Trx-fold [4]. The Trx-fold consists of a five β sheet strand core surrounded by four α helices and a conserved catalytic domain [4]. This structural similarity is due to the fact that all thioredoxins from humans to Archea share a 27-69% sequence similarity to the *E. coli* Trx1 [5]. The conserved succession of the secondary elements or Trx fold is as follows: β1, α1, β2, α2, β3, α3, β4, β5, α4 [5]. The active catalytic site is found in the loop between the β2 and α2 structural units (see Figure 1).

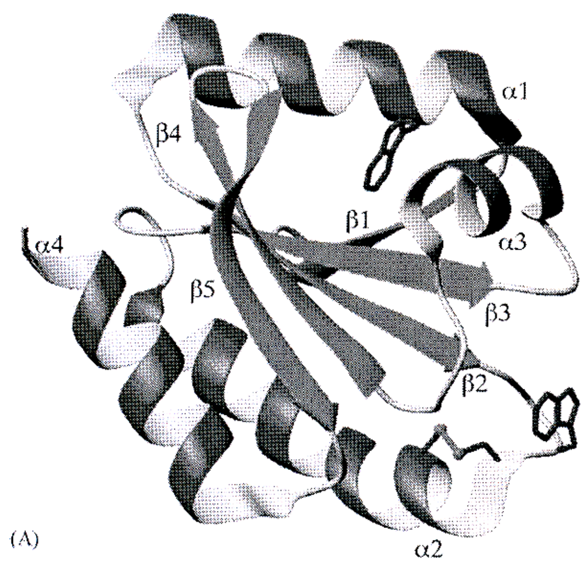

Figure 1. The crystallographic structure of thioredoxin is shown in the strand and ribbon representation. The active site of the protein is located between the β2 and α2 structural units (lower right corner of Figure 1) This site is highly conserved across species and contains the two important Cys residues [5].

The conserved active site consists of a five amino acid sequence: – Trp – Cys – Gly – Pro – Cys [1]. The two most important amino acids within this sequence are the two Cys residues. The first Cys, recognized as Cys^{32}, and the second Cys, recognized as Cys^{35}, interact and form a disulfide bond (This process is covered in detail in the Biochemical section) [1].

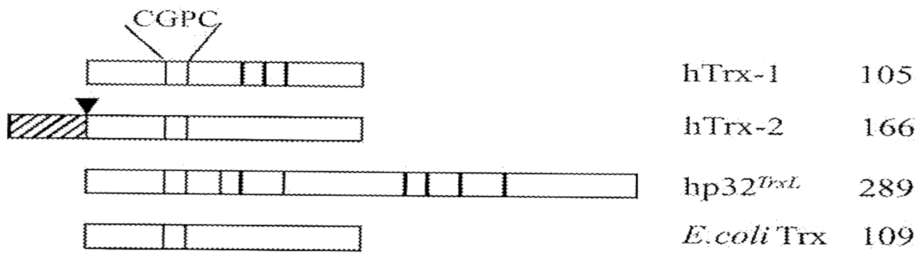

Figure 2. Trx family members are very similar in size and share a common active site denoted by 'CGPC'. The numbers at the right indicate the number of amino acids contained in the protein. The shaded area illustrates a mitochondrial import sequence and the arrow denotes the cleavage site (h = human, hp32TrxL is a Trx-like protein that shares catalytic homology, but not size homology) [1].

An interesting aspect of Trx is that the Trx gene or a Trx pseudogene may be found on multiple chromosomes. For instance, the human Trx1 gene was originally reported on chromosome 3 at band 3p11-p12, but is now thought to lie on chromosome 9 at band 9q32 and a Trx pseudogene has been identified on chromosome 10 at 10q25.2-q25.3 [2]. The shear number of Trx genes plus the existence of Trx pseudogenes has made mapping a difficult task.

