

Ongoing Applicative Studies of Plant Thioredoxins

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ABSTRACT Studies triggered by the discovery of the function of thioredoxin (Trx) in photosynthesis have revealed its role throughout biology. Parallel biochemical and proteomic analyses have led to the identification of its numerous putative targets. Recently, to verify the biological significance of these targets, *in vivo* studies using transformants in which Trx is overexpressed or suppressed are in progress, and the transformants themselves that are being used in such studies show their potential applicative values. Moreover, Trx's mitigation of allergenicity for some proteins offers promising prospects in the food industry. Practical studies based on redox regulation, once only on the horizon, are now achieving new dimensions. This short review focuses on the industrial applications of Trx studies, the current situation, and future perspectives. The putative targets obtained by the proteomics approach in comparison with *in vivo* observations of the transformants are also examined. Applicative studies of glutathione, a counterpart of Trx, are also discussed briefly.

Key words: allergen; food; redox regulation; target; thioredoxin.

INTRODUCTION

Thioredoxins (Trxs) are regulatory proteins widely distributed throughout most living cells. The redox-active disulfide group of Trxs controls the thiol–disulfide exchange of the target proteins functional in various biological processes. In other words, Trxs are disulfide reductases, and the oxidized Trxs are re-reduced by NADPH-dependent Trx reductases. The pioneering work of Reichard, Holmgren and co-workers with *Escherichia coli* led to the identification and characterization of Trx and, further, the elucidation of its function as an electron donor to ribonucleotide reductase (Reichard, 1968; Holmgren, 1985).

Since the regulatory function of Trx was discovered in photosynthesis (Wolosiuk and Buchanan, 1977), biochemical analyses gradually increased the number of known Trx-linked enzymes. Then, at the beginning of the twenty-first century, the concept of proteomics was introduced to Trx studies (Motohashi et al., 2001; Yano et al., 2001b). With the use of several upgraded techniques (Maeda et al., 2004; Marchand et al., 2004; Hagglund et al., 2008), proteomic approaches have accelerated the comprehensive target analyses of Trxs, and have contributed to the impressive listing of targets (Montrichard et al., 2009). The role of Trx, first discovered in the context of photosynthesis, is being elucidated throughout biology.

Meanwhile, biochemical/proteomics approaches have identified 'putative' targets of Trx, and have helped us speculate about the reactions in which they are involved. For example, it is not easy to reproduce, in a test tube, the exact

physiological conditions that occur in a plant system. Thus, experimentally obtained data do not necessarily reflect biological reactions. In this context, Shahpiri et al. (2009) noted that, during germination, the pH of the starchy endosperm becomes as low as 5 (Dominguez and Cejudo, 1999) and, at such a low pH, the thiolate anion, required for nucleophilic attack during the disulfide exchange between Trx and the target (Kallis and Holmgren, 1980), may not be formed. Although they confirmed that the barley NTR/Trx system was active *in vitro* at pH 5 (Shahpiri et al., 2008), they stressed the importance of determining whether the NTR/Trx h system was functional under experimental conditions in which putative targets were identified. The significance of setting the *in vitro* experimental conditions was also pursued by Traverso et al. (2010), who developed a 'stringent' column to capture targets of Trx and successfully identified distinctive respective targets of pea Trx isoforms, PsTrxh1 and PsTrxh2.

Accordingly, the increasing number of putative targets increases the demand to confirm whether the targets function *in vivo*. To address this issue, verification studies, such as those using transformants in which Trx was overexpressed or suppressed, are now in progress. These studies not only help

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to confirm the role of Trx suggested by the *in vitro* experiments, but also lay out the possibilities for the industrial use of the transformants themselves. This research area includes boosting the starch yield in tobacco leaves for bioethanol production and lessening acrylamide in French fries, as well as suppressing pre-harvest sprouting. What is more, applicative studies of Trx especially in the food industry are in progress.

This short review introduces ongoing studies from a practical application perspective. We also seek to verify the putative targets obtained by biochemical/proteomics approaches with the behavior of transformants. In addition, the latter part of this review covers the progress in applicative studies of glutathione, a counterpart of Trx. Together, the results obtained thus far help us confront important issues such as the growing numbers of people with allergies, the changing climate, and the increasing demand for energy. Applicative studies on redox regulation, which Dr. Bob Buchanan once mentioned as 'on the horizon' (Buchanan and Balmer, 2005), are now under way. To avoid overlapping content with other papers in this special issue, this review focuses on the industrial applications of the redox biology of plants—a door that was opened by the discovery of Trx's role in photosynthesis.

INDUSTRIAL APPLICATIONS OF THIOREDOXINS

The First Trial to Overexpress Trx in Plants

Trx research stemmed from the studies of its regulatory function in photosynthesis. Trx is now known to work not only in the Fdx/Trx system, but also in the NADPH/Trx system in the cytosol, endoplasmic reticulum, mitochondria, and throughout the whole plant system. These experimental observations have attracted researchers who want to study the effects of overexpression or suppression of Trx in plants. Thus, Dr. Buchanan's group, in cooperation with Dr. Peggy Lemaux's group, has developed effective transformation techniques to control the expression of Trx in plants (Cho et al., 1999, 2004; Li et al., 2009).

Overexpression of Trx in plants was first tried in barley (Cho et al., 1999). Using the B₁-Hordein promoter as well as signal peptides targeted to the protein body, Trx h was overexpressed (×30) in barley endosperm. The transformant had several unique features. First, it germinated earlier than the wild-type. Second, enzymes involved in starch degradation appeared earlier. Third, the amount of gibberellic acid (GA) increased to 1.6–2.8-fold. Finally, the solubility of some storage proteins increased (Wong et al., 2002). Activation of enzymes involved in the germination (Kobrehel et al., 1991) and solubilization of storage protein (Kobrehel et al., 1992) in the transformant were expected in light of the previous *in vitro* experimental data. However, these findings comprised the first discovery showing that the control of Trx expression affects plant physiological processes such as germination. As the acceleration of barley seed germination reduces

production costs and improves beer quality (Bamforth, 2000), this was the first evidence suggesting the possibility of the industrial application of Trx-transformants.

The transformants also showed interesting features. When the de-embryonated transgenic grain was incubated with GA, the activity of the amylase, which is synthesized in the aleurone layer, increased 1 d earlier compared to the wild-type. Thus, it was suggested that information in the starchy endosperm was transmitted to aleurone layers (Wong et al., 2002); that is, Trx h of the starchy endosperm communicates with adjoining tissues, thereby regulating their activities, notably by accelerating the germination of the embryo and the appearance of α -amylase released by the aleurone. This interesting hypothesis was strengthened later by the discovery of a unique transmittable Trx (Meng et al., 2010) which is discussed later in this review.

Moreover, as germination is not interfered with in the presence of Se, the transformants may be useful in bio-remediation studies (Kim et al., 2003). Wei et al. (2008) also overexpressed Trx S in barley, and the transgenic seeds showed increased activity levels of α -amylase, free β -amylase, and limit dextrinase. A higher sulfhydryl content of protein in the transgenic seeds was also confirmed.

Alleviation of Allergenicity by Trx

Wong et al. (2002) reported that, relative to null segregant controls lacking the transgene, homozygotes overexpressing Trx h caused a 35% increase in the ratio of the relative reduction (abundance of SH) of the propanol-soluble proteins (hordein I fraction) as well as 5–12% and 11–35% increases in extractable and soluble proteins, respectively. As evidence accumulates that disulfide bonding, protease resistance, and allergenicity are interrelated, the obtained knowledge opens up the opportunity to develop less-allergenic cereals.

Now, the correlation between disulfide and the allergenicity of a protein is introduced briefly. We have been studying the detailed mechanisms underlying the degradation of storage proteins in seed germination (Yano et al., 2001a; Yano and Kuroda, 2006). Most storage proteins are located in the protein body, and they achieve a compact structure by forming intra- and/or intermolecular disulfide bonds. On the other hand, in germination, they are promptly degraded into amino acids to serve as pieces for the construction of roots and shoots. Here, plants have a unique system to make both of them compatible. Trx 'unfolds' the compact structure of the storage protein by cleaving its disulfide bonds (Figure 1) (Yano, 2009), and it also activates enzymes to degrade the protein. In contrast, when animals eat seeds or crops, they just try to digest the protein with proteases without unfolding it. Here, allergy problems occur. In some cases, the region around the disulfide bond is protease-resistant. Thus, when the protein is attacked by a protease, the protease-resistant region survives and occasionally becomes an allergen (Sen et al., 2002; Yano et al., 2006). Dr. Buchanan's group treated

