Korean J Biol Sci 8: 105-109, 2004

Cloning and Expression of a cDNA *AAPT3* Encoding Aminoalcoholphosphotransferase Isoform from Chinese Cabbage

Kwang Soo Kim, Jong Ho Park and Sung Ho Cho*

Department of Biological Sciences and Institute of Molecular Cell Biology, College of Sciences, Inha University, Incheon 402-751, Korea

Key Words:

Aminoalcoholphosphotransferase
Chinese cabbage
Low temperature
Phosphatidylcholine
Phosphatidylethanolamine

Aminoalcoholphosphotransferase catalyzes the synthesis of phosphatidylcholine and phosphatidylethanolamine from diacylglycerol plus a CDP-aminoalcohol such as CDP-choline or CDP-ethanolamine. Previously we suggested the presence of possible isoforms of this enzyme from Chinese cabbage roots and now report the cDNA cloning and expression analysis of *AAPT3* encoding a third isoform of aminoalcoholphosphotransferase (AAPT3). *AAPT3* contains an open reading frame of 1,176 bp coding for a protein of 392 amino acids. It shares 96 and 95% identity with Chinese cabbage *AAPT1* and *AAPT2*, respectively, at the deduced amino acid level. The results from reverse transcriptase-polymerase chain reaction analysis indicate that expression of *AAPT3* is up-regulated by low temperature as well as *AAPT1* and *AAPT2*.

Phosphatidylcholine and phosphatidylethanolamine are abundant phospholipids, comprising more than 80% of the total in most eukaryotic membranes. They are mainly synthesized *de novo* by homologous nucleotide pathways consisting of three consecutive reactions (Kennedy and Weiss, 1956; Harwood, 1979; Moore, 1982; Vance, 1989; Kinney, 1993). The terminal step of each pathway involves the conversion of diacylglycerol to phospholipid using cytidine diphosphate (CDP)-choline or -ethanolamine as the source of the head group (Weiss et al., 1958). In yeast, the enzymes responsible for this reaction, collectively called aminoalcoholphosphotransferases, are separate; cholinephosphotransferase for phosphatidylcholine biosynthesis and ethanolaminephosphotransferase for phosphatidylethanolamine biosynthesis (Hjelmstad and Bell, 1991). In plants, however, it was speculated that both enzyme activities are catalyzed by a single aminoalcoholphosphotransferase with dual substrate specificity (Macher and Mudd, 1974; Lord, 1975; Sparace et al., 1981; Justin et al., 1985; Dewey et al., 1994). It was demonstrated that the product of a single gene is responsible for both cholinephosphotransferase and ethanolaminephosphotransferase activities in soybean (Dewey et al., 1994), in *Arabidopsis thaliana* (Goode and Dewey, 1999), and recently, in *Brassica napus* (Qi et al., 2003), although in this case the gene product BnAAPT1 exhibited a preference for CDP-choline over CDP-ethanolamine.

It has been well documented that the metabolism of plant lipids is affected by temperature (Harwood et al., 1994; Harwood, 1998). Our laboratory has been interested in the molecular aspects of the effects of temperature on metabolism of phosphatidylcholine in plants, especially in relation to its biosynthesis. There have been reports describing the effects of temperature on phosphatidylcholine synthesis in various plants. For example, in rye roots the incorporation of choline into phosphatidylcholine was higher in 5°C-grown than 20°C-grown roots (Kinney et al., 1987). All three enzymes of the nucleotide pathway, including cholinephosphotransferase, showed higher activities in roots grown at low temperature. In soybean the activity of cholinephosphotransferase was higher in 20°C-grown than in 35°C-grown cotyledons (Cho and Cheesbrough, 1990). It was suggested that the higher enzyme activity at lower temperature was the result of increased synthesis of the enzyme rather than to the involvement of isozymes or metabolic effectors. In contrast, however, in young wheat leaves a temperature rise from 20°C to 24°C caused a marked increase in the rate of phosphatidylcholine synthesis, apparently at the expense of diacylglycerol (Williams and Harwood, 1997). Since there is a paucity of data on molecular aspects of the temperature control mechanism, we have undertaken a series of experiments to study the effects of temperature on phosphatidylcholine biosynthesis in various plants. We previously reported the cloning of cDNAs encoding

*To whom correspondence should be addressed.
Tel: 82-32-860-7696, Fax: 82-32-874-6737
E-mail: shcho@inha.ac.kr

isoforms of aminoalcoholphosphotransferase, AAPT1 and AAPT2, from Chinese cabbage (Min et al., 1997; Choi et al., 2000). It was found that expression of *AAPT2* is up-regulated by low temperature (Choi et al., 2000). We now report the cloning of another aminoalcoholphosphotransferase cDNA, *AAPT3*, from Chinese cabbage and compared its expression pattern with that of AAPT1 and AAPT2 in response to low temperature.

