Plant Physiol. (1992) 99, 919-924
0032-0889/92/99/0919/06/$01.00/0

Received for publication October 3, 1991
Accepted December 30, 1991

# Specific Reduction of Wheat Storage Proteins by Thioredoxin *h*[1]

**Karoly Kobrehel[2], Joshua H. Wong, Árpád Balogh[3], Ferenc Kiss[3], Boihon C. Yee, and Bob B. Buchanan***

*Department of Plant Biology, University of California, Berkeley, California 94720*

## ABSTRACT

**Gliadins and glutenins, the major storage proteins of wheat endosperm (*Triticum durum*, Desf. cv Monroe), were reduced *in vitro* by the NADP/thioredoxin system (NADPH, NADP-thioredoxin reductase and thioredoxin; in plants, the *h* type) from either the same source or the bacterium *Escherichia coli*. A more limited reduction of certain members of these protein groups was achieved with the reduced form of glutathione or glutaredoxin, a protein known to replace thioredoxin in certain bacterial and mammalian enzyme systems but not known to occur in higher plants. Endosperm extracts contained the enzymes necessary to reduce NADP by the oxidative pentose phosphate pathway (hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase). The gliadins and glutenins were also reduced *in vivo* during germination—an event that accompanied their proteolytic breakdown. The results suggest that thioredoxin, reduced by NADPH generated via the oxidative pentose phosphate pathway, functions as a signal in germination to enhance metabolic processes such as the mobilization of storage proteins and, as found earlier, the activation of enzymes.**

Thioredoxin is known to be involved in two types of enzyme systems in plants. Chloroplasts contain an Fd/thioredoxin system comprised of Fd, Fd-thioredoxin reductase, and thioredoxins *f* and *m* that links light to the regulation of enzymes of photosynthesis (4, 23). The other system is analogous to the one established for animals and most microorganisms, in which thioredoxin (*h* type) is reduced by NADPH and the enzyme, NTR[4] (Eq. 1) (8, 11, 29). Current evidence suggests that the NADP/thioredoxin system is widely distributed in plant tissues and is housed in the mitochondria, ER, and cytosol (2, 18).

$$\text{NADPH} + \text{H}^+ + \text{Thioredoxin } h_{ox} \xrightarrow{NTR} + \text{Thioredoxin } h_{red} \quad (1)$$

The seed is the only tissue for which the NADP/thioredoxin system has been ascribed physiological activity in plants. Thioredoxin *h* reduces members of several different soluble seed proteins—thionins, α-amylase, and trypsin inhibitors (11, 14)—and also reductively activates an enzyme of carbohydrate metabolism (PPi fructose-6-P, 1-phosphotransferase, or PFP) (13). The results (14) suggest that the inhibitor proteins, long known to be active in bioprotection, may function within the seed to link thioredoxin to the regulation of yet-to-be identified target enzymes (*cf.* 9, 16, 21).

The question arises as to whether thioredoxin can reduce other types of seed proteins. Quantitatively, the most important group is comprised of storage proteins, which account for up to 80% of the total protein of the seed (12, 20). In the case of plants such as cereals, these proteins are insoluble in aqueous solutions and are chemically inert until they are reduced. It is not known how these proteins are mobilized during germination, and a physiological agent capable of their reduction has not been described.

To help fill this gap, we have undertaken a study with wheat, a cereal with well-characterized seed proteins. We now report that representatives of the major wheat (*Triticum durum*) storage proteins—the gliadins and glutenins—are specifically reduced by thioredoxin. The results provide evidence that the NADP/thioredoxin system functions in the reduction of the principal seed proteins, thereby increasing their proteolytic susceptibility and making amino acids (nitrogen and sulfur) available during germination. Taken together with our recent work (14), the new findings suggest that thioredoxin functions as a signal to enhance metabolic processes associated with seed germination. A preliminary account of this work has been published (31).

## MATERIALS AND METHODS

### Plant Material

Seeds and semolina of durum wheat (*Triticum durum*, Desf. cv Monroe) were kind gifts of Dr. K. Kahn.

### Germination of Wheat Seeds

Twenty to thirty seeds were placed in a plastic Petri dish on three layers of Whatman No. 1 filter paper moistened with 5 mL of deionized water. Germination was carried out for up to 4 d at room temperature in a dark chamber.

[1] This research was supported by grants from the Cellular Biochemistry and U.S.-Hungary Program of the National Science Foundation. K.K. also acknowledges support of a North Atlantic Treaty Organization Fellowship.

[2] Present address: Laboratoire de Technologie de Céréales, INRA-2, Place Viala, 34060 Montpellier Cedex 01, France.

[3] Present address: György Bessenyei College, Sóstói ut 31B, Nyiregy háza, 4401 Hungary.

[4] Abbreviations: NTR, NADP-thioredoxin reductase; mBBr, monobromobimane.

### Reagents/Fine Chemicals

Biochemicals and lyophilized coupling enzymes were obtained from Sigma. *Escherichia coli* thioredoxin and NTR were purchased from American Diagnostica, Inc. (Greenwich, CT). Wheat thioredoxin *h* and NTR were isolated from wheat germ, following the procedures developed for spinach leaves (8). *E. coli* glutaredoxin was a kind gift of Professor A. Holmgren. Reagents for SDS-PAGE were purchased from Bio-Rad. mBBr (Thiolite) was obtained from Calbiochem. Aluminum lactate and methyl green were products of Fluka Chemicals (Buchs, Switzerland).

### Gliadins and Glutenins

For isolation of insoluble storage proteins, semolina (0.2 g) was extracted sequentially with 1 mL of the following solutions for the indicated times at 25°C: 50 mM Tris-HCl, pH 7.5 (20 min); 70% ethanol (2 h); and 0.1 M acetic acid (2 h) (20). During extraction, samples were placed on an electrical rotator and, in addition, occasionally agitated with a vortex mixer. After extraction with each solvent, samples were centrifuged (12,000 rpm for 5 min) in an Eppendorf microfuge, and supernatant fractions were saved for analysis. In between each extraction, pellets were washed with 1 mL of water and collected by centrifugation as before, and the supernatant wash fractions were discarded. By convention, the fractions are designated albumin/globulin, gliadin, and glutenin.

### *In Vitro* mBBr Labeling of Proteins

Reactions were carried out in 100 mM Tris-HCl buffer, pH 7.9. As indicated, 0.7 $\mu$g of NTR and 1 $\mu$g of thioredoxin (both from *E. coli* unless specified otherwise) were added to 70 $\mu$L of this buffer containing 1 mM NADPH and 30 $\mu$g of target protein (final volume, 100 $\mu$L). When thioredoxin was reduced by DTT, NADPH and NTR were omitted and DTT was added to 0.5 mM. Assays with reduced glutathione were performed similarly but at a final concentration of 1 mM. After incubation for 20 min, 100 nmol of mBBr was added, and the reaction was continued for another 15 min. To stop the reaction and derivatize excess mBBr, 10 $\mu$L of 10% SDS and 10 $\mu$L of 100 mM $\beta$-mercaptoethanol were added, and the samples were then applied to the gels. For reduction by glutaredoxin, the thioredoxin and NTR were replaced by 1 $\mu$g of *E. coli* glutaredoxin, 1.4 $\mu$g of glutathione reductase (purified from spinach leaves), and 1.5 mM NADPH.

### *In Vivo* mBBr Labeling of Proteins

At the indicated times, the dry seeds or germinating seedlings (selected on the basis of similar radicle length) were removed from the Petri dish, and their embryos or germinated axes were removed. Five endosperms from each lot were weighed and then ground in liquid $N_2$ with a mortar and pestle. One milliliter of 2.0 mM mBBr in 100 mM Tris-HCl, pH 7.9, buffer was added just as the last trace of liquid $N_2$ disappeared. The thawed mixture was then ground for 1 min and transferred to a microfuge tube. The volume of the suspension was adjusted to 1 mL with the appropriate mBBr or buffer solution. Protein fractions of albumin/globulin, gliadin, and glutenin from endosperm of germinated seedlings were extracted as described above. The extracted protein fractions were stored at −20°C until use. A buffer control was included for each time point.

### SDS-PAGE

SDS-PAGE of the mBBr-derivatized samples was performed in 15% gels at pH 8.5 as described by Laemmli (15). Gels of 1.5 mm thickness were developed for 16 h at a constant current of 9 mA.

### Native Gel Electrophoresis

To resolve the different types of gliadins, native PAGE was performed in 6% gels of 3 mm thickness (a procedure designed to separate gliadins into the four types) as described by Bushuk and Zillman (5) and modified for vertical slab gels by Sapirstein and Bushuk (22). A gel solution in a final volume of 100 mL contained 6.0 g of acrylamide, 0.3 g of bisacrylamide, 0.024 g of ascorbic acid, 0.2 mg of ferrous sulfate heptahydrate, and 0.25 g of aluminum lactate. The pH was adjusted to 3.1 with lactic acid. The gel solution was degassed for 2 h on ice, and then 0.5 mL of 3% hydrogen peroxide was added as a polymerization catalyst. The running buffer, also adjusted to pH 3.1 with lactic acid, contained 0.5 g of aluminum lactate/L. Duration of electrophoresis was approximately 4 h, with a constant current of 50 mA. Electrophoresis was terminated when the solvent front, marked with methyl green tracking dye, migrated to about 1 cm from the end of the gel.

### mBBr Removal/Fluorescence Photography

Following electrophoresis, gels were placed in 12% (w/v) TCA and soaked for 4 to 6 h with one change of solution to fix the proteins; gels were then transferred to a solution of 40% methanol/10% acetic acid for 8 to 10 h to remove excess mBBr. The fluorescence of mBBr, both free and protein bound, was visualized by placing gels on a light box fitted with a UV light source (365 nm). After excess (free) mBBr was removed, gels were photographed with Polaroid Positive/Negative Landfilm, type 55, through a yellow Wratten gelatin filter No. 8 (cutoff = 460 nm) with an exposure time of 25 to 60 s at f4.5 (6).

### Protein Staining/Destaining/Photography

SDS gels were stained with Coomassie brilliant blue R-250 in 40% methanol/10% acetic acid for 1 to 2 h and destained overnight as described before (6). Aluminum lactate native gels were stained overnight in a filtered solution containing 0.1 g of Coomassie brilliant blue R-250 (dissolved in 10 mL of 95% ethanol) in 240 mL of 12% TCA. Gels were destained overnight in 12% TCA (5, 22).

Protein-stained gels were photographed with Polaroid type 55 film to produce prints and negatives. Prints were used to determine band migration distances and loading efficiency.

### Scanning of Gels

The Polaroid negatives of fluorescent gels and prints of wet protein-stained gels were scanned with a laser densitometer (Pharmacia-LKB UltroScan XL). Fluorescence was quantified by evaluating peak areas after integration with GelScan XL software.

### Enzyme Assays

The following activities were determined in crude extracts with previously described methods: hexokinase (1), glucose 6-phosphate dehydrogenase (25), 6-phosphogluconate dehydrogenase (25), glutathione reductase (8), NTR (8), and thioredoxin *h* (8).

### Protein Determination

Protein concentrations were determined by the Bradford method (3), with Bio-Rad reagent and BSA as a standard.

### Subunit Molecular Mass Determination

The subunit molecular mass of gliadins and glutenins was estimated on SDS-PAGE gels by using two sets of molecular mass standards. The first set consisted of BSA (66 kD), ovalbumin (45 kD), Kunitz soybean trypsin inhibitor (20.1 kD), myoglobin (17 kD), Cyt *c* (12.4 kD), and aprotinin (6.5 kD). The other set was the Bio-Rad prestained low SDS-PAGE standards: phosphorylase b (110 kD), BSA (84 kD), ovalbumin (47 kD), carbonic anhydrase (33 kD), Kunitz soybean trypsin inhibitor (24 kD), and lysozyme (16 kD).

## RESULTS

As a result of the pioneering contributions of Osborne and Voorhees (20) a century ago, seed proteins can be fractionated on the basis of their solubility in aqueous and organic solvents. In the case of wheat, preparations of endosperm (flour or semolina) are historically sequentially extracted with four solutions to yield the indicated protein fraction: (a) water, albumins; (b) salt water, globulins; (c) ethanol/water, gliadins; and (d) acetic acid/water, glutenins. A wide body of evidence has shown that different proteins with specific characteristics are enriched in each fraction. For example, the albumin and globulin fractions contain numerous enzymes, and the gliadin and glutenin fractions are enriched in the storage proteins required for germination. It is generally considered that gliadins are monomeric proteins, whereas glutenins are polymeric (12, 26).

Our laboratory has previously shown that a number of water-soluble seed proteins (albumins/globulins) are reduced by the NADP/thioredoxin system, derived from either the seed itself or *E. coli* (11, 14). The ability of the system to reduce insoluble storage proteins from wheat seeds, viz., representatives of the gliadin and glutenin fractions, is described below.

### Reduction of Gliadins

Based on its reactivity with mBBr, the gliadin fraction was extensively reduced by thioredoxin (Fig. 1). The major members undergoing reduction showed a molecular mass ranging from 30 to 50 kD. As seen previously with the seed $\alpha$-amylase and trypsin inhibitor (14) proteins, the gliadins were reduced by either native *h*- or *E. coli*-type thioredoxin (both homogeneous); NADPH (and NTR) or DTT could serve as the reductant for thioredoxin. Much less extensive reduction was observed with glutathione and glutaredoxin—a protein able to replace thioredoxin in certain *E. coli* and mammalian enzyme systems but not known to occur in higher plants.

