The NADPH-thioredoxin reductase/Thioredoxin System

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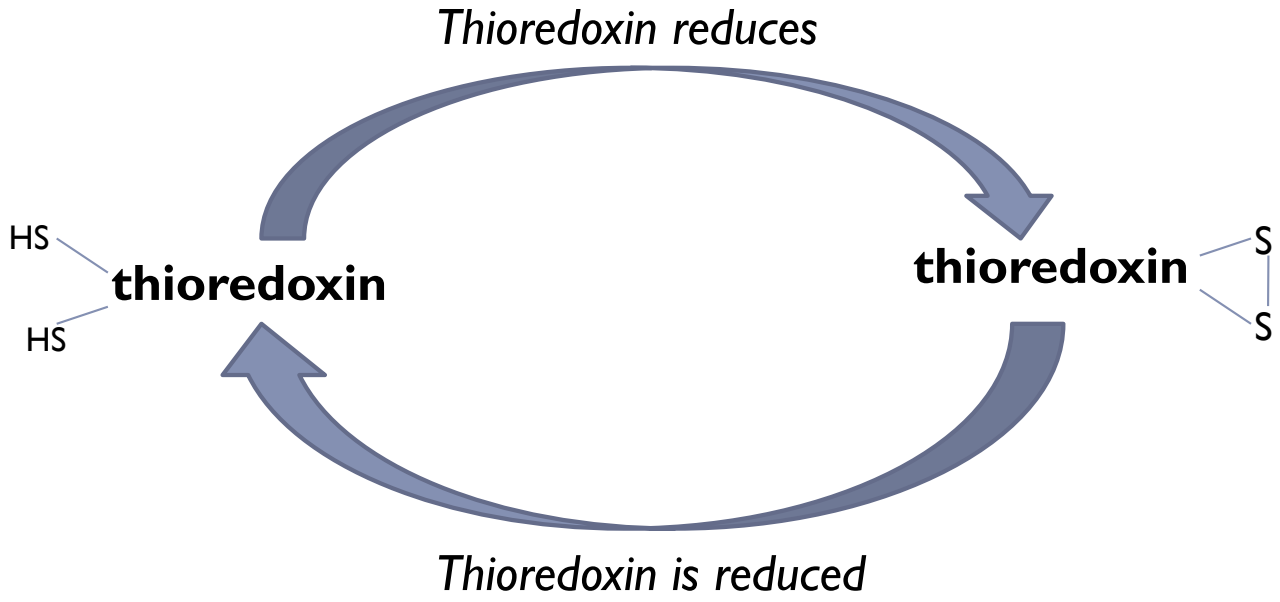
Overview of Thioredoxin

- Thioredoxin is a small, soluble disulfide-containing protein

- The function of thioredoxin is to reduce disulfide bonds

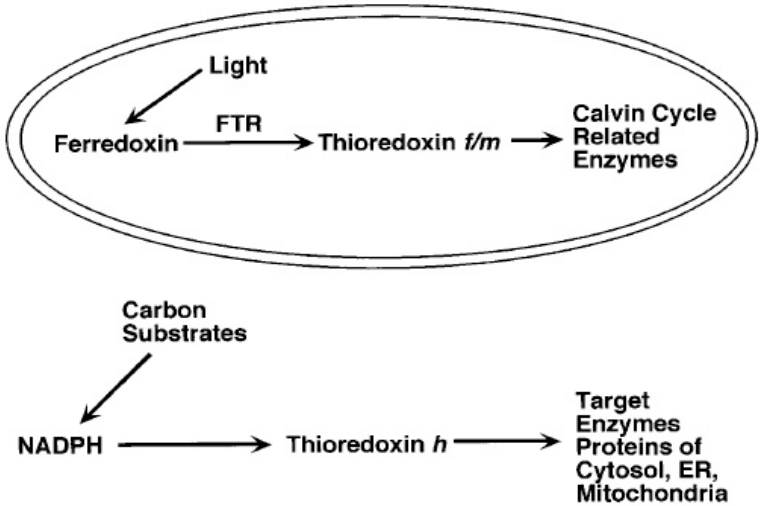


- There are various different isoforms of thioredoxin in both plants and animals.

Classification of thioredoxins

- Classification is based on the primary structure of the thioredoxin protein and its cellular localization.
  - Trx *f, m, x, y* are found in chloroplasts
  - Trx *o, h* are found in the cytosol and mitochondria

- Depending on the type of thioredoxin and where it is localized, it participates in different reduction reactions in varying systems.
  - FTR/thioredoxin system (ferredoxin-thioredoxin reductase)
  - NTR/thioredoxin system (NADPH-thioredoxin reductase)



NADPH-thioredoxin reductase (NTR)

- Flavoprotein – protein that contains a nucleic acid derivative of riboflavin such as FAD or FMN.
- NTR is a homodimer with subunits of ~35kDa
- Each subunit has 2 subdomains
  - 1. central NADPH binding domain
  - 2. FAD binding domain
- A conformational change is needed to accommodate Trx

The NTR/thioredoxin system and Trxh

- The tissue type in which NTR/Trx*h* effects its physiological activity is in seeds.

- The oxidative pentose phosphate pathway in the seed produces NADPH for reduction of thioredoxin.
  - Endosperm is metabolized to produce NADPH via this pathway, and in turn the reducing power of thioredoxin activates enzymes that further metabolize the endosperm for seed nourishment.

- NTR/Trx*h* affects seed germination and development
  - Influences metabolism in the seed by increasing the availability of carbon and nitrogen

NTR/Trx*h* regulation of protein activity

- Carbohydrate mobilization
  - NTR/Trx system inactivates inhibitory enzymes of α-amylase
  - It also activates pullulanase (limit dextrinase)
- Protein mobilization
  - Trx*h* inactivates inhibitory enzymes of trypsin
  - Reduces storage proteins such as prolamins and glutelins.
  - Activates thiocalsin, a seed-specific protease

Pullulanase (Limit Dextrinase)

Overexpression of thioredoxin *h* leads to enhanced activity of starch debranching enzyme (pullulanase) in barley grain
Cho et al.

- Two DNA constructs with B_1-hordein promoter and wTrxh, one with a signal peptide sequence targeting the protein body and one without
- Results showed that Trxh levels in transgenic lines were many times greater than the nontransformed control.
- Pullulanase activity was also greater in lines that overexpressed Trxh.
  - Activity of thioredoxin on pullulanase was not clear

Prolamins and glutelins

- In wheat:
  - Primary storage proteins:
    - Gliadin (prolamin)
    - Glutenin (glutelin)
- In sorghum:
  - Primary storage proteins:
    - Kafirins (prolamins)

Recap

- There are different kinds of thioredoxin that are differentiated by their primary protein structure and their cellular localization
- One of these is Trxh which is part of the NTR/Trxh system and exhibits its activity in seed tissue.
- In the seed tissue, the system regulates enzymes and proteins that increase the mobility of carbon and nitrogen in the seed.
- In cases such as with pullulanase, the NTR/Trxh system does not necessarily control activity of a protein by regulating its inhibitor.

Sources

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