Materials and Methods

Plant material and isolation of RNA

Seeds of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) were germinated on MS Duchefa agar medium in sterile Erlenmyer flasks, and plants were grown for one week. Shoots and roots were collected into liquid nitrogen and stored at -70°C prior to use. Total RNA was extracted from leaves of normal (25°C) or low temperature-treated (5°C) plants with RNeasy Plant Mini Kit (Qiagen).

Rapid amplification of cDNA ends (RACE)

The 5' end of aminoalcoholphosphotransferase cDNA was obtained by RACEs (Frohman et al., 1988; Belyavsky et al., 1989) using 5'/3' RACE Kit (Boehringer-Mannheim) according to the instructions of the manufacturer. Based on the sequence revealed from a 3'-RACE product from the previous experiment (Min et al., 1997), 5'-RACE was performed using a gene specific primer, 5'-GTCCATATCGATCATTCAAC-3'. The product of this reaction was cloned into TOPO vector (Invitrogen) and sequenced.

Sequence analysis

Hydropathy analysis of the deduced polypeptide was performed according to Kyte and Doolittle (1982). The prediction of protein secondary structure was based on the Chou-Fasman algorithm (Chou and Fasman, 1978).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

In order to study the expression pattern of *AAPT1, AAPT2* and *AAPT3*, reverse RT-PCR was performed. Using 2 μg total RNA as templates, reverse transcription was performed using the M-MuLV reverse transcriptase (Roche) for 1 h at 42°C and 10 min at 65°C. Using 5 μl of total 20 μl reaction mixture as templates, PCR was performed using forward primer, 5'-ACCGGATCCGTATAGTAGAGTTGG-3', and reverse primer, 5'-CACCCTCGAGGTTTCTTCTTCAAG-3', to synthesize a 1,219 bp fragment corresponding nucleotide sequence 60-1,278 of the *AAPT1* cDNA, forward primer, 5'-GATGCGGTTGATGGGAAGCAAGCCA-3', and reverse primer, 5'-GTAGATTCCGAGTGCAGTTGTGATCTCA-3', to synthesize a 858 bp fragment corresponding nucleotide sequence 425-1,282 of the *AAPT2* cDNA, and forward primer, 5'-GAACTCGAGACTCTTGTCTCTTTT-3', and reverse primer, 5'-GGTCCATCTAGATCATTCAACGTA-3', to synthesize a 1,351 bp fragment corresponding nucleotide sequence 33-1,383 of the *AAPT3* cDNA. The PCR reaction mixture (25 μl) contained 500 ng template, 2.5 μl 10x PCR buffer (15 mM $MgCl_2$, 500 mM KCl, 100 mM Tris-HCl, pH 8.3), 50 μM primers, 200 μM dNTP's, and 2.5 units of *Taq* DNA polymerase (*TaKaRa Ex Taq*). Reactions were annealed for 50 sec at 60°C for *AAPT1*, for 30 sec at 60.2°C for *AAPT2*, and for 40 sec at 62°C for *AAPT3*, extended for 1 min at 72°C and denatured for 40 sec at 94°C, and repeated for 25 cycles.

Results

The sequence of the Chinese cabbage aminoalcoholphosphotransferase cDNA *AAPT3* is 1,524 bp and contains an open reading frame of 1,176 bp coding for a protein of 392 amino acids with a molecular mass of 43.7 kDa. The length of the coding sequence is 12 bp longer than that of all other known aminoalcoholphosphotransferase cDNAs (Fig. 1). The hydropathy profile revealed from Kyte and Doolittle analysis (1982) shows a pattern very similar to those of the soybean and *A. thaliana* enzymes (Fig. 2). All sequences contain seven membrane-spanning helices, demonstrating that aminoalcoholphosphotransferase is an integral membrane protein.