The gliadin fraction is made up of four different protein types, designated $\alpha$, $\beta$, $\gamma$, and $\omega$, according to their mobility when separated by native PAGE under acidic conditions (5, 12, 22, 30). Except for the $\omega$-gliadins, each species contains cystine (S-S) groups and thus has the potential for reduction by thioredoxin.

When the thioredoxin-reduced gliadin fraction was subjected to native gel electrophoresis, the proteins found to be most specifically reduced by thioredoxin were recovered in the $\alpha$ fraction (Fig. 2). There was active reduction of the $\beta$- and $\gamma$-gliadins, but, as evident from the densitometer results summarized in Table I, the reduction within these groups was nonspecific, *i.e.* relatively high levels of reduction were

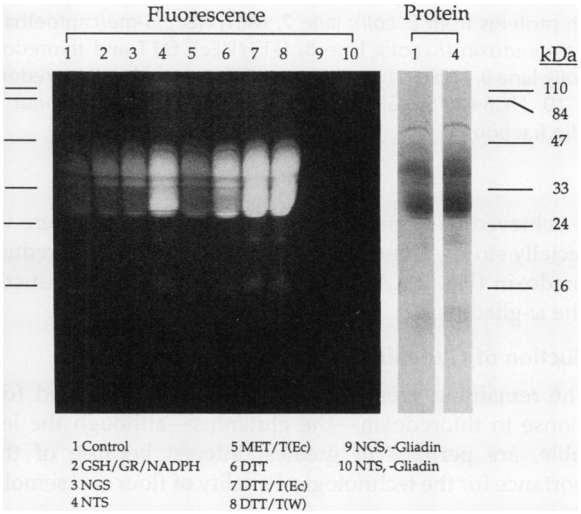


**Figure 1.** Thioredoxin-linked reduction of gliadins determined by an SDS-PAGE/mBBr procedure. Following incubation with the indicated additions, the gliadin proteins were derivatized with mBBr, and fluorescence was visualized after SDS-PAGE. The ratio of fluorescence to protein (Coomassie blue) of the gliadin fraction, determined by scanning the fluorescence negative and the protein print, is indicated in brackets after each treatment. Lane 1, Control: no addition [1.1]; lane 2, GSH/GR/NADPH: GSH, glutathione reductase (from spinach leaves), and NADPH [1.2]; lane 3, NGS: NADPH, GSH, glutathione reductase (from spinach leaves), and glutaredoxin (from *E. coli*) [1.6]; lane 4, NTS: NADPH, NTR, and thioredoxin (both proteins from *E. coli*) [3.9]; lane 5, MET/T(Ec): $\beta$-mercaptoethanol and thioredoxin (*E. coli*) [1.8]; lane 6, DTT [2.5]; lane 7, DTT/T(Ec): DTT and thioredoxin (*E. coli*) [3.0]; lane 8, DTT/T(W): same as lane 7 except with wheat thioredoxin *h* [3.6]; lane 9, NGS,−Gliadin: same as lane 3 except without the gliadin protein fraction [0]; lane 10, NTS,−Gliadin: same as lane 4 except without the gliadin protein fraction [0].

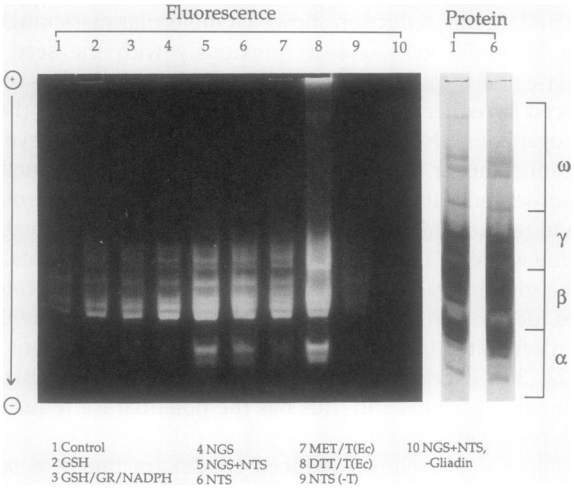


**Figure 2.** Thioredoxin-linked reduction of the different types of gliadins determined by an acid PAGE/mBBr procedure. Following incubation with the indicated additions, proteins were derivatized with mBBr, and fluorescence was visualized after acidic PAGE. Lane 1, Control: no addition; lane 2, GSH; lane 3, GSH/GR/NADPH: GSH, glutathione reductase (from spinach leaves), and NADPH; lane 4, NGS: NADPH, GSH, glutathione reductase (from spinach leaves), and glutaredoxin (from *E. coli*); lane 5, NGS+NTS: combination of lanes 4 and 6; lane 6, NTS: NADPH, NTR, and thioredoxin (both proteins from *E. coli*); lane 7, MET/T(Ec): β-mercaptoethanol and thioredoxin (*E. coli*); lane 8, DTT/T(Ec): DTT and thioredoxin (*E. coli*); lane 9, NTS(−T): same as lane 6 except without thioredoxin; lane 10, NGS+NTS,−Gliadin: same as lane 5 except without the gliadin fraction.

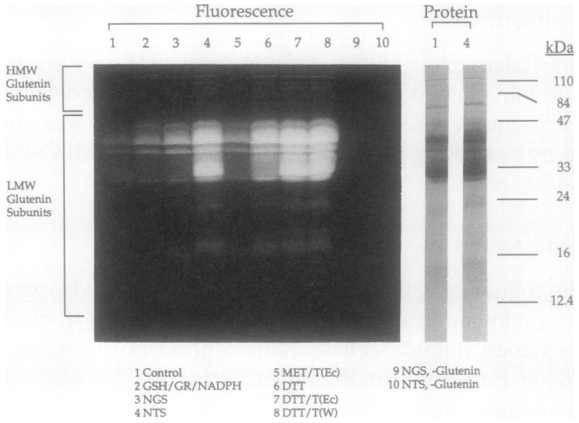


**Figure 3.** Thioredoxin-linked reduction of acid-soluble glutenins determined by an SDS-PAGE/mBBr procedure. Treatments and abbreviations were the same as in Figure 1. The ratios of fluorescence to protein (Coomassie blue) of the glutenin fraction, determined by scanning the fluorescence negative and protein print, are as follows. Lane 1, 0.5; lane 2, 0.6; lane 3, 0.8; lane 4, 1.4; lane 5, 0.8; lane 6, 1.0; lane 7, 1.4;. lane 8, 1.2; lane 9, 0; lane 10, 0.

also achieved with glutathione and glutaredoxin. There was especially strong reduction of the γ-gliadins by DTT-reduced thioredoxin (Fig. 2). As anticipated, there was no reduction of the ω-gliadins.

## Reduction of Glutenins

The remaining group of seed proteins to be tested for a response to thioredoxin—the glutenins—although the least soluble, are perhaps of greatest interest because of their importance for the technological quality of flour and semolina (17).

As shown in Figure 3, glutenins were reduced by thioredoxin. The most extensive reduction was observed in the low molecular mass range (30–55 kD). The reduction observed in the higher molecular mass range was less pronounced but still obvious, especially in the high molecular mass, 100 kD, region and above. Like the gliadins, certain of the glutenins were appreciably reduced by glutathione and glutaredoxin. However, in all cases, reduction was greater with thioredoxin and, in some cases, specific to thioredoxin (Table II, note proteins in the 30- to 40- and 60- to 110-kD range). As observed with the other wheat proteins tested, both the native *h* and *E. coli* thioredoxins were active and could be reduced with either NADPH and the corresponding NTR or with DTT. Thus, as found for the gliadins, glutenins were reduced *in vitro* by thioredoxin; some of them were reduced specifically, whereas others were reduced, albeit less effectively, by glutathione and glutaredoxin.

## *In Vivo* Reduction Experiments

The above findings demonstrate that thioredoxin specifically reduces components of the wheat gliadin and glutenin

**Table I.** *Reductant Specificity of the Different Types of Gliadins*

The area under α, β, and γ fluorescence peaks following reduction by the NADP/thioredoxin system were 1.2, 4.8, and 2.2 absorbance units times millimeters, respectively. These combined areas were about 65% of those observed when thioredoxin was reduced by DTT. Reaction conditions were as described in Figure 2.

| Reductant | Gliadin | | |
|---|---|---|---|
| | α | β | γ |
| | % relative reduction | | |
| None | 17 | 35 | 27 |
| Glutathione | 12 | 58 | 36 |
| Glutaredoxin | 11 | 83 | 64 |
| Thioredoxin | 100 | 100 | 100 |

