Note

Protein Disulfide Isomerase Activity of Some Plant Seeds

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Received July 23, 1997

The activity of protein disulfide isomerase, in the extracts of several dormant seeds including soybean, rice, wheat, and maize was assayed. The activity was higher in the extracts of beans than in those of the other seeds. A correlation was significant (R = 0.95 and 0.93; p < 0.01) between the PDI activity and the concentration of protein soluble in a salt solution.

Key words: PDI; protein disulfide isomerase; folding; seed

Inter- and intra-molecular disulfide bonds of protein are important for the formation of secondary and tertiary structure needed for proteins in cell to have biochemical functions. Anfinsen suggested that some accelerators in the cell speed protein folding.1) Protein disulfide isomerase (PDI) may be such an accelerator, catalysing disulfide bond formation.2) PDI has been purified from mammalian cells, bacteria, and a yeast, and its activity and structure has been studied in some detail.3-10 cDNAs encoding PDI have been cloned from various animal organs11 and refs. therein) and also from some plants including soybeans.7,9 Little is known about plant PDI, although it is believed to participate in the formation of the higher structure of many plant proteins, including enzymes, receptors, and storage proteins.

Plant PDI has been purified from soybean seeds.9 We found two APWCGHCK motifs in the amino acid sequence, like those of animal enzymes, and showed that the motifs were the active site by synthesizing peptides with PDI activity on multiple antigen peptide resin.10 PDI seems to be important in plants as well as in animals and microorganisms.

While studying changes in PDI activity during germination and maturation of soybean seeds, we found high PDI activity in dormant seeds and at an early stage of germination; the activity was not affected by cycloheximide (Kainuma et al., manuscript in preparation). In this study, the PDI activity of dormant seeds of several species including beans was compared in relation to their protein contents.

Cultivars of beans and other seeds studied were as follows: Enrei (soybean), PI165486 (cowpea), Erimoshozu (redbean), Osakaryokuzu (mungbean), Koshikkari (rice), Misato Golden, Sanukihadaka, and Kashimamugi (all barley), ICW, ASW, and VW (all wheat), and Hidaka (oat). Seeds were supplied by the National Agricultural Research Center (Tsukuba, Japan). Honey Bantam (maize) was purchased from Atariya Co., Ltd. (Chiba, Japan). Bovine pancreatic RNase A was purchased from Sigma Chemical Co. (St. Louis, MO). RNA was purchased from Kojin (Tokyo, Japan). All other reagents were of analytical grade from Nakalai Tesque (Kyoto, Japan).

Dormant seeds were soaked and imbibed in distilled water in the presence of 35 \( \mu \text{M} \) cycloheximide for 12 h at 25°C or at room temperature. Samples were homogenized in 3 vol. (w/v) of buffer A (50 mm Sodium-phosphate pH 7.5, 35.5 \( \mu \text{M} \) cycloheximide, 10 mm EDTA, 5 mm DTT, 2 mm PMSF, 50 mm NaN, and 0.1% Triton X-100) on ice. The homogenates were centrifuged at 15,000 \( \times \) g for 30 min at 4°C. The supernatant was dialyzed against buffer B (50 mm sodium-phosphate, pH 7.5, 10 mm EDTA, and 50 mm NaN) with membrane with a cut-off at the molecular weight of 3,500 or 25,000 to remove thioredoxin (Mw, 13,000) and low-molecular-weight reductive compounds. The proteins were assayed by the method of Lowry and others with bovine serum albumin as a standard.13

PDI activity was measured by the reactivation of scrambled RNase by the method of Hillson et al.12 Scrambled RNase was prepared as follows. Bovine pancreatic RNase A was reduced and denatured with 130 mm DTT and 9 mm urea in 50 mm Tris-HCl buffer, pH 8.6, for 1 h at 35°C. The solution of denatured RNase was adjusted to pH 4.0 with glacial acetic acid and put on a Sephadex G-25 column equilibrated with 0.1 \( \text{M} \) acetic acid. Fractions giving protein peaks all were collected. By gel filtration, the protein peaks were detected by the absorbance at 280 nm or by the Lowry method. The pooled protein fraction was adjusted to pH 8.5 with solid Tris and diluted to a concentration of 0.5 mg/ml protein with 0.1 \( \text{M} \) acetic acid. The reduced and denatured RNase A was reoxidized by oxidation in the air in the presence of 10 mm urea at 25°C until the concentation of free thiol groups decreased below 0.1 mmol per mole of RNase by Ellman's method.14 After this reoxidation, urea was removed with Sephadex G-25 in 0.1 \( \text{M} \) acetic acid. The scrambled RNase A obtained was lyophilized and stored at -20°C.