Analysis of the primary sequence of Chinese cabbage AAPT3 also indicated a very close homology to the known sequences of aminoalcoholphosphotransferases. It shares 96 and 95% identity and 97% similarity with Chinese cabbage AAPT1 and AAPT2, respectively, at the deduced amino acid level (Min et al., 1997; Choi et al., 2000). It also has 87% similarity with soybean AAPT1 (Goode and Dewey, 1999), 94 and 92% with *A. thaliana* AAPT1 (Dewey et al., 1994) and AAPT2 (Goode and Dewey, 1999), respectively, 90% with *Pimpinella brachycarpa* AAPT1 (Lee et al., 2001), and 63% with *Brassica napus* AAPT1 (Qi et al., 2003).

The CDP-aminoalcohol binding domain spanning amino acids 69-139, as revealed by studies with chimeric enzymes of cholinephosphotransferase and ethanolamine-phosphotransferase from yeast (McMaster and Bell, 1997), shares 73.2% identity among different plant species. This site is believed to confer substrate specificity to the enzymes for CDP-choline or CDP-ethanolamine.

An amphipathic helix with highly asymmetric distribution of hydrophilic and hydrophobic residues was identified within the CDP-aminoalcohol binding domain (Fig. 3). The polar face of the helix was heavily charged with two Asp (–) and Glu (–) residues each and one His (+) residue. It was hypothesized that the amphilicity of this helix is

```
Br3   MGYIGAHGAAALHRYKYSGEDHSYLAKYLLNPFWTREVKVFPLWMPPNMITLMGFMFLVT  60
Br2   MGYIGAHGAAALHRYKYSGEDHSYLAKYLLQPFWTREVKVFPLWMPPNMITLMGFMFLVT
Br1   MGYIGAHGVAALHRYKYSGVDHSYLAKYVLQPFWTREVKVFPLWMPPNMITLMGFMFLVT
At1   MGYIGAHGVAALHRYKYSGVDHSYLAKYVLQPFWTREVKVFPLWMPPNMITLMGFMFLVT
At2   MGYIGAHGVAALKKHKYSGVDHSYLAKYVLQPFWNREVKIFPLWMPPNMITLMGFMFLLT
Gm1   MGYIGTHGVAALHRYKYSGVDHSYVAKYVLQPFWSREVNFFPLWMPPNMITLVGSMFLVT
Pb1   MGYIGAHGVAALHKHKYSGVDHSYLAKYVLQPFWTKCVTFFPLWMPPNMITLVGSMFLVT
Bn1   MGYIGAHGVAALHRHKSSGVGSLLPCQIRPPTFWNREVKIFPLWMPPQHDNTYGVHVSHH

Br3   SSLLGYIYSPQLDSPPPRWVHFAHGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD 120
Br2   SSLLGYIYSPQLDSPPPRWVHFAQGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD
Br1   SSLLGYIYSPQLDSPPPRWVHFAHGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD
At1   SSLLGYIYSPQLDSPPPRWVHFAHGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD
At2   SALLGYIYSPKLDSPPPRWVHFAHGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD
Gm1   SAALGFIYSPHLDSPPPRWVHVAHGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD
Pb1   SAALGFIYSPHLDSPPPRWVHVAHGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD
Bn1   ICALRLCILTSVGFSSSSMVHLAHGLLLFLYQTFDAVDGKQARRTNSSSPLGELFDHGCD

Br3   ALACAFEAMAFGSTAMCGRDTFWFWVISAVPFYGATWEHYFTNTLILPVINGPTEGLALI 180
Br2   ALACALEAMAFGSTAMCGRETFRSWVISAIPLQGATWEHYFTNTLILREINGPTEGLALI
Br1   ALACAFEAMAFGSTAMCGRDTFWFWVISAIPFYGATWEHYFTNTLILPVINGPTEGLALI
At1   ALACAFEAMAFGSTAMCGRDTFWFWVISAIPFYGATWEHYFTNTLILPVINGPTEGLALI
At2   ALGCALETMAYGSTAMCGRDTFWFWVISAVPFFGATWEHYFTNTLTLPVVNGPTEGLALI
Gm1   ALACTFEALAFGSTAMCGRTTFWWWLISAITFYGATWEHYFTNTLILPVVNGPTEGLMII
Pb1   ALACTFEALAFGSTAMCGKDTFWFWVISAVPFYGATWEHFFTNTLILPVVNGPTEGLMLI
Bn1   ALACAFETMAYGSTAMCGRNTFWFWIISAIPFTGSTWETYFTNILTLPVVNGPTESPCTY

Br3   YVSHFFTALCGSEWWAQQLGESIPLFSWVPFVNAIQTSRAVLYMMIAFAVIPTVAINVSN 240
Br2   YVSHLFTALVGAEWWAQQLGQSIPLFSWVPFVNAIQTSRAVLYMMIAFAVIPTVAINVSN
Br1   FVSHFFTAIVGAEWWAQQLGQSIPLFSWVPFVNAIQTSRAVLYMMIAFAVIPTVAFNVSN
At1   FVSHFFTAIVGAEWWAQQLGQSIPLFSWVPFVNEIQTSRAVLYMMIAFAVIPTVAFNVTN
At2   YCGHFFTAIVGAEWWAQPFGKSIPLFSWVPFLNEMQMSRTILFSMIFFAVIPTLAINTSN
Gm1   YTCHFFTAIVGAEWWVQQFGKSLPFLNWLPYLGGIPTFKAILCLMIAFGVTPTVTCNVSN
Pb1   YVGHIFTAIVGAEWWVHQFGKSVPFLSWVPILSEVPTYRAVLYLMIAFAVIPTLTFNVQN
Bn1   ILWSLLPQPLLVLNGGLSSWRVNPLFSWGAFLKEITTSRVVLITDGCFCCYTNTCIQRVQ

Br3   VYKVVQSRKGSMVLALAMLYPFVVLLGGVLIWDYLSPINLIETYPHLVVLGTGLAFGFLV 300
Br2   VYKVVQSRKGSMLLALAMLYPFVVLLGGVLIWDSLSPINLIETYPHLVVLGTGLAFGFLV
Br1   VYKVVQSRKGSMLLALAMLYPFVVLLGGVLIWDYLSPINLIFTYPHLVVLGTGLAFGFLV
At1   VYKVVRSRNGSMVLALAMLYPFVVLLGGVLIWDYLSPINLIATYPHLVVLGTGLAFGFLV
At2   VYKVVHSRNGSMLLALAMLYPLVTLIAGVLIWDYLSPIDLIRNYPHLVVLGTGLAFGFLV
Gm1   VYKVVKGKNGSMPLALAMLYPFVVLVGGVLVWDYLSPSDIMGKYPHLVVIGTGLTFGYLV
Pb1   VYKVVQARKGSMLLALAMLYPFVVLMAGILIWDYLSPYDIMVNYPYMVVLGTGLAFGFLV
Bn1   CIQSYTAKKRKHVCSIIMLFPFVGLLAGVLIWDYLSPTDLIRNYPHLVVLGTGLAFGFIV

Br3   GRMILAHLCDEPKGLKTNMCTSLVYLPFALANALTARLNNGVPLVDELWVLLGYCIFTVS 360
Br2   GRMILAHLCDEPKGLKTNMCMSLLYLPFALANALTARLNAGVPLVDELWVLLGYCIFTVS
Br1   GRMILAHLCDEPKGLKTNMCLSLVYLPFALANALTARLNDGVPLVDELWVLLGYCIFTVS
At1   GRMILAHLCDEPKGLKTNMCMSLLYLPFALANALTARLNAGVPLVDELWVLLGYCIFTVS
At2   GRMILAHLCDEPKGLKTNMCMSLLYLPFALANALTARLNDGVPLVDEFWVLLGYCIFTLS
Gm1   GRMILAHLCDEPKGLKTGMCMSLMFLPLAIANVLASRLNDGVPLVDERLVLLGYCAFSVT
Pb1   GRMVLAHLCDEPKGLKTNMCLSLLYLPFAIANTLTARLNDGVPLVEEKWVLLGYCVYTGA
Bn1   GRIILAHICDEPKGLKTNMCMSLLYLPFALANALTARLNNGVALVDEFGASWLLYIQQWH

Br3   LYLHFATSVIHEITAALGIYCFRITKKLEKKP                            392
Br2   LYLHFATSVIHEITAALGIYCFRITRKEA
Br1   LYLHFATSVIHEITTALGIYCFRITRKEA
At1   LYLHFATSVIHEITEALGIYCFRITRKEA
At2   LYAHFATSVIHEITTALGIYCFRITRKEA
Gm1   LYLHFATSVIHEITNALGIYCFRITRKEA
Pb1   LYLHFATSVIHEITTALGIYCFRITRKEA
Bn1   YNMHFATSVIHEITTALGIYCFRITRKEA
```

Fig. 1. Alignment of amino acid sequence of Chinese cabbage AAPT3 with other AAPT sequences. CDP-aminoalcohol phosphotransferase motif (98-120) is boxed. The conserved amino acid residues in the motif are bolded. Br3, Chinese cabbage AAPT3 (GenBank accession no. AF446089). Br2, Chinese cabbage AAPT2 (GenBank accession no. AF183933). Br1, Chinese cabbage AAPT1 (GenBank accession no. U96713) (Min et al., 1997). At1, *Arabidopsis thaliana* AAPT1 (GenBank accession no. AF091843) (Goode and Dewey, 1999). At2, *A. thaliana* AAPT2 (GenBank accession no. AF091844) (Goode and Dewey, 1999). Pb1, *Pimpinella brachycarpa* AAPT1 (GenBank accession no. U96439) (Lee et al., 2001). Gm1, soybean AAPT1 (GenBank accession no. U12735) (Dewey et al., 1994). Bn1, *Brassica napus* AAPT1 (GenBank accession no. AY179560) (Qi et al., 2003).

required to allow interfacing of the hydrophilic CDP-aminoalcohol substrate with that of the hydrophobic diacylglycerol (Mancini et al., 1999). A conserved motif of phospholipid synthesizing enzymes catalyzing CDP-alcohol phosphotransferase reactions, Asp Gly X_2 Ala Arg X_8 Gly X_3 Asp X_3 Asp (Williams and McMaster, 1998; Qi et al., 2003), was also identified in this catalytic domain (Fig. 1). All the plant aminoalcoholphosphotransferases known so far have exactly the same amino acid sequences in this region.

In order to study the response of *AAPT3* to low temperature and to compare its expression pattern with

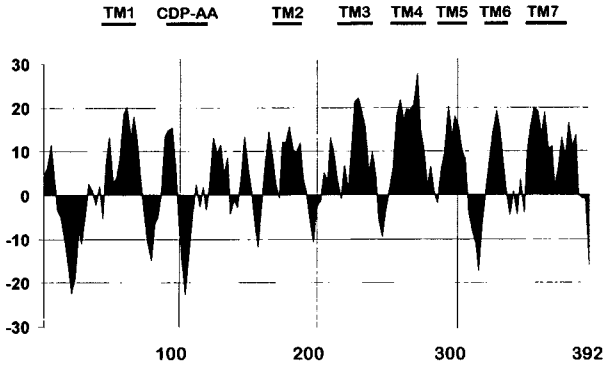

Fig. 2. Hydropathy plot of the predicted amino acid sequence of Chinese cabbage AAPT3. The horizontal scale indicates the number of amino acid residues and the vertical scale indicates the free energy in kcal/mole/amino acid for transfer from a hydrophobic to hydrophilic environment. TM1-TM7, transmembrane spanning regions; CDP-AA, CDP-aminoalcohol phosphotransferase motif.

AAPT1 and *AAPT2*, RT-PCR was performed. As shown in Fig. 4, *AAPT1*, *AAPT2* and *AAPT3* are expressed equally well in shoots of 5-d old seedlings grown at 25°C. The expression was also noticed in roots of 5-d old seedlings. The plants were then either maintained in the same condition or transferred to a growth chamber of 5°C and further grown for 2 d. The level of mRNA of all three isoforms slightly increased after two days at 25°C in both shoots and roots. A marked increase of the transcripts was noticed in both shoots and roots of seedlings transferred to low temperature.

Discussion

Aminoalcoholphosphotransferase sequences are well

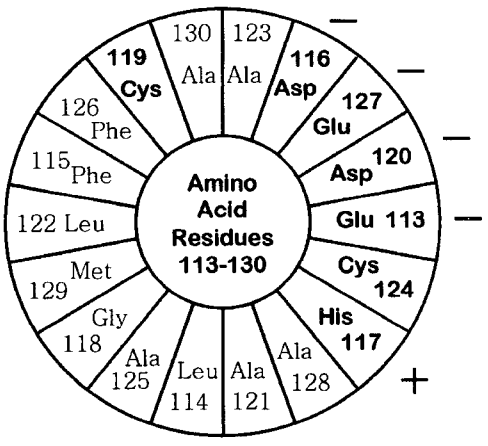

Fig. 3. The positioning of amino acid residues for Chinese cabbage AAPT3 in a predicted amphipathic helix within the catalytic domain. The aspartic acid residues (116, 120) of the CDP-aