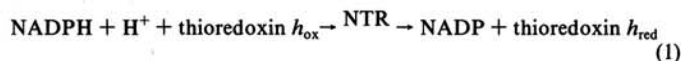


## Thioredoxin and Bread Wheat

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Thioredoxins are small (12 kDa) thiol redox carrier proteins that play a regulatory role in a number of cellular processes (Holmgren 1985, Buchanan 1991, Scheibe 1991). Two thioredoxin systems occur in plants. In chloroplasts, the ferredoxin-thioredoxin system, comprised of ferredoxin, ferredoxin-thioredoxin reductase, and two types of thioredoxin (*f* and *m*), provides a means by which light is linked to the regulation of selected enzymes of photosynthetic CO<sub>2</sub> assimilation. The other thioredoxin system—the nicotinamide adenine dinucleotide phosphate (NADP)-thioredoxin system—utilizes reduced NADP (NADPH) generated in the oxidation of carbon substrates to reduce a specific thioredoxin (designated the *h*-type) via a flavin enzyme, NADP-thioredoxin reductase (NTR) (equation 1). The NADP-thioredoxin system, localized in the cytosol, mitochondria, and endoplasmic reticulum, has been identified in all plant tissues so far examined including wheat endosperm (Suske et al 1979, Johnson et al 1987, Marcus et al 1991).



We have found previously that thioredoxin *h* reduces low-molecular-weight soluble proteins of seeds—thionins,  $\alpha$ -amylase inhibitors, and trypsin inhibitors (Wada and Buchanan 1981, Johnson et al 1987, Kobrehel et al 1991). Moreover, the NADP-thioredoxin system functions in the reduction of members of the major (insoluble) storage proteins of durum wheat—gliadins and glutenins. The *in vivo* reduction of these storage proteins is linked to their proteolytic breakdown during germination (Kobrehel et al 1992).

The storage proteins studied so far have been restricted to durum wheat. Here we report that thioredoxin reduces glutenins as well as the other major protein classes of bread wheat (cv. Scout 66). Reduction of the flour proteins was accompanied by dough strengthening as determined by microfarinograph tests.

## Reduction of Glutenins

Among the flour proteins, glutenins are perhaps of greatest interest because of long-standing recognition of their particular importance to dough formation. We, therefore, tested the ability of thioredoxin and reduced glutathione to reduce this family of proteins from bread wheat.

Glutenins were extracted from flour with 0.1M acetic acid after its sequential extraction with 0.5M NaCl and 70% ethanol. Reduction of glutenins was determined by the sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-monobromobimane (mBB) procedure (Kobrehel et al 1992). There were five different treatments: 1) control (no addition); 2) reduced glutathione; 3) reduced glutathione, glutathione reductase (from spinach leaves), and NADPH; 4) NADPH, NTR, and thioredoxin (both proteins from *Escherichia coli*); and 5)  $\beta$ -mercaptoethanol. Reactions were carried out in 30 mM Tris-HCl buffer, pH 7.9, at 25°C. As indicated, 0.8  $\mu$ g of NTR and 0.7  $\mu$ g of thioredoxin (both from *E. coli*) were added to 70  $\mu$ l of this buffer containing

1 mM NADPH and approximately 40  $\mu$ g of glutenin protein fraction. When assayed with either reduced glutathione or  $\beta$ -mercaptoethanol, NADPH and NTR were omitted, and each of these reductants were added to 2 mM to give a constant sulfhydryl concentration. As indicated in treatment three, a saturating level (1.4  $\mu$ g) of glutathione reductase from spinach leaves (Florencio et al 1988) was added to the reaction mixture. After incubation for 20 min, 400 nmol of mBB was added and the reaction was continued for another 15 min. To stop the reaction and derivatize excess mBB, 10  $\mu$ l of 10% SDS and 10  $\mu$ l of 100 mM  $\beta$ -mercaptoethanol were added. After adding 40  $\mu$ l of tracking dye (bromophenyl blue in 40% glycerol), samples were applied to SDS-polyacrylamide gels (10%, 1.5 mm thick, pH 8.5) that were developed at a constant current of 8mA (Kobrehel et al 1991; 1992).

The results showed that flour glutenins, extracted with acetic acid, were reduced by thioredoxin (Fig. 1). Although the most extensive reduction was observed in the low-molecular-weight range, the high-molecular-weight glutenin subunits also were reduced. The glutenin fraction was less extensively reduced by glutathione (maintained in the reduced state by NADPH and glutathione reductase). For all glutenin fractions, reduction was greatest with thioredoxin, and some of them were specific to thioredoxin. Similar findings were made earlier with glutenins from durum wheat (Kobrehel et al 1992). The other major protein groups of flour—albumins, globulins, and cysteine-containing gliadins—also were reduced by thioredoxin with results similar to those obtained with semolina (compare Kobrehel et al 1992).