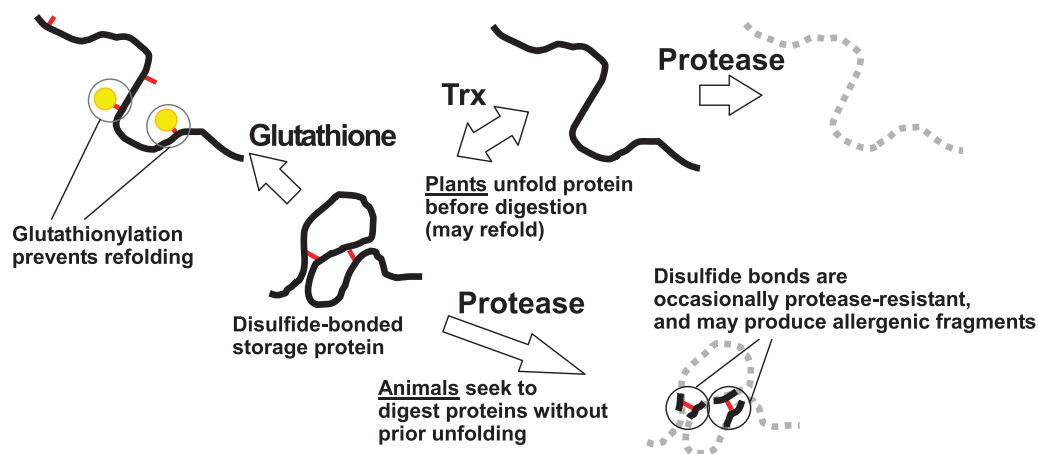


Figure 1. A Model Illustrating the Disulfide-Bond Formation of Protein and Its Protease Resistance.

In seed germination, disulfide bonds of storage protein are reduced by Trx before protease digestion. However, animals seek to digest protein without prior unfolding, and this occasionally leads to the generation of protease-resistant fragments. Glutathionylation of the exposed SH groups prevents refolding. Trx, thioredoxin.

such allergens with Trx, and they demonstrated that the cleavage of the disulfide bonds, namely the unfolding of the protease-resistant region, improved the protease digestibility of the allergens (Buchanan et al., 1997; del Val et al., 1999). Moreover, to prevent the recovery of disulfide-bond formation, they capped (glutathionylated) the free SH groups using glutathione or cystamine (Morigasaki et al., 2001, 2003).

In those studies, Dr. Buchanan's group also used a unique high IgE-producing canine model (Ermel et al., 1997; Buchanan and Frick, 2002) to estimate the allergenicity of a protein. The dogs were descendants of high IgE-producing spaniel/retriever/basenjis, and were maintained at the University of California, Davis. In short, the dogs were previously sensitized to the native proteins. Prior to skin testing, each dog received Evans blue dye solution through a cephalic vein to enhance the assessment of the cutaneous IgE antibodies. Allergen samples were then injected intradermally on the abdominal skin, and the allergic response was determined by measuring the blue wheal reaction (maximum length and width). When Trx-treated allergen was applied as a sample, it showed decreased allergenicity, apparently as a result of changes in IgE epitope accessibility (Buchanan et al., 1997).

Moreover, Trx treatment alleviated the dogs' clinical symptoms such as vomiting, diarrhea, and constipation. To evaluate allergenicity, Western blotting using patient sera is generally conducted. However, although specific IgE is indispensable to the induction of an allergic reaction, its presence in the serum does not necessarily indicate that the patient is allergic clinically to the protein (Thomas et al., 2008). In contrast, the canine test can be used not only to conduct comparative studies of known allergens, but also to estimate the allergenicity of unknown foods by sensitizing dogs to them. The profiles of allergenic foods as well as the responses to individual allergens and the clinical reactions

of dogs are similar to those observed in humans. However, the immune responses of dogs, such as the secretion of cytokines and chemokines, are not yet as well characterized as those of mice or rats. The advantages and disadvantages of evaluating the allergenicity of a protein by several animal models are discussed comparatively elsewhere (Kimber et al., 2003).

As issues surrounding food allergies are increasing (Burks et al., 2012), the development of less-allergenic foods is becoming more important. Recent studies on Trx and foods are thus transitioning from experimental to practical projects. The immunoreactivity of gliadin was reduced without losing the dough rheological properties of winter wheat (Waga et al., 2008). An edible yeast extract in which Trx was concentrated was applied for the mitigation of food allergens (Taketani et al., 2011) as well as for the development of functional foods (Nakajima et al., 2012). The use of canine and other models for the precise evaluation of the allergenicity of foods is becoming increasingly important before the initiation of human clinical trials.

