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The Expression of a Cytosolic Fructose-1,6-Bisphosphatase, a Key Enzyme in Sucrose Biosynthesis, Gene was Diurnally Fluctuated and Increased in Cold Acclimated Leaves of Chinese Cabbage

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Abstract

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is one of the most important vegetable crops in Korea and other East Asian countries. Cytosolic fructose-1,6-bisphosphatase (cytFBPase) is a key enzyme in sucrose biosynthesis, which controls the sucrose levels as well as the productivity of plants. The Chinese cabbage cytFBPase gene, *BrFBPase*, encodes the 340 amino acid polypeptide, giving a theoretical molecular weight of 37.2 kD and a isolectric point of 5.4. *BrFBPase* showed high sequence identity with *Brassica* homologs and its functional domains, such as F2,6P_2 binding site or active site and F6P binding site, were highly conserved in diverse sources of organisms. Although the genome of Chinese cabbage seemed to be triplicated, *BrFBPase* appears to be a single copy gene. The expression of *BrFBPase* was examined at transcript and protein levels under various conditions. *BrFBPase* expression was observed only in photosynthetic source tissue, not in sink tissue. The expression was slightly higher during the day than at night, and it showed a diurnal cycle with circadian rhythmicity. Short-term exposure to low temperature inhibited the expression of the *BrFBPase*, while long-term exposure increased the expression, supporting that sugar levels are high in late autumn when temperatures are low.

Key words: Chinese cabbage; *BrFBPase*; gene expression; low temperature; diurnal fluctuation; sugar content

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Introduction

Fructose-1,6-bisphosphatase (FBPase; D-fructose-1,6-bisphosphate 1-phosphohydrolase, EC 3.1.3.11) is a key regulatory enzyme of the gluconeogenic pathway (Hodgson et al. 1998) and sucrose biosynthetic pathway (Huber et al. 1985; Daie 1988; Stitt 1988; Stitt and Quick 1989). The enzyme is present in microorganisms, animals, and plants. Despite the same catalytic activity, the enzyme is associated with several quite different metabolic pathways: in microorganisms and mammalian tissues, it catalyzes a reaction essential for gluconeogenesis, and in plants, it is a key enzyme in sucrose biosynthesis as well as in the reductive pentose phosphate pathway of photosynthetic CO_2 fixation (Nel and Terblanche 1992). In all eukaryotes, FBPase is found in the cytosol, but in plants, FBPase is also located in the plastid (Hodgson et al. 1998).

In plants, two FBPase isoenzymes are necessary for photosynthesis to take place. One form which is localized in the cytosol (cytosolic FBPase, cytFBPase) is involved in sucrose synthesis from triose phosphates exported from the chloroplasts (Kelly et al. 1982). The chloroplast FBPase (cpFBPase) which is the other form takes part in the regeneration of RuBP in the photosynthetic carbon reduction cycle (Kelly et al. 1982). These two forms are distinguishable with respect to enzyme kinetics andexpression characteristics. The cytFBPase catalyses the first irreversible step in sucrose biosynthesis, starting from triose-P exported from the chloroplast *via* the phosphate translocator. This enzyme occupies a strategic position at the branch point between sucrose synthesis and the retention of triose-P in the chloroplasts for regeneration of RuBP or

starch synthesis. Thus, cytosolic FBPase is strictly regulated not only in response to the rate of photosynthesis and Pi (inorganic phosphate) requirement in chloroplast, but also in response to the rate of sucrose synthesis and sink demand (Stitt 1987). The key regulatory steps in sucrose synthesis are the conversion of fructose 1,6-bisphosphate (FBP) to fructose 6-phosphate (F6P) catalyzed by a cytFBPase, and the formation of sucrose 6-phosphate from UDP-glucose and F6P catalyzed by sucrose-phosphate synthase (SPS) (Daie 1993; Huber and Huber 1992). Thus, understanding the regulatory role of cytFBPase is very important.

The exposure of warm temperature-grown plants to low temperature for a short-period of time inhibits the activities of sucrose biosynthetic enzymes (Stitt 1990; Weiner et al. 1992), resulting from the accumulation of soluble sugars (Goldsmith and Huber 1992). However, long-term exposure over 10 days results in the increase in the total activities of enzymes both in photosynthetic carbon reduction cycle and in sucrose biosynthesis, including cytFBPase (Holaday et al. 1992). It has been suggested that the accumulation of soluble carbohydrate would be associated with plants' cold acclimation. In cereals (barley, wheat, and rye), freezing tolerance is strongly correlated with the capacity to increase soluble carbohydrate concentrations during cold acclimation (Olien and Clark 1993; Tognetti et al. 1990). The accumulation of simple sugars in the cytosol during cold acclimation (Koster and Lynch 1992) suggests that they might act as cryoprotectants (Archordoguy et al. 1987; Carpenter and Crowe 1988). Chinese cabbage would be considered as a cold-tolerant vegetable because it can survive temperature below -5℃ Hence, it is an excellent sample in studying on relationship between cytFBPase expression and low temperature.