**Table II.** *Reductant Specificity of Glutenins*

Reaction conditions were the same as described in Figure 3. Area under the fluorescence peaks of the three molecular mass classes (from high to low) following reduction by the NADP/thioredoxin system were 0.2, 4.2, and 3.6 absorbance units times millimeters, respectively.

| Reductant | Glutenin | | |
|---|---|---|---|
| | 60–110 kD | 40–60 kD | 30–40 kD |
| | % relative reduction | | |
| None | 12 | 32 | 24 |
| Glutathione | 28 | 55 | 27 |
| Glutaredoxin | 46 | 72 | 36 |
| Thioredoxin | 100 | 100 | 100 |

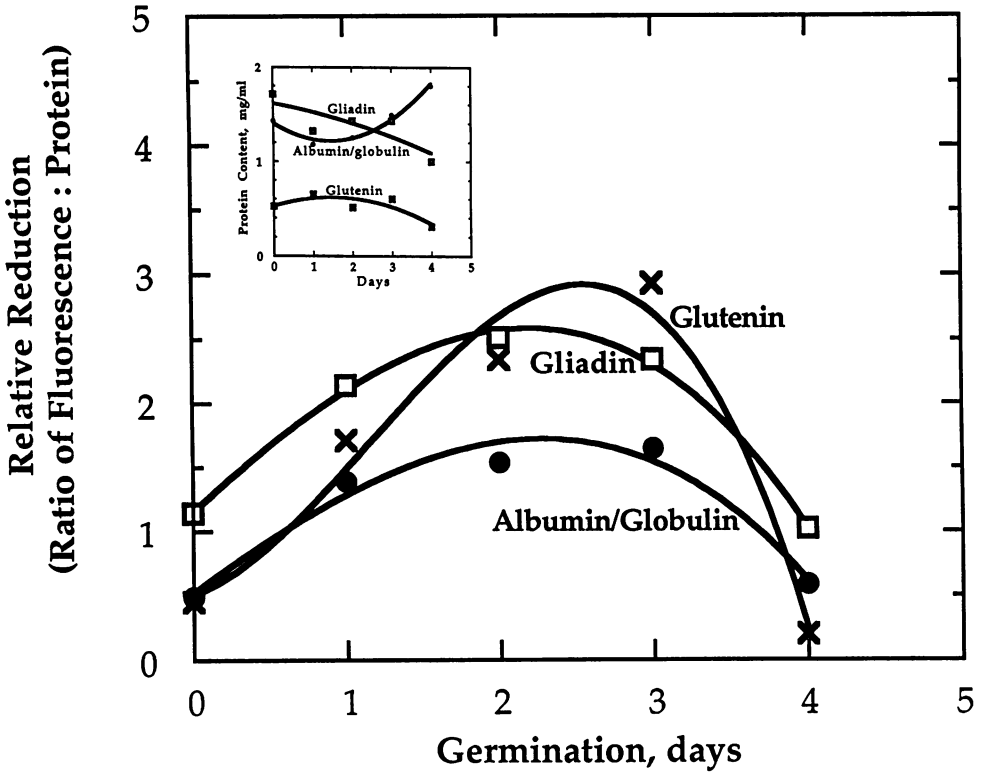


**Figure 4.** *In vivo* reduction status of seed protein fractions during germination.

fractions when tested *in vitro*. The results, however, provide no indication as to whether these proteins are reduced *in vivo* during germination—a question that, to our knowledge, has not been previously addressed (*cf.* 27).

To answer this question, we applied the mBBr/SDS-PAGE technique to monitor the reduction status of proteins in the germinating seed. We observed that reduction of components in the Osborne fractions increased progressively with time and reached a peak after 2 to 3 d germination (Fig. 4). The observed increase in reduction ranged from twofold with the gliadins to threefold with the albumin/globulins and fivefold with the glutenins. The net redox change was most apparent in the gliadins and glutenins found to undergo maximal reduction by thioredoxin, *i.e.* the 30- to 50-kD gliadins and 30- to 60-kD glutenins (*cf.* Figs. 1 and 3). The redox changes taking place in these fractions during germination are shown in Figure 5.

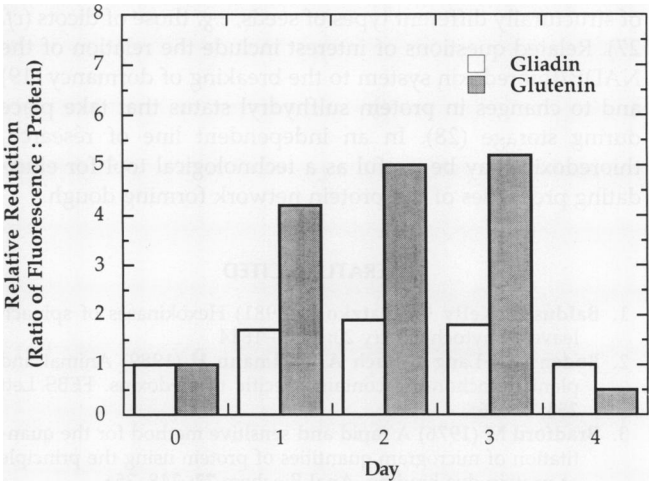


**Figure 5.** Reduction of principal thioredoxin-linked gliadins and glutenins during germination. Principal thioredoxin-linked gliadins were those in the molecular mass range from 30 to 50 kD (see Fig. 1) and glutenins were those in the molecular mass range from 30 to 60 kD (see Fig. 3).

### Enzyme Measurements

One question not answered by the above results is the source of NADPH needed for the NTR-linked reduction of thioredoxin *h*. To gain information concerning this point, we analyzed semolina for enzymes that function in the generation of NADPH, notably dehydrogenases of the oxidative phosphate pathway. The results summarized in Table III confirm earlier evidence (30) that endosperm extracts contain the enzymes needed to generate NADPH from glucose via this pathway: hexokinase, glucose 6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. The glucose 6-phosphate dehydrogenase activity (Table III) was insensitive

**Table III.** *Activities of Enzymes Effecting the Reduction of Thioredoxin h in Semolina (Glucose → Glu-6-P → 6-Gluconate → NADP → Thioredoxin h)*

| Protein | Activity |
|---|---|
| | *nkat/mg protein* |
| Hexokinase | 0.28 |
| Glucose-6-P dehydrogenase | 0.45 |
| 6-P-Gluconate dehydrogenase | 0.39 |
| NTR | 0.06 |
| Thioredoxin *h* | 0.35 |

to reduced thioredoxin (data not shown). In this respect the endosperm enzyme resembles its cytosolic (7) rather than its chloroplast (4, 24) counterpart from leaves (7).

As anticipated from earlier results with flour (10, 29), semolina also contained thioredoxin *h* and NTR (Table III). Based on activity measurements, NTR appeared to be a rate-limiting component in preparations from the cultivar examined.

## CONCLUDING REMARKS

The present results provide evidence that thioredoxin *h* functions as a signal to enhance metabolic processes associated with the germination of wheat seeds. Following reduction by NTR and NADPH (generated via the oxidative pentose phosphate pathway), thioredoxin *h* appears to function not only in the previously recognized activation of enzymes but also in the mobilization of storage proteins. It remains to be seen how thioredoxin relates to other germination signals, such as hormones, and to the mobilization of storage proteins of structurally different types of seeds, *e.g.* those of dicots (*cf.* 27). Related questions of interest include the relation of the NADP/thioredoxin system to the breaking of dormancy (19) and to changes in protein sulfhydryl status that take place during storage (28). In an independent line of research, thioredoxin may be useful as a technological tool for elucidating properties of the protein network forming dough.