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Abbreviations: PDI, protein disulfide isomerase; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; ER, endoplasmic reticulum.
Fig. 1. PDI Activity and Water-soluble Protein of Crude Extracts from Seeds.
Left, dialyzed with membrane filtering Mr of 3,500; right, dialyzed with membrane filtering Mr of 25,000. Enrei, soybean; PI165486, cowpea; Ermosouzu, red bean; Osakarokuzu, mung bean; Koshikari, rice; Misato Golden, Sanukihadaka, and Kashimamugi, all barley; ICW, ASW, WW, all wheat; Hidakal, oat; and Honey Bantam, maize.

Fig. 2. Correlation Between PDI Activity and Water-soluble Protein of the Extracts from Seeds.
PDI activity and protein concentration are expressed as U/wet weight in gram and mg/wet weight in gram, respectively. Enrei, 1; PI165486, 2; Ermosouzu, 3; Osakarokuzu, 4; Koshikari, 5; Misato Golden, 6; Sanukihadaka, 7; Kashimamugi, 8; ICW, 9; ASW, 10; WW, 11; Hidakal, 12; Honey Bantam, 13.

The enzyme activity was assayed in terms of the rate of regeneration of scrambled RNase A to the native active form. Various amounts of crude extracts were incubated with scrambled RNase in the presence of 9 μM DTT and 2.5 mM EDTA in 50 mM Na-phosphate buffer, pH 7.5, for 10 min at 30°C. Twenty microliters of the mixture was added to 3 ml of RNA solution in 50 mM Tris-HCl buffer, pH 7.5, containing 25 mM KCl and 5 mM MgCl₂. The extent of RNase reactivation was measured by monitoring of changes in absorbance at 260 nm with a spectrophotometer (Hitachi U-3210). One unit of PDI activity was defined as an increase in absorbance by 1 unit at 260 nm per minute. Reactivation of the denatured enzyme by DTT in the absence of PDI was measured as a negative control. The value, which was generally low, was subtracted from the value in the presence of PDI.

The extracts of various seeds were dialyzed against the dialyzing buffer with membrane cut-off at the molecular weight of 3,500 or 25,000 for estimation of the contribution of thioredoxin (Mr, 13,000) to the activity. After dialysis, the activity of the inner solution of the former was from PDI and thioredoxin (if any), and that of the latter arose from PDI alone. Little difference in the PDI activity between the two dialyzates was found (Fig. 1). The activity of undialyzed extracts was similar to those of the dialyzed ones (data not shown). These results showed that the enzyme activity arising from protein with molecular weight of 25,000 or more was mostly that of PDI, and the contribution of thioredoxin and other low molecular-weight compounds was low.

The PDI activity of bean extracts was generally higher than that of the other seeds. Osakarokuzu (mung bean) had the highest activity, Ermosouzu (red bean) was the second highest, and Enrei (soybean) was the third highest. The PDI activity of Honey Bantam (maize) was higher than ICW, ASW, and WW (wheat) and also Hidakal (oat). Koshikari (rice) and Misato Golden, Sanukihadaka, and Kashimamugi (barley) had the lowest PDI activities.

The soluble protein concentrations also were shown in Fig. 1. The correlation between the PDI activity and the soluble protein concentration of the different seeds was significant with both dialysis membrane. When the cut-off was 3500, R=0.95, and when the cut-off was 25,000, R=0.93 (Fig. 2). The significant correlation (both p<0.01) suggests that seeds with more protein, (at least proteins soluble in salt solution) contain higher PDI activity, needed for structure formation and ac-
cumulation of proteins in seeds.

These results may simply reflect how abundant and stable the enzyme has been at the end of seed maturation. A recent report by Grimwade et al.\textsuperscript{14} has already showed that biosynthesis of PDI and Bip is high in the early stage of maturation of wheat seeds.

Our results are studies limited in terms of the number and species of seeds, but they suggest that seeds rich in protein have high PDI activity, and that high PDI activity in dormant seeds is related to possible protein synthesis during maturation and germination.

References