Challenges in the Development of Useful Plants

This section introduces studies on the usefulness of transformants in which Trx is overexpressed or suppressed (Table 1).

Prevention of Pre-Harvest Germination

Climate change, especially global warming, greatly affects both the yield and the quality of crops. Trx studies are being used for the development of plants that can cope with these changes. Dr. Buchanan's group first overexpressed Trx in wheat endosperm and confirmed the increased solubility and digestibility of the storage proteins, the minimized allergenicity of the gliadin fraction, and the improved quality of

Table 1. Effects of overexpression and underexpression of Trx on plants and the industrial implications.

Promoter	Trx type	Effect	Industrial meaning	Reference
OVEREXPRESSION				
<i>Barley</i>				
B ₁ -hordein	Trx h5	Early germination/ α -amylase release Higher pullulanase activity Increase in extractable and soluble protein	Effective beer production Less-allergenic cereal	Cho et al. (1999) Wong et al. (2002)
<i>Wheat</i>				
B ₁ -hordein	Trx h5	Higher gliadin solubility Lowered gliadin allergenicity	Less-allergenic cereal	Li et al. (2009)
<i>Tobacco</i>				
PrnG10L	Trx f	Leaf starch accumulation (x7) Release of fermentable sugar (x5)	Biofuel production	Sanz-Barrio et al. (2013)
<i>Arabidopsis</i>				
CaMV35S	TDX	Heat shock-induced holdase chaperone activity	High-temperature-tolerant plant	Lee et al. (2009)
UNDEREXPRESSION				
<i>Wheat</i>				
Alpha-gliadin	Trx h9	Retarded germination up to a week Slightly lowered pullulanase	Suppression of pre-harvest sprouting	Li et al. (2009)
<i>Potato</i>				
35S CaMV	Trx h1	Prevention of cold-induced sugar accumulation	Less acrylamide in processed food	He et al. (2012)

bread dough made from the transformant (Li et al., 2009). They also reduced the expression of Trx h9 using its antisense RNA and opened a new possibility for applicative studies of Trx.

Reflecting increasing health concerns, the consumer demand for high-fiber whole-wheat breads is growing (Slavin, 2004). White wheat has become increasingly popular in making those breads, because it is naturally white and does not need to be bleached. However, white wheat is vulnerable to pre-harvest sprouting (PHS)—a condition where grains germinate on the spike before harvest. The resulting harvest losses reduce crop yields and reduce the quality of seeds and flour, resulting in significant financial losses for farmers. Prolonged or untimely rainfalls or high humidity occurring late in the growing season causes PHS, and global climate change may increase the prevalence of such occurrences. Although some genes to regulate PHS have been identified (Nakamura et al., 2011), basic studies have not yet led to control over PHS. Dr. Buchanan's group reduced the expression of Trx h9 in starchy endosperm, and they found that the suppression led to improved PHS resistance of the transformants (Li et al., 2009). They also found that the crop yield and flour quality were not reduced (Ren et al., 2012).

Besides increasing the industrial usefulness of white wheat, the discoveries described above provide new scientific knowledge, namely a clue to answer the question posed previously: How is the information in the starchy endosperm transmitted to the embryo and aleurone? It was found that

Trx h9 is a unique homolog, in that it is a membrane-associated protein and transmittable between cells (Meng et al., 2010). While green fluorescent protein (GFP)-modified Trx2, expressed with the SCARECROW promoter, was observed in the root endodermal cells, the Trx h9 counterpart was observed throughout the root. This means that Trx h9 can move from cell to cell; that is, it can migrate from its original expression site in the endodermal cells to other cell layers of the root. Conclusively, the results suggest a role for this Trx in cell-to-cell communication, relaying information to maintain cellular redox balance and possibly functioning in redox signaling.

Starch Accumulation in Leaves

The studies by Dr. Buchanan's group described above successfully proved the biological behavior of transformants predicted by prior biochemical observations. The following research shows the difficulty of estimating *in planta* reaction based on *in vitro* data.

Alternatives to petroleum-derived transport fuels are being sought in order to diminish the world's dependence on non-renewable resources, which are of limited availability and contribute to global warming. Most current bioethanol production relies on plant starch, and thus extensive studies have been done to increase its yield. Much effort has been centered on manipulating ADP-glucose pyrophosphorylase (AGPase), because this enzyme catalyzes the first committed

step of starch synthesis, namely the conversion of glucose 1-phosphate and ATP to ADP-glucose and PPi. However, limited success has been obtained so far in raising the yield of starch (Smith, 2008). Another study suggested that the synthesis/degradation of starch is subjected to complex redox regulations (Kotting et al., 2010). Moreover, the small subunit of the AGPase was reported to be a putative target of Trx f and m (Ballicora et al., 2000). Based on the notable influence of redox regulation on photosynthetic and carbon metabolism enzymes, Sanz-Barrio et al. (2013) overexpressed Trx f and Trx m, respectively, in the tobacco leaf to explore whether the rate of starch synthesis could be increased. The outcome was impressive. When Trx f was overexpressed in tobacco leaves, the plant showed an increase of up to seven-fold in leaf starch accumulation, accompanied by increased biomass yield. Moreover, 5.5 times more fermentable sugar was obtained in comparison to the wild-type. Sanz-Barrio et al. estimated that, in theory, 40 l of bioethanol per fresh ton of transformants could be obtained, which is 10 times the amount obtained from the wild-type. Conclusively, genetically engineered tobacco could be an excellent alternative feedstock for biofuel production.

Meanwhile, the work underscores anew the deep divisions between biochemical observations and plant biology. First, contrary to expectations based on the previous *in vitro* observations, the light redox activation of AGPase in the leaves was not affected by the overexpression of Trx f. It is thus unlikely that the higher starch accumulation is due to an increase in AGPase redox activation. The mechanism by which the Trx f-overexpressing plants come to show their markedly starchy phenotypes remains unknown. It is possible that impaired sucrose export exerted by the overexpressed Trx f (Thormahlen et al., 2013) contributes to the increase in sucrose content in the leaves, which in turn exert feedback control resulting in the starchy phenotype. Another possibility is the activation of starch synthase III (Li et al., 2011). As several enzymes involved in the synthesis/degradation of starch are known to be redox-regulated (Glaring et al., 2012), the possibly complex mechanism underlying these actions should be elucidated in further studies. Second, whereas the overexpression of Trx f was effective in increasing the starch yield, the counterpart Trx m was not. Although both Trx f and m function as messengers in the ferredoxin/Trx system by transmitting the redox signal from ferredoxin/Trx reductase to target enzymes, they differ in phylogenetic origin (Hartman et al., 1990) as well as in primary/tertiary structures and target specificity (well discussed in Schürmann and Buchanan, 2008). Their distinct *in vivo* behavior demonstrates difficulties in predicting the Trx-dependent *in planta* reactions from biochemical observations.

Prevention of Acrylamide Formation in Fried Potato

The biological synthesis and degradation of starch also significantly affects a familiar dish: French fries. After harvesting, potato tubers are often stored at low temperature,

mainly to prevent sprouting. However, the cold storage leads to the accumulation of reducing sugar, in a process called cold-induced sweetening (CIS). The accumulation of sucrose leads to increased cytochlema concentrations to cope with the cold. Subsequently, a proportion of the sucrose may be hydrolyzed to glucose and fructose, resulting in CIS (Malone et al., 2006).

Acrylamide formation in food processing is a significant problem in the food industry. Acrylamide is generated by the reaction of reducing sugar and amino acids during frying. Thus, the prevention of CIS has been considered an effective strategy for reducing acrylamide in fried potatoes (Vinci et al., 2012). He et al. (2012) cloned a novel isoform of the Trx h group, *SbTRXh1*, which was up-regulated early in the 4°C storage of potato tubers of the cold-resistant cultivar (cv.) *Solanum berthaultii*. Using RNA interference, He et al. then inhibited the expression of the *SbTRXh1* gene in the potato cv. E-potato 3 (E3). The transcripts of *SbTRXh1* in the two selected transformants were decreased by 66.1% and 88.2%, respectively. Moreover, the proportion of reduced sugar was 33.7–65.2% in the wild-type when the tubers were stored at 4°C. These data suggested that *SbTRXh1* functions in the sucrose-reducing sugar pathway and regulates the CIS of potato tubers.

Both the synthesis and degradation of starch are affected by redox regulation (Schürmann and Buchanan, 2008). After detachment from the mother plant, AGPase is considered a key enzyme for the regulation of starch synthesis in potato tubers (Tiessen et al., 2002). The enzyme catalyzes the first committed step of starch synthesis Trx-dependently (Ballicora et al., 2000; Tiessen et al., 2002). Other enzymes in the starch-sucrose pathway are putative targets of Trx: phosphoglucosyltransferase (Lindahl and Florencio, 2003) and UDP-glucose pyrophosphorylase (Wong et al., 2004; Alkhalfioui et al., 2007). Further research should elucidate the detailed mechanism by which RNA interference with *SbTRXh1* decreased the production of reducing sugars. As the potato Trx group have also succeeded in decreasing reduced sugar by suppressing acid vacuolar invertase (Li et al., 2013), a combinational work will lead to the effective development of potato tubers for acrylamide-free processed foods, such as French fries.

Heat-Resistant Plants

In contrast to cold-temperature studies, interesting results have been reported on Trx-dependent heat resistance. Lee et al. (2009) found that TDX, a Trx-like protein, has both disulfide reductase and foldase chaperone activities at moderate temperatures. At high temperature, TDX forms high-molecular-weight polymers to achieve holdase chaperone activity. Thus, the oligomerization status is reversibly regulated by heat shock, which causes a transition from a disulfide reductase/foldase chaperone to a holdase chaperone. When TDX was overexpressed in *Arabidopsis*, the transgenic plant recovered from heat shock faster than the wild-type.

Although the molecular mechanism has yet to be clarified, this result suggests the possible contribution of this Trx-like protein in producing crops that can cope with the changing environment.

As observed above, controlling Trx expression in plants has led to the development of plants with useful potency in various industrial aspects. The effective utilization of plant Trxs can be expected to increase in the future.

INDUSTRIAL APPLICATIONS OF GLUTATHIONE

Development of Foods with New Properties

Disulfide bonds affect critical characteristics of food other than allergenicity (Vasbinder et al., 2003; Visschers and de Jongh, 2005). If not for the unique disulfide-network formation of gluten from gliadin and glutenin (Barak et al., 2013), wheat would not have been a principal crop worldwide. Moreover, disulfide plays significant roles in the production of distinctive foods such as tofu (Kohyama et al., 1995) and yuba (also known as tofu skin) (Kunisho et al., 2010). Control over disulfide cross-linking will make it feasible to develop unique and novel foods. Although commonly used chemicals such as dithiothreitol or mercaptoethanol are not suitable for food production, Trx and glutathione are excellent candidates for a controller. Glutathione is a ubiquitous peptide existing in most plant and animal tissues (Jones, 1995) and is present in a wide range of foods (Wierzbicka et al., 1989). Here, we introduce examples in which glutathione is used in food and plant development.

Gluten is the primary allergenic substance in wheat allergy (Varjonen et al., 2000) as well as celiac disease (Green and Cellier, 2007). Thus, demand for the development of gluten-free foods, especially bread, is growing (do Nascimento et al., 2013). We have sought to produce bread using rice flour, which does not contain gluten. We first tried to make a gluten-like viscous protein out of rice flour. Glutathione was applied, aiming to transform intramolecular proteins in rice flour into intermolecular proteins to form a gluten-like network. Both reduced glutathione (GSH) and oxidized glutathione (GSSG) were found effective in swelling the gluten-free rice batter (Yano, 2010, 2012). However, contrary to our expectation, the swelling occurred based on a new mechanism rather than by the formation of gluten-like protein. Microstructure and biochemical analyses suggested that glutathione cleaved the disulfide-linked polymers surrounding the starch granule (Figure 2). The polymer has been suggested to work as a barrier to hinder the adsorption of water when water was added to the flour (Hamaker and Griffin, 1993). Degradation of the barrier may make the batter more consistent and viscous, thereby improving its gas-holding capacity in yeast fermentation. While protease digestion of the polymer was tried in other studies (Hamada et al., 2013; Kawamura-Konishi et al., 2013), the specific volume of the

bread was lower than that obtained with the disulfide cleavage of the polymer. Therefore, glutathione should be a useful tool with which to develop food with new properties.

The biological behavior of the disulfide-linked polymer in seed germination has not well been elucidated. Thus, the present status of the research suggests that there are several unknown/unutilized structures in food material such as cereals or meats that affect food properties. Some of them may be controllable by a cleavage of the disulfide bonds by the addition of Trx or glutathione.

Development of Useful Plants

Recent studies have advanced the use of glutathione in controlling plant physiology. Keeping imbibed seeds at low temperature for a certain period, so-called vernalization (Chouard, 1960), promotes seed germination and subsequent flowering in various plant species. In the rosette plant *Eustoma grandiflorum*, vernalization is a prerequisite in the transition from the vegetative rosette stage to the reproductive growth stage (bolting). Dr. Ken'ichi Ogawa's group in Okayama, Japan, found that, when cysteine, a precursor for glutathione biosynthesis, was added to the growth medium, the transition from the vegetative rosette stage to bolting occurred without vernalization (Yanagida et al., 2004). The addition of buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, interfered with this effect. Moreover, the effect was recovered by the addition of GSH but not by that of cysteine. Thus, Yanagida et al. concluded that GSH biosynthesis is involved in vernalization-induced bolting, and that it is regulated specifically by GSH. Also, the overexpression of *GSH1*, involved in the synthesis of GSH, exerted effects similar to vernalization on gene expression patterns (Hatano-Iwasaki and Ogawa, 2012).

Such new knowledge of glutathione-dependent agricultural benefits is not limited to vernalization. Ogawa's group found that spraying glutathione solution onto leaves or adding it to growth medium allowed structural changes of plastids and increased yields of corn (Ogawa, 2012). Although further verification studies are required, these observations anticipate the usefulness of glutathione in improving plant/crop quality (Ogawa, 2005, 2012).

In comparison to the advances in applicative studies of Trx, the studies of glutathione are less developed. However, their rapid progress—briefly introduced in this article—demonstrates their great potential.

CONCLUSIONS

Redox regulation controls plant physiology through complex and minute biological networks. The study of the redox biology of plants, originating in studies on photosynthesis, has now expanded to research of promising industrial value: the development of useful plants to overcome recent challenges such as climate change, increasing demand for food

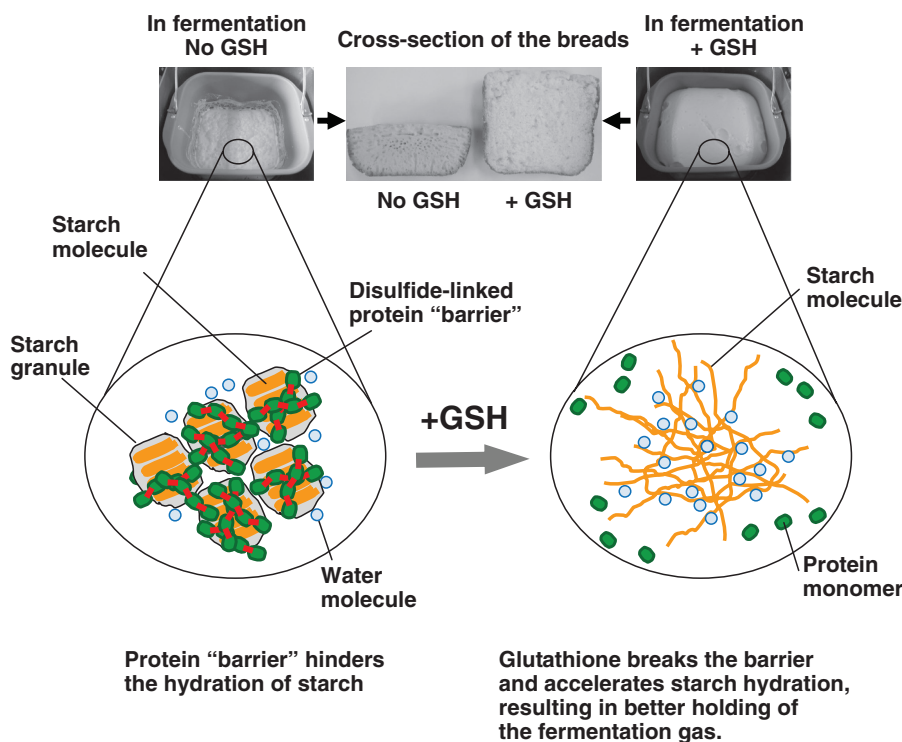


Figure 2. The Correlation between Disulfide-Bonded Proteins and Hydration of Starch Molecules based on the Unproven Barrier Theory. GSH, glutathione. Modified from Yano (2010), Kawakami and Yano (2012) with permission.

and energy, and allergy issues. These studies are taking Trx research to a new dimension in moving plants from the laboratory to the field. Trx, a multifunctional regulatory small protein, is expected to contribute significantly to a broad range of industries.

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