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is one of the most important vegetable crops in Korea and other East Asian countries. The Chinese cabbage gene for cyt-FBPase which is designated as *BrFBPase* was isolated from a leaf cDNA library constructed in the Chinese cabbage inbred line 'Chiifu'. In the present study, we examined the changes in *BrFBPase* transcript and protein levels under various conditions in order to provide information on the role of BrFBPase in Chinese cabbage.

Materials and Methods

Plant materials

The *Brassica rapa* L. ssp. *pekinensis* inbred line, Chiifu, used in this experiment was grown in the field from September to November in 2004 in South Korea as well as in a growth chamber at 23±2℃ vith light/dark cycles of 16/8 h under a light intensity of 150 μmol $m^{-2}s^{-1}$. Heat shock and light-chilling treatments were carried out with plants grown in the environmental growth chamber, while other experiments were done on with field-grown Chinese cabbage. Light- chilling treatments were performed by exposing 1-cm^2 leaf discs to 100 μmol photons $m^{-2}s^{-1}$ at 4℃ or 1-12 h. Heat shock was also applied to 20 leaf discs (1 cm in diameter) under the same light conditions at 32℃ or 1-12 h. To examine the expression of *BrFBPase* during development, two distinct plants before and after leafy head formation were selected from the field grown ones. Before leafy head (BH) formation was restricted to 45-day-old Chinese cabbage (mid-October), while after leay head (AH or 90 days old) formation confined plants after 1 week exposure to frost (daily low temperatures of below 2℃ when the content of soluble sugar was drastically increased (mid-November). The samples for examining the daily change of *BrFBPase* expression were harvested at 09:20, 13:20, 17:20, 21:20, 01:20, 05:20, and 09:20 from the outdoor-grown Chinese cabbage in five-inch pots. For continuous darkness treatment, Chinese cabbage plants in the pots were transferred to the dark chamber and were sampled at indicated times under the green safety light. Harvested samples were quickly frozen in liquid nitrogen and were stored at -70℃ ıntil use.

Isolation and sequence analysis of the *BrFBPase* gene

BrFBPase was selected from the microarray experiment with cDNA chip with respect to heat shock and light-chilling treatments (GenBank accession number, CO750359) (Unpublished data). The full-length *BrFBPase* gene was isolated from a leaf cDNA library with the EST sequence and was subjected to sequence analysis using the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov) with the BLAST search program.

Genomic Southern hybridization

Genomic DNA was extracted from Chinese cabbage leaves through the CTAB precipitation method (Saghai-Maroof et al. 1984). For Southern blot analysis, the genomic DNA was digested with *Bam*HI, *Eco*RI and *Hind*III, electrophoresed on a 0.8% agarose gel, transferred to Nylon membranes (Schleicher & Schuell) using the VacuGene™ XL Vacuum Blotting System (Pharmacia Biotech) and the membranes were fixed by UV crosslinking. Hybridization was performed with ^{32}P-labeled *BrFBPase* cDNA (4×10^6 cpm/ml) at 42℃ or 12 hours in a hybridization buffer (6× SSPE, 5× Denhardt's reagent, 0.1% SDS, 50% formamide). The membranes were washed twice with 6× SSPE and 0.1% SDS for 5 min at room temperature, followed by two washes in 1× SSPE/ 0.1% SDS at 60℃ or 30 min, and then exposed on Personal Molecular Imager FX screens (BIO-RAD).

Northern blot analysis

Northern hybridization with the total RNA isolated using Trizol Reagent was performed according to a standard protocol (Sambrook and Russell 2001). Equal amounts of RNA were fractionated by electrophoresis through a 1% agarose-formaldehyde gel using MOPS buffer. After electrophoresis, the RNAs were transferred to a Nylon membrane (Schleicher & Schuell) using the VacuGene^{TM} XL Vacuum Blotting System (Pharmacia Biotech). Hybridization was carried out using ^{32}P-labeled *BrFBPase* cDNA probes. Autoradiography was carried out by exposure of the membrane to Personal Molecular Imager FX screens (BIO-RAD).

Western blot analysis

The sample powder prepared by grinding in liquid nitrogen was well suspended in the extraction buffer (50 mM potassium phosphate buffer, pH 7.8, 0.5 mM PMSF, 0.05% β-mercaptethanol, 0.5% ascorbic acid), and the supernatant was saved by microcentrifuge at the maximum speed at 4℃ Protein concentration was then measured using BCA kit (PIERCE) with BSA as standard. Equal amounts of proteins (40 μg) were fractionated on 10% SDS-PAGE and transferred nitrocellulose membranes (Schleicher & Schuell). The blot was blocked with 3% bovine serum albumin (BSA, Sigma A7030) in TBS (10mM Tris-HCl, 150mM NaCl, pH 7.2). Then, the membrane was incubated with 1,000 dilutions of the anti-cytosolic FBPase antiserum (purchased from AgriSera, Sweden) in TBS containing 0.3% BSA. For detection, the membrane was incubated with Protein A- conjugated alkaline phosphatase (PIERCE) (1:3,000 dilution) in TBS containing 0.3% BSA. The membrane was developed using BCIP and NBT (PIERCE), as color developing substrates for alkaline phosphatase.

Measurement of total soluble sugars

The leaves were ground to fine powder in liquid nitrogen. Three grams of the powder were suspended into 20 ml of 80% ethanol, incubated for 30 min at 60℃ and centrifuged for 10 min at 20,000 rpm. The supernatant was transferred to a new tube and the pellet was reextracted with 80% ethanol. Some of the total 50 ml extracts were diluted up to 20 times with 80% ethanol and 0.5 ml of the diluted extract was mixed with an equal amount of phenol. To the mixture, 2.5 ml of sulfuric acid was added, and this was cooled down to room temperature. OD_{490nm} was measured, and the concentration was calculated with glucose as a standard. The measurements were carried out at least with three replicates.

Chlorophyll fluorescence measurements

The activity of the PSII complex in the detached leaves was measured as the ratio of the variables *Fv* and *Fm* by a fluorometer (PAM2000; Walz, Effeltrich) in pulse amplitude modulation mode (Schreiber et al., 1986). The variable for fluorescence, *Fv*, was calculated as (*Fm* -*Fo*). *Fo*, the minimal fluorescence, was determined with a modulated beam, whereas *Fm*, the maximal fluorescence, was determined with a saturated light pulse. The ratio, *Fv*/*Fm*, was normalized with the concentration of chlorophyll and was interpreted as the functional state of the PSII complex.

Results

Chinese cabbage cytosolic FBPase (*BrFBPase*) gene

A full-length *Brassica rapa* L. ssp. *pekinensis* cytosolic FBPase (*BrFBPase*) cDNA consisted of 1,377 bp nucleotides that contained 223 bp of 5'-UTR, 131 bp of 3'-UTR including the poly (A) tail, and 1,023 bp of the coding sequence. The sequence was deposited in GenBank (accession number, DQ165552). The deduced amino acid sequence contained 340 amino acids, giving a theoretical molecular weight of 37.2 kD and a isolectric point of 5.4. The total number of negatively charged residues (Asp + Glu) is 45 (13%).

The comparison of the deduced amino acid sequence of the cDNA with other gluconeogenic FBPases from diverse sources is shown in Figure 1A. The identities of amino acid sequence were 94%, 85% and 84% with Crucifrae enzyzmes (canola; BnFBPase and *Arabidopsis*; AtFBPase), with three dicot enzymes (sugar beet; BvFBPase, potato; StFBPase, and spinach; SoFBPase) and with two monocot enzymes (rice; OsFBPase and sugarcane; ShFBPase), respectively. BrFBPase showed 60% and 58% identities with worm (CeFBPase) and human (HsFBPase) enzymes, respectively. The highest sequence identity was with canola enzyme at 98%. Amino acid sequence identities were also proved by the neighbor-joining tree (Figure 1B). Despite the diverse levels of sequence identities, there are highly conserved regions among all FBPases, suggesting similar properties in the structural stability and the regulatory mechanism for functions. Particularly, some important residues, such as $F2,6P_2$ (fructose-2,6-bisphosphate, a potent inhibitor of cytFBPase) binding site or active site and F6P binding site, proposed by Marcus et al. (1982) were completely identical (Figure 1A).

For Southern blot analysis of the Chinese cabbage genomic DNA, several restriction endonucleases were selected on the bases of the restriction map of the *BrFBPase* cDNA: *Hind*III as an enzyme having one

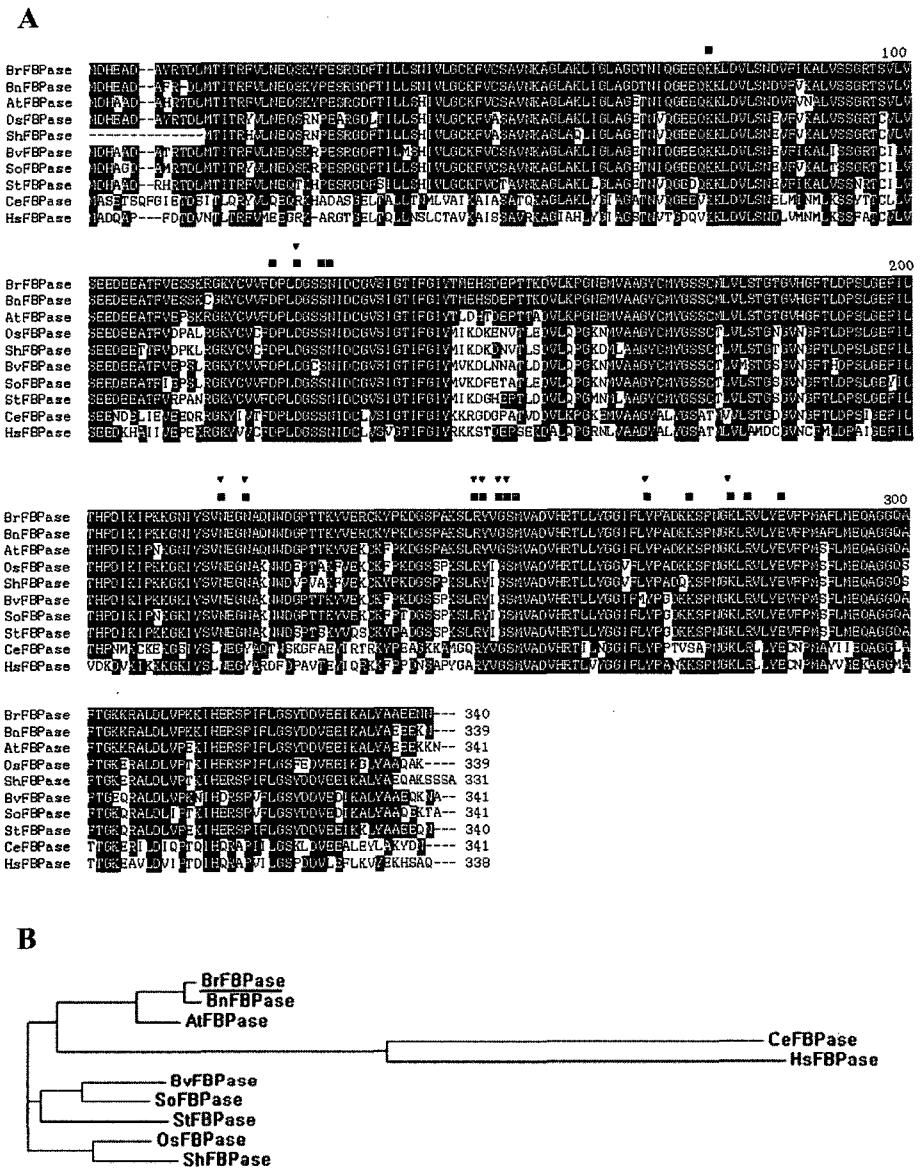

Figure 1. Comparision of amino acid sequences and phylogenetic relationship among cytosolic FBPases. **A.** Alignment of the deduced amino acid sequence of *BrFBPase* with *Brassica napus* (BnFBPase, U20179), *Arabidopsis thaliana* (AtFBPase, BT000470), *Oryza sativa* (OsFBPase, AB007193), *Saccharum* hybrid cultivar (ShFBPase, X89006) *Beta vulgaris* (BvFBPase, AF317553), *Spinacia oleracea* (SoFBPase, X61690), *Solanum tuberosum* (StFBPase, X76946), *Caenorhabditis elegans* (Ce FBPase, NM_058603) and *Homo sapiens* (HsFBPase, NM_000507) FBPases. The numbers in parenthesis indicate either the GenBank or EMBL accession number. The amino acid sequences are given a one letter code and have been aligned by the introduction of gaps (---) to maximize homology. The sequence displayed as reverse print is identical amino acids with BrFBPase. The symbols above the amino acid residues represent the location of reactive sites in pig kidney FBPase: dark rectangles for $F2,6P_2$ binding residues or active site and reverse triangles for F6P binding residues. **B.** Neighbor-joining tree representing phylogenetic relationship among FBPases. The alignment of amino acid sequences was performed using CLUSTAL W (1.82) multiple sequence alignment program.

internal site, and *Bam*HI and *Eco*RI as enzymes having no internal restriction site. As shown in Figure 2, the hybridization pattern implies the presence of a single gene family encoding the cytosolic FBPase in Chinese cabbage.

Change in *BrFBPase* expression upon short-term exposure to light-chilling and heat shock conditions

To examine the *BrFBPase* expression upon short-term exposure to low and high temperatures, we prepared 1-cm^2 leaf discs from Chinese cabbage leaves grown at 23℃ nd subjected to 100 μmol photons $m^{-2}s^{-1}$ at 4℃ ınd 32℃ or light-chilling and heat shock treatments, respectively. The treatments were sustained for 12 h, and 15 discs were taken up at the indicated time. *BrFBPase* transcript levels by exposure to light-chilling were dramatically decreased for the first two hours, and then they gradually returned to the normal level (Figure 3A, left). Similar pattern was observed in the level of polypeptide as revealed by western blotting

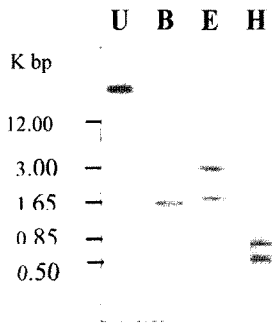

Figure 2. Genomic DNA Southern blot analysis of *BrFBPase*. The genomic DNA isolated from *Brassica rapa* L. ssp. *pekinensis* leaf tissue was digested with *Bam*HI, *Eco*RI and *Hind*III. The digested DNA was separated on 0.8% agarose gel, blotted onto the nylon membrane. The full-length *BrFBPase* cDNA, which is labeled with ^{32}P-dCTP, was used as the probe. **U**, uncut genomic DNA; **B**, *Bam*HI digested DNA; **E**, *Eco*RI digested DNA; **H**, *Hind*III digested DNA.

(Figure 3C, left). However, the expression pattern of *BrFBPase* upon heat shock treatment was different from that in the low temperature one. As shown in Figure 3A and C (right), the levels of *BrFBPase* transcript and polypeptide were slightly increased for the first two hours and then decreased after. The result of the light-chilling treatment somewhat affirms previous reports that sucrose synthesis is restricted at low temperature (Stitt and Grosse 1988), but there is no heat shock-related information with respect to cytFBPase.

Change of *BrFBPase* expression during two distinct growing periods

There are several characteristics of the Chinese cabbage as compared with other higher plants. The mature leaf can be divided into two parts, the green photosynthetic tissue and white the non-photosynthetic tissue. After leafy head formation, the inner non-photosynthetic leaves become storage organs for essential nutrients. It was also reported that the exposure of warm-grown plants to low temperature (10℃ for 10 days resulted in the increase in the total activities of enzymes both in photosynthetic carbon reduction cycle and in sucrose biosynthesis, including cytFBPase (Holaday et al. 1992). From these properties, what happens to the *BrFBPase* expression during the long term exposure to low temperature is the question that needs to be addressed. First of all, we checked the total soluble sugar contents from 45-day-old leaf before leafy head formation (the daily low temperature was around 14℃ and the 90-day-old leaf after leafy head formation when the daily low temperature was below 2℃ Figure 4A). As shown in Figure 4A, the total sugar levels in leaves after leafy head formation was almost tripled, implying that cold temperature increased sucrose synthesis and accumulation. This increased sugar content was also observed in inner non-photosynthetic leaf (data not shown), indicating that sink demand might be high. Similarly, the levels of transcript and polypeptide for BrFBPase were higher in leaves after leafy head formation than in leaves before that (Figure 4B and C). This result indicates that an increase in the sugar content leads to increased sucrose biosynthesis due to an increase in the expression of enzymes involved in the pathway, like cytFBPase. This implication can be supported by results in Figures 4D and E in which neither the transcripts nor the polypeptide for BrFBPase was detected in non-photosynthetic inner leaves (a sink organ) where no sucrose biosynthesis occurs. In the photosynthetic outer leaves of the Chinese cabbage, both *BrFBPase* transcript and its polypeptide levels were

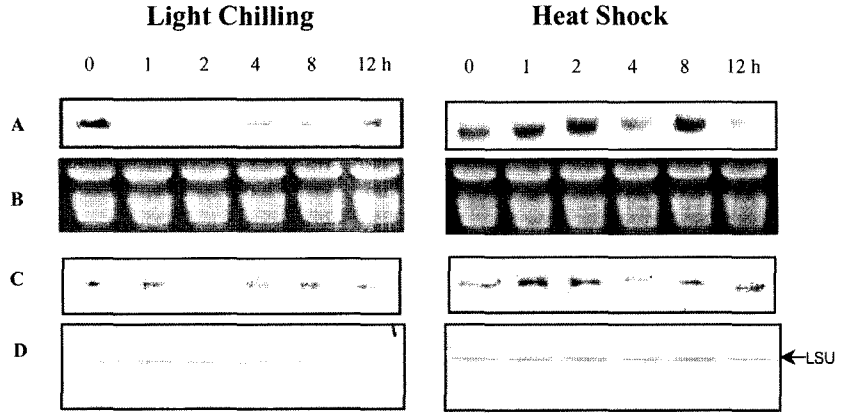

Figure 3. Expression of *BrFBPase* under light-chilling (left) and heat-shock (right) stresses. The total RNAs were isolated from the 10 leaf discs (1 cm in diameter) which were exposed to 100 μmolm^{-2}s^{-1} at 4℃ light chilling) or 37℃ heat shock) for 1 to 12 h. Twenty micrograms of the total RNAs were subjected to RNA gel-blot hybridization. The full-length *BrFBPase* cDNA was used as the probe. Soluble proteins were extracted from 20 leaf discs which were treated with the same condition and 40 μg of proteins were fractionated on 10% SDS-PAGE. **A** and **C** represent the levels of *BrFBPase* transcript and BrFBPase protein, respectively. **B** and **D** represent methylene blue-stained blot and Coomassie blue-stained gel showing the calculated amounts of RNA and protein to be transferred, respectively. LSU indicates the large subunit of Rubisco protein.

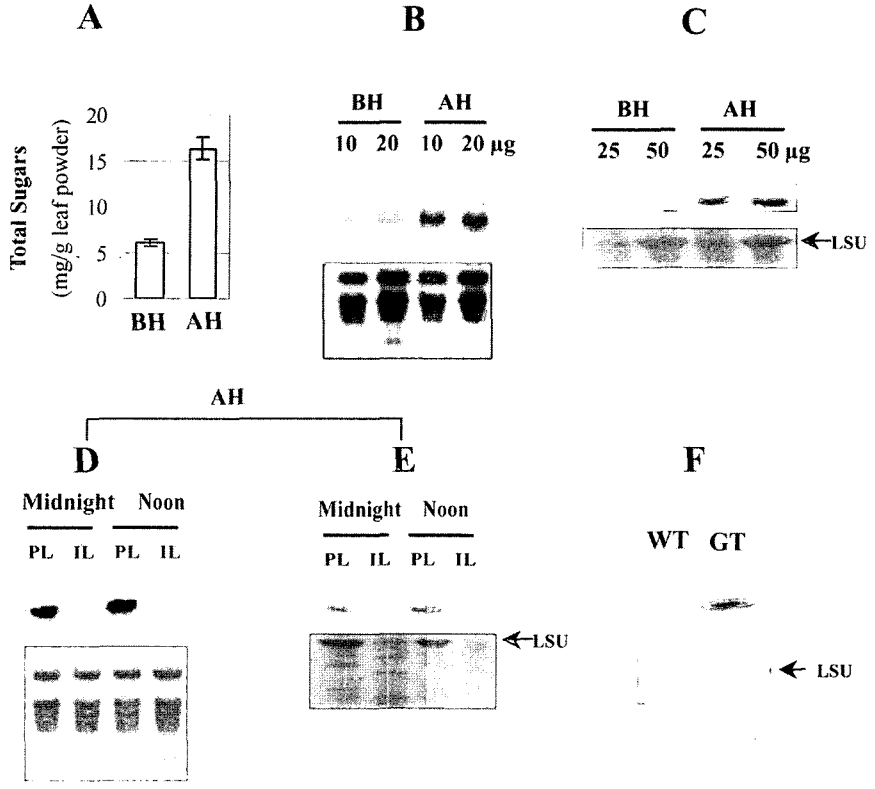

Figure 4. Sugar contents (**A**), the expression of cytosolic *BrFBPase gene* (**B-E**) in relation to leafy head formation, and the protein level in different tissues of a leaf (**F**). The levels of transcript (**B**) and protein (**C**) were measured with leaf samples collected either before (BH) or after (AH) leafy head formation in field grown Chinese cabbage. **D** and **E** represent the levels of *BrFBPase* transcript and BrFBPase protein in photosynthetic leaves (source tissue, PL) and non-phtosynthetic inner leaves (sink tissue, IL) of Chinese cabbage in that leafy head-formation was completed in field. Samples were also collected from either photosynthetic (noon) or non-photosynthetic (midnight) conditions. **F** shows the BrFBPase polypeptide level in the white tissue (WT) and the green tissue (GT) of a mature leaf. The photos of B and D at bottom represent a methylene blue-stained blot showing the calculated amounts of RNA to be transferred. Likewise, the photos of C, E and F at the bottom represent a Coomassie blue-stained gel showing equal amounts of protein to be transferred. LSU indicates the large subunit of Rubisco protein.

slightly higher at noontime when high photosynthesis is expected than at midnight when no photosynthesis is expected. In this study, we did not consider the leafy head formation process but only the content of soluble sugar. The fact that *BrFBPase* will not be expressed in sink tissue was also supported by the result in Figure 4F in which BrFBPase protein was detected only in photosynthetic green tissue but not in non-photosynthetic white tissues of the same leaf.

Change of *BrFBPase* expression during the day and the extended dark period

As shown in Figure 4D, the levels of BrFBPase protein were slightly higher during daytime than midnight. To elucidate whether the expression of *BrFBPase* is the light dependent manner, we examined its expression of a 45-day-old Chinese cabbage for a day, the time of which was before head (BH) formation (mid-October). Since transcript levels were correlated to protein levels in Figures 3 and 4, we only examined the protein level for *BrFBPase* expression study. PSII activities were almost same throughout the day (Figure E), whereas the level of BrFBPase protein was high at 3 p.m. (Figure 5B and D). BrFBPase levels were relatively low at night and high during a day, implying a diurnal fluctuation of its protein levels. It is very interesting that the highest level of BrFBPase was observed at 3 p.m. despite the highest light intensity at noon. This result may imply that the high light intensity is not adequate for maximum photosynthesis. Since BrFBPase levels showed a diurnal cycle in Figure 5, we examined the protein levels during the extended dark period for 48 h (Figure 6). As shown in Figure 6, the levels of BrFBPase protein seemed to change with circadian rhythm; high during daytime when photosynthesis might be high, but low during the night time. However, the levels gradually decreased during the extended darkness. This will be the first report to reveal that cytFBPase expression has circadian rhythmicity.

Discussion

BrFBPase showed 98% identity to canola enzyme, whereas 58-60% identities to animal ones. However, the reactive sites identified by the pig kidney FBPase study,

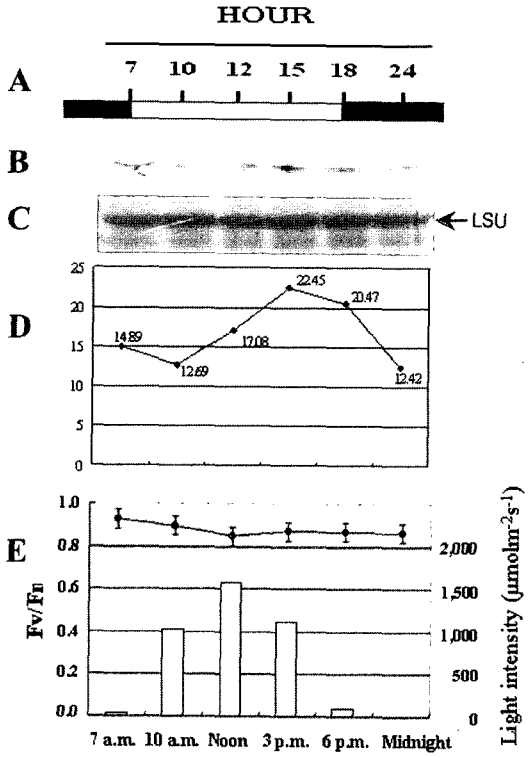

Figure 5. Change in BrFBPase protein during a day. Soluble proteins were extracted from Chinese cabbage leaves harvested in Korea at the indicated time in October 21. Forty micrograms of proteins were fractionated on 10% SDS-PAGE, transferred to NC papter, and immunoblotted with an anti-FBPase serum. **A** represents the photoperiod, **B**, the western blot analysis, **C**, the Coomassie blue-stained gel showing equal amounts of protein to be loaded, **D**, the relative density of cytosolic FBPase polypeptides, and **E**, the light intensities (square graph) and PSII activity (• LSU indicates the large subunit of Rubisco protein.

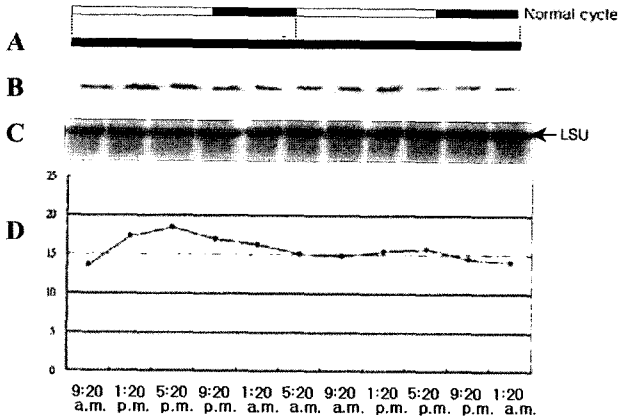

Figure 6. Expression of BrFBPase protein during an extended dark period. Chinese cabbage grown during a normal day/night cycle was kept in the darkness for two days, and the leaves were harvested at the indicated time. Forty micrograms of proteins were fractionated on 10% SDS-PAGE, transferred to NC paper, and immunoblotted with anti-FBPase serum. A-D are the same as the legend in Figure 5.

such as $F2,6P_2$ binding residues or active site and F6P binding residues, were highly conserved, indicating that same regulatory mechanism will be present among all eukaryotic enzymes. Unexpectedly, *BrFBPase* turned out to be a single copy gene (Figure 2). Since the Chinese cabbage genome has been considered as triplicated one, the *BrFBPase* gene which is very important in plant metabolism would be expected to be one of a multigene family. To be able to further probe on this, a comparative genomic study between *Arabidopsis* and other *Brassica* species is required.