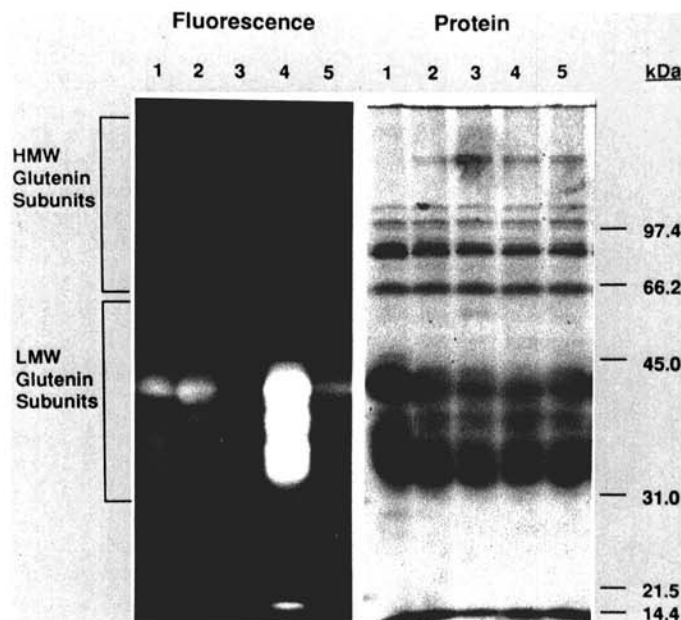


Fig. 1. Thioredoxin-linked reduction of glutenins. The five treatments were 1) control (no addition); 2) reduced glutathione; 3) reduced glutathione, glutathione reductase (from spinach leaves), and reduced nicotinamide adenine dinucleotide phosphate (NADPH); 4) NADPH, NADP-thioredoxin reductase, and thioredoxin (both proteins from *Escherichia coli*); and 5)  $\beta$ -mercaptoethanol. HMW = high molecular weight; LMW = low molecular weight.

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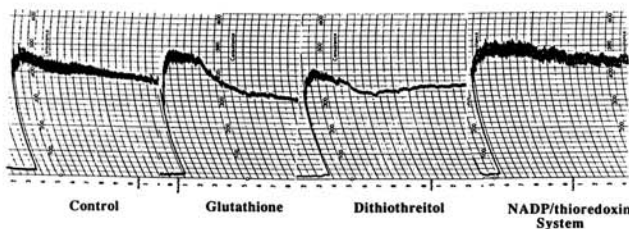


Fig. 2. Contrasting effects of sulfhydryl reductants on dough prepared from poor-quality soft wheat flour (microfarinograph assay). NADP = nicotinamide adenine dinucleotide phosphate.

### Influence on Dough Formation and Quality

It is generally thought that disulfide proteins, especially glutenins, play a primary role in dough formation. The question arises as to whether, in its role as a reductant of specific disulfides, thioredoxin can intervene in this process and enable the free-protein sulfhydryl (SH) groups to interact during mixing.

As shown in Figure 2, the NADP-thioredoxin system strengthened dough prepared from poor-quality soft wheat based on microfarinograph measurements. For the microfarinograph assays, 10 g of flour (Apollo) was added to the mixing compartment of a Brabender microfarinograph. In the control sample, 5.25 ml of 30 mM Tris-HCl buffer, pH 7.9, was added to the flour at 0 time, the chamber was covered, and mixing was carried out for 15 min at 30°C. Farinograph measurements were recorded for at least 10 min. With the NADP-thioredoxin system, 2 µg of *E. coli* thioredoxin, 1 µg of *E. coli* NTR, and 500 nmol of NADPH were added to 100 µl of the above buffer. After stirring, the solution was diluted to 5.25 ml with additional buffer. At 0 time, the total enzyme mixture was added to the 10 g of flour and processed as above. With the sulfhydryl compounds, procedures were as with the control except that the buffer contained either 1 mM reduced glutathione or 0.5 mM dithiothreitol.

The microfarinograph reading was 375 BU 7 min after dough formation with the control (no added enzyme system) vs. 450 BU for the same flour treated with components of the NADP-thioredoxin system. To enhance enzyme activity by using higher hydration, no attempt was made to obtain the peak value of 500 BU. As expected from earlier studies, the dough was weakened by the addition of either dithiothreitol or reduced glutathione. The effects of thioredoxin thus differ from those of other thiols. A possible explanation for this difference lies in the specificity of thioredoxin, which as exemplified by extensive studies with chloroplast enzymes, reduces only specific protein disulfides. The results suggest that, during mixing, groups, generated from intramolecular disulfides by thioredoxin, interact with certain disulfide groups of other subunits to form intermolecular disulfide linkages between proteins, thereby contributing to the formation of the

network. Because thioredoxin cannot effectively reduce the new intermolecular disulfide bonds, it enhances network formation and thereby strengthens the dough product. On the other hand, in the cases of reductants such as dithiothreitol and reduced glutathione, the newly formed disulfide bonds can be reduced again because of the lack of specificity of these reductants.

### CONCLUSIONS

The present findings demonstrate that thioredoxin, reduced by NADPH and NTR, is a specific reductant for the glutenins and other protein families of bread wheat. The results suggest that reduction by thioredoxin is of technological significance as determined by microfarinograph tests. Baking tests currently in progress support this conclusion.

### ACKNOWLEDGMENTS

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