## LITERATURE CITED


1. **Baldus B, Kelly GJ, Latzko E** (1981) Hexokinases of spinach leaves. Phytochemistry **20:** 1811–1814
2. **Bodenstein-Lang J, Buch A, Follmann H** (1989) Animal and plant mitochondria contain specific thioredoxins. FEBS Lett **258:** 22–26
3. **Bradford M** (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Anal Biochem **72:** 248–256
4. **Buchanan BB** (1991) Regulation of $CO_2$ assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspective on its discovery, present status and future development. Arch Biochem Biophys **288:** 1–9
5. **Bushuk W, Zillman RR** (1978) Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Can J Plant Sci **58:** 505–515
6. **Crawford NA, Droux M, Kosower NS, Buchanan BB** (1989) Evidence for function of the ferredoxin/thioredoxin system in the reductive activation of target enzymes of isolated intact chloroplasts. Arch Biochem Biophys **271:** 223–239
7. **Fickenscher K, Scheibe R** (1986) Purification and properties of the cytoplasmic glucose-6-phosphate dehydrogenase from pea leaves. Arch Biochem Biophys **247:** 393–402
8. **Florencio FJ, Yee BC, Johnson TC, Buchanan BB** (1988) An NADP/thioredoxin system in leaves: purification and characterization of NADP/thioredoxin reductase and thioredoxin *h* from spinach. Arch Biochem Biophys **266:** 496–507
9. **Garcia-Olmedo F, Rodriguez-Palenzuela P, Hernández C, Ponz F, Maraña C, Carmona MJ, Lopez-Fando J, Fernandez JA, Carbonero P** (1989) The thionins: a protein family that includes purothionins, viscotoxins and crambins. Oxf Surv Plant Mol Cell Biol **6:** 31–60
10. **Johnson TC, Cao RQ, Kung JE, Buchanan BB** (1987) Thioredoxin and NADP-thioredoxin reductase from cultured carrot cells. Planta **171:** 321–331
11. **Johnson TC, Wada K, Buchanan BB, Holmgren A** (1987) Reduction of purothionin by the wheat seed thioredoxin system. Plant Physiol **85:** 446–451
12. **Kasarda DD, Bernardin JE, Nimmo CC** (1976) Wheat proteins. Adv Cereal Sci Technol **1:** 158–236
13. **Kiss F, Wu M-X, Wong JH, Balogh A, Buchanan BB** (1991) Redox active sulfhydryls are required for fructose-2,6-bisphosphate activation of plant PFP (PPi fructose-6-phosphate 1-phosphotransferase). Arch Biochem Biophys **287:** 337–340
14. **Kobrehel K, Yee BC, Buchanan BB** (1991) Role of the NADP/thioredoxin system in the reduction of α-amylase and trypsin inhibitor proteins. J Biol Chem **266:** 16135–16140
15. **Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
16. **Laskowski M Jr, Kato I** (1980) Protein inhibitors of proteinases. Annu Rev Biochem **49:** 593–626
17. **MacRitchie F, du Cros DL, Wrigle CW** (1990) Flour polypeptides related to wheat quality. Adv Cereal Sci Technol **10:** 79–145
18. **Marcus F, Chamberlain SH, Chu C, Masiarz FR, Shin S, Yee BC, Buchanan BB** (1991) Plant thioredoxin *h*: an animal-like thioredoxin occurring in multiple cell compartments. Arch Biochem Biophys **287:** 195–198
19. **Mayer AM, Poljakoff-Mayber A** (1989) The Germination of Seeds, Ed 4. Pergamon Press, New York, p 160
20. **Osborne TB, Voorhees CG** (1893) The proteids of the wheat kernel. Am Chem J **15:** 392–471
21. **Ryan CA** (1981) Proteinase inhibitors. *In* PK Stumpf, E Conn, eds, Biochemistry of Plants, Vol 6. Academic Press, New York, pp 351–370
22. **Sapirstein HD, Bushuk W** (1985) Computer-aided analysis of gliadin electrophoregrams. I. Improvement of precision of relative mobility determination by using a three reference band standardization. Cereal Chem **62:** 372–377
23. **Scheibe R** (1991) Redox-modulation of chloroplast enzymes. A common principle for individual control. Plant Physiol **96:** 1–3
24. **Scheibe R, Geissler A, Fickenscher K** (1990) Chloroplast glucose-6-phosphate dehydrogenase: $K_m$ shift upon light modulation and reduction. Arch Biochem Biophys **274:** 290–297
25. **Schnarrenberger C, Oeser A, Tolbert NE** (1973) Two isoenzymes each of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in spinach leaves. Arch Biochem Biophys **154:** 438–448
26. **Shewry PR, Miflin BJ** (1985) Seed storage proteins of economically important plants. Adv Cereal Sci Technol **7:** 1–83
27. **Shutov AD, Vaintraub IA** (1987) Degradation of storage proteins in germinating seeds. Phytochemistry **26:** 1557–1566
28. **Stevanov BZ, Dencheva AV** (1984) Physiological and biochemical basis of seed viability. II. Content of sulfhydril [sic] groups in maize seed reserve proteins related to changes in their viability due to storage conditions. Fiziol Rast **10:** 67–72
29. **Suske G, Wagner W, Follmann H** (1979) NADPH thioredoxin reductase and a new thioredoxin from wheat. Z Naturforsch **34:** 214–221
30. **Tatham AS, Shewry PR, Belton PS** (1990) Structural studies of cereal prolamins, including wheat gluen. Adv Cereal Sci Technol **10:** 1–78
31. **Wong JH, Kobrehel K, Yee BC, Kiss F, Balogh A, Shin S, Wyss-Benz M, Buchanan BB** (1991) Thioredoxin as a reductant for seed proteins (abstract No. 902). Plant Physiol **96:** S-136