The expression of *BrFBPase* was reduced upon short-term exposure to low and high temperatures (Figure 3), whereas it was increased when the leaves were exposed to low temperature for a long period of time (Figure 4B and C). This result was similar to a previous report in which the exposure of warm-grown spinach and bean (27℃ to low temperature (10℃ for 10 days resulted in the increase in the total activities of enzymes both in photosynthetic carbon reduction cycle and in sucrose biosynthesis, including cytFBPase (Holaday et al. 1992). The short-term exposure to low temperature led to the accumulation of soluble carbohydrates (Goldsmith and Huber 1992), which in turn led to the inactivation of SPS by phosphorylation (Weiner et al. 1992). SPS inactivation caused the accumulation of F6P which triggers the synthesis of F2,6BP, a potent inhibitor of cytFBPase (Stitt 1990). The inhibition of cytFBPase caused Pi limitation in chloroplast, thereby inhibition of photosynthesis. However, long-term exposure like during late autumn will confer an advantage to plants. In cereals, such as barley, wheat, and rye, freezing tolerance is strongly correlated with the capacity to increase soluble carbohydrate concentrations during cold acclimation (Olien and Clark 1993; Tognetti et al. 1990). The accumulation of simple sugars in the cytosol during cold acclimation (Koster and lynch 1992) suggests that they might act as cryoprotectants (Archordoguy et al. 1987; Carpenter and Crowe 1988). The experiment similar to our study was reported by Strand et al. (1997) using *Arabidopsis*. The shift (3 days to 21 days) of warm-grown *Arabidopsis* plants to low temperature (5℃ caused reduction in the expression of chloroplast genes, such as *cpFBPase* and *AGPase*, whereas *cytFBPase* and *SPS* expressions were greatly increased. They explained that since the increased amount of transcript correlated with increased enzyme activity, it was likely associated with increased amounts of the enzyme. However, *CytFBPase* and *SPS* transcript showed the highest amount of transcript in cold-developed leaves. Both increase in the expression of *BrFBPase* and the total sugar contents in Chinese cabbage leaves after leafy head formation would be explained as a phenomenon of cold acclimation or freezing tolerance.

In sugar beet, the *cytFBPase* transcript and protein levels were low in immature (sink) leaves, but were increased in photosynthetically active mature (source) leaves (Pitcher and Daie 1991). The expression pattern

was correlated to photosynthetic rates and sucrose content (Daie 1993). However, we could not detect any BrFBPase protein in the sink tissues of the Chinese cabbage, such as the inner leaves after leafy head formation and the white tissues in a leaf. This result indicates that no gluconeogenesis will occur in the sink tissues of the Chinese cabbage.

Chueca et al. (1984) first reported that chloroplast FBPase activity sharply increased in etiolated spinach seedlings following *de novo* synthesis of the enzyme under light conditions. However, the effect of light on the expression of *cytFBPase* is inconsistent: both enzyme activity and expression of spinach *cytFBPase* were constant under light or dark conditions (Hur and Vasconcelos 1998), while the activity and transcript levels of sugarbeet *cytFBPase* gradually increased under continuous white light (Khayat et al. 1993) and pea *cytFBPase* expression was light inducible (Lee and Hahn 2003). In Chinese cabbage, *cytFBPase* expression was slightly of a light-dependent manner, even with the diurnal cycle. It was reported that sucrose synthesis during the night was active using maltose and glucose resulting from starch degradation in the chloroplast (Niittyla et al. 2004; Schleucher et al. 1998; Weise et al. 2005). However, this pathway would not utilize cytFBPase-catalyzed reaction. The diurnal cycle of *BrFBPase* expression somewhat indicated circadian rhythmicity, which is the first report in this study.

In summary, the expression of *BrFBPase* gene that encodes a key enzyme in sucrose biosynthesis showed a light-dependent manner with diurnal cycle and circadian rhythmicity. Its expression was restricted to source tissues and increased upon long-term exposure to low temperature, resulting in the increase in sugar contents. These results may suggest that the overexpression of *BrFBPase* in concert with the overexpression of *SPS* will increase sugar contents in Chinese leaves.

Acknowledgments

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