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The thioredoxin *h* system: potential applications

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Abstract

The thioredoxin *h* system has the specific capability to reduce intramolecular disulfide bonds of proteins, thereby modifying their tertiary structure. It is involved in many processes: in the activation or deactivation of enzymes and enzyme inhibitors and in the germination process. This system can be used to improve the breadmaking quality of wheat by strengthening the dough. It can also decrease the epitope accessibility, then modifying the response of the IgE immune system. Transgenic barley and wheat have been created to confirm the functionality of the NADP-dependent thioredoxin *h* system.

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Keywords: Thioredoxin; Seed germination; Food improvement; Allergenicity

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1. Introduction

Thioredoxins are small ubiquitous proteins characterized by a conserved dicysteine active site. They participate in dithiol/disulfide exchange reactions with a large range of substrates. In plants, two thioredoxin systems have been characterised, one localised in chloroplasts (*f* and *m* thioredoxins) involved in photosynthesis regulation and the other one in the cytosol (thioredoxin *h*). This latter system is analogous to the one present in animals and microorganisms. To operate, thioredoxin *h* needs to be reduced by the NADP thioredoxin reductase.

In this paper, we will review the different implications of the thioredoxin *h* system during seed germination, for improvement of dough for breadmaking quality and for modification of allergens or venoms properties.

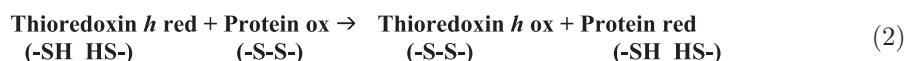
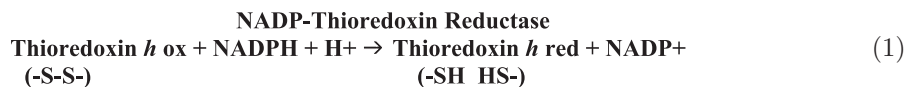
1.1. Thioredoxin *h* and seed germination

Thioredoxin *h*, a 12-kDa protein with a catalytically active disulfide group, is ubiquitous in the plant kingdom and is localized in the cytosol, the endoplasmic reticulum and mitochondria.

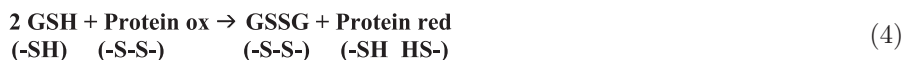
Storage proteins, which are synthesized and stored within seeds, serve as the main source of nitrogen and as a primary source of carbon for the germination and growth of seedlings. When conditions are favourable for germination, these proteins are mobilized and proteolytically degraded. In most kind of seeds, the storage proteins are, even after maturity, enclosed within a membrane, thereby creating a structure known as a protein body. In several cereals, such as wheat, barley, rye, triticale, the protein body membrane is disrupted during maturation and drying of the grain, thereby exposing the storage proteins to endogenous proteases of the endosperm. However, in typically nonglutinous cereals such as corn, rice, sorghum and millet, storage proteins remain in protein bodies in the mature grains.

Several investigators have shown that, akin to the larger insoluble storage proteins, small cysteine-rich proteins of seeds are degraded during germination. Our laboratory has uncovered evidence that both protein groups undergo reduction in conjunction with their degradation. This finding is consistent with the observation that these two groups of proteins are resistant to proteolysis in the oxidized (S-S) state. The NADP/thioredoxin *h* system (NTS) appears to play a role in the reduction of critical disulfide bonds of seed proteins (S-S→2SH) and, thereby, as seen below, triggers germination (Wong et al., 1995).

Thioredoxin *h* is reduced via NADPH by the flavin enzyme NADP–thioredoxin reductase (NTR) (Eq. (1)). The reduced thioredoxin *h* reduces disulfide bonds on target proteins (Eq. (2)).



There are some evidences that glutathione, maintained in reduced (SH) form by NADPH and glutathione reductase, may also act in the reduction of these proteins (Eqs. (3) and (4)), particularly after thioredoxin *h* has initiated the reduction process.



Much of the evidence for the role of thiols in seed germination had come from collaborative studies between Buchanan's laboratory (USA) and our laboratory. We demonstrated that seed storage proteins undergo redox changes during grain formation and development as well as during germination. Thioredoxin *h* was also found to reduce specifically the intramolecular disulfide bonds of cysteine-rich proteins.

In wheat, thioredoxin *h* acts as an early wake-up call in seed germination, facilitating the mobilisation of nitrogen and carbon through the activation of a protease (thiocalsin) (Besse et al., 1996), inactivation of proteinaceous inhibitors such as α -amylase and trypsin inhibitors and reduction of disulfide bonds of storage proteins (gliadins and glutenins) thereby increasing hydrolysis (Kobrehel et al., 1992). The abundance of thioredoxin *h* in the endosperm is controlled by the embryo via hormones. Gibberelins enhance the disappearance of thioredoxin *h*, whereas abscisic acid shows the opposite effect (Lozano et al., 1996).

Wheat seeds contain at least four thioredoxin *h* isoforms and analysis of different cultivars revealed important variations in the relative abundance of these isoforms (Runquist et al., 1999).

2. Potential applications of the NTS

2.1. Thioredoxin *h* and breadmaking quality

Disulfide bonds are one of the main stabilizing forces of storage proteins (glutelins and prolamins) in dough. In cereals, the formation of disulfide bonds appears to increase structural stability on the one hand and to decrease solubility on the other hand. Both of these features provide protection against proteolysis. The NTS target proteins include typically large, insoluble storage proteins (60–100 kDa), as well as generally less abundant low molecular weight soluble proteins (5–30 kDa) that are rich in disulfide bonds (e.g. α -amylase and protease inhibitors). Except for the α -amylase/subtilisin inhibitor of barley and other cereals, which inhibits endogenous α -amylases, the physiological function of these small cysteine-rich proteins within the seed remains unclear. It has long been considered that proteins of this type play a bioprotection role by blocking digestive enzymes of invading pests.

It has been shown that addition of the NTS components to poor quality flour strengthens the dough as determined by farinograph measurements and increases loaf volume and viscoelasticity (Wong et al., 1993; PCT US 92/08595). Therefore, it could be

possible to improve the breadmaking quality of wheat cultivars that are now used as forage crops.

This goal could be achieved either by addition of the different components of the NTS, but it will not be economically profitable, or by a transgenic approach consisting in the expression of the NTS to proper levels to make improved baked products.

2.2. Thioredoxin: allergies and toxins

Because of its specific property to reduce intramolecular disulfide bonds, the thioredoxin system modifies the tertiary structure of various cysteine-rich proteins. Resulting consequences could be the loss (or gain) of biochemical activity, an increase in susceptibility to proteolysis and/or to other parameters (heat, pH, etc.) and eventually a decrease in allergenicity. With respect to the latter, Buchanan's team was able to demonstrate that the effect of major allergens of wheat grain (gliadins and glutenins) was decreased after reduction with thioredoxin *h* (Buchanan et al., 1997). It seems also to operate with proteins from milk such as β -lactoglobulin (US patent 6,190,723 B1).

Many venom toxins from bees, snakes or scorpions are small cysteine-rich proteins. Then, once again, the action of the NTS on the disulfide bonds of these toxic proteins could result potentially in their inactivation (PCT US 92/08595).

*2.3. Transformation of cereals with thioredoxin *h* gene*

In previous works, we isolated clones encoding wheat thioredoxin *h*. Procedures have been described to produce them in a heterologous system in order to enhance the economics of applications of thioredoxin *h* (patent no. 94 09235 FR 55581 E). Later on, a gene coding for a wheat thioredoxin *h* has been isolated and regions that drive tissue- and temporal-specific expression were identified (PCT no. WO 00/70065).

The team of Peggy Lemaux (USA) used one of our clones (Gautier et al., 1998) coding for wheat thioredoxin *h*, to create transgenic barley and wheat in which the thioredoxin *h* is preferentially accumulated in grain endosperm. Homozygous transformants of barley and wheat showed enrichment up to 30-fold in the content of biochemically active thioredoxin *h* relative to null segregants.

The characterization of these transgenic grains showed that the overexpression of thioredoxin *h* enhances the activity of starch-debranching enzyme and accelerates the emergence of the radicle during germination.

3. Conclusions

The thioredoxin *h* system plays important roles during the germination process of seeds. Furthermore, because of its properties and specificity of action in reducing intramolecular disulfide bonds, critical disulfide groups of target proteins participating in grain maturation and drying appear to become reversibly reduced, thereby either suppressing activity of enzymes or enzyme inhibitors, or lowering enzymatic accessibility (storage proteins). Due to the participation of the thioredoxin system in dithiol/disulfide

exchange reactions with a large range of cellular substrates, the NTS is able to modify the function of many proteins. Wheat thioredoxin is of particular interest owing to its potential application in the improvement of foods by enhancing dough quality and lowering allergenicity. In the future, the identification of new proteins targeted by thioredoxin will improve our understanding of its function and extend its technological and medical applications.

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