Trx has the capability to form homodimers. A specific example of this is human Trx1. Dimer formation occurs readily at high concentrations of Trx1 or when it is in the presence of an oxidant. The formation of a Trx homodimer is the result of three interactions: an 1100-angstrom patch of 12 hydrophobic amino acids associate; five hydrogen bonds form between the two molecules; and the two Cys^{73} residues are covalently linked by a disulfide bond [2]. When the linkage is noncovalent, the dissociation constant is between 6 and 166 μM at pH levels of 3.8 to 8.0 [2]. When the linkage is covalent the hydrophobic interaction excludes all water molecules and the dimer is locked together by the Cys^{73} - Cys^{73} disulfide bond. This information was obtained under experimental conditions and the exact concentration of Trx in human tissue is unknown, so it is difficult to access the biological relevance to this data [2]. It is very difficult to determine exact Trx concentrations, but it has been determined that Trx concentrations in bovine tissues is approximately 0.5 - 5.0 μM [2]. However, a couple of interesting facts have arisen from this experiment. Dimerization has been observed in vertebrate species, but not lower species indicating this may be a recent evolutionary event. Also it is known that the dimerized form of Trx can no longer be reduced by TrxR (described in Biochemical section) and it does not stimulate growth like the single protein form [2].

Biochemical Reactions

The primary function of Trx is to reduce Cys residues on proteins. The active agent in this process is the Cys at the N-terminus. This residue, Cys^{32}, processes a low pK_a value (7.0-7.5) that allows it to act as an attacking nucleophile in the disulfide reduction of proteins [4,6]. While the pK_a value of Cys^{32} has been determined, there is still some uncertainty about the pK_a of Cys^{35} because it is buried within the active site (most data suggests it is only slightly higher that Cys^{32} and less than 8.0) [4,6]. When Trx reduces a protein it is oxidized and a disulfide bond is formed between the Cys^{32} and Cys^{35} residues. The oxidation and subsequent disulfide bond produces a conformational change in the Trx protein. Oxidized Trx is inactive and unable to perform protein reductions, so it must be recycled to its reduced form to regain its activity. Trx is able to help itself ensure a reduced conformation is favored through a low redox potential. For instance, the redox potential to Trx1 is –270 mV, which makes the reduced form of Trx the favorable and major dithiol reductant in the cytoplasm [4]. However, when Trx is oxidized by its activity, the only known substance that is able to reduce Trx is TrxR [4]. TrxR is able to obtain reducing equivalents from NADPH and pass them to the oxidized form of Trx. This series of reactions is depicted below {X indicates a protein substrate; -$(SH)_2$ indicates the reduced/active form of Trx; -S_2 indicates the oxidized/inactive form of Trx} [2].

$$\text{Trx} - (\text{SH})_2 + \text{X} - \text{S}_2 \quad \Leftrightarrow \quad \text{Trx} - \text{S}_2 + \text{X}\,(\text{SH})_2 \qquad (1)$$

$$\text{Trx} - \text{S}_2 + \text{NADPH} \quad \overset{\text{TrxR}}{\Leftrightarrow} \quad \text{Trx} - (\text{SH})_2 + \text{NADP}^+ \qquad (2)$$

This series of reactions creates a unique pathway that allows Trx to be regenerated. In this pathway the regeneration of Trx is dependent on TrxR and NADPH. This pathway and Trx dependence on TrxR and NADPH for its activity is illustrated in Figure 3.

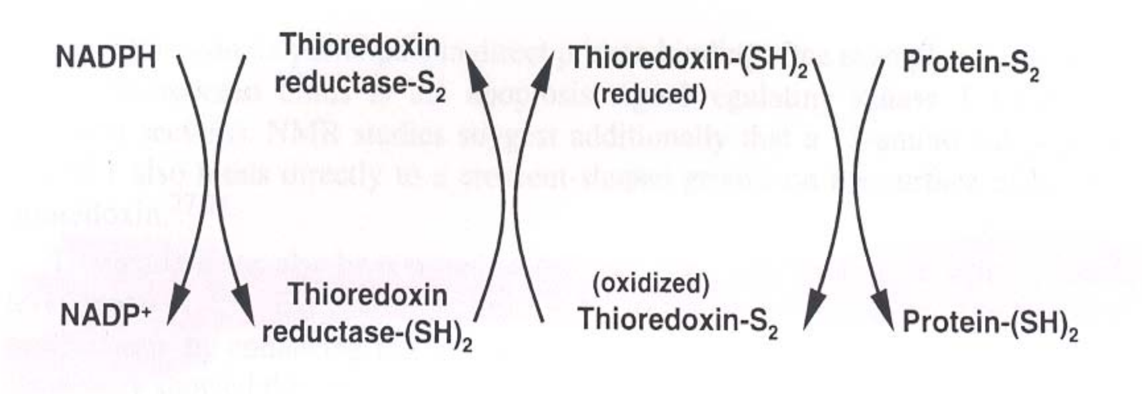

Figure 3. This illustration shows the pathway of action of Trx. NADPH and TrxR act to reduce Trx, which in turn reduces Cys residues on proteins. The picture also depicts how each component can be recycled with the reduced forms on the top and the oxidized forms on the bottom [7].

Biological Activities

Thioredoxin is involved in many different biological activities. In mammals it can play a role in regulation of apoptosis, pregnancy, and redox regulation of transcription factors. In many organisms Trx plays a role as an antioxidant. Trx reacts directly with hydrogen peroxide to form molecular oxygen and water [2].

$$Trx-(SH)_2 + H_2O_2 \rightarrow O_2 + H_2O + Trx-S_2 \quad (3)$$

As mentioned Trx can play a role as a transcriptional regulator, one example of this is AP-1. It has been shown experimentally that the overexpression of Trx in cells induces a three-fold increase in the DNA binding activity of AP-1 [8]. An even greater increase in response to IR and the overexpression of a mutant/inactive form of Trx prevents the induction of AP-1 in the response to IR [8]. Trx does not regulate AP-1 directly, but it does so through the Ref-1 protein. As in the previously mentioned biochemical pathway of Trx, electrons are passed from NADPH to TrxR to Trx and in this case are then passed to Ref-1, which relays them to AP-1 activating its DNA binding activity and stimulating transcription [8].

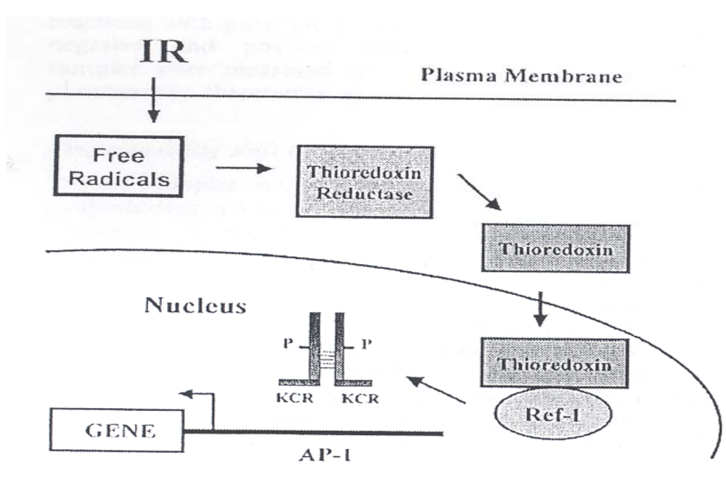

Figure 4. Illustration of how Trx is able to regulate transcription and Ap-1 activity through the Ref-1 protein [8].

Summary

Thioredoxin is a small globular redox protein whose primary function is to reduce Cys residues on proteins. Through this activity it involves itself in a vast array of biological activities. Among its many activities are antioxidant capabilities and transcriptional regulation, which were mentioned in this short review. All members of the Trx family have a highly conserved active domain that contains the two critical active Cys residues. This sequence has been conserved throughout evolution among all Trx containing species. While it is known that Trx is involved in many different activities, all of the details and intricacies of this protein and its processes have yet to be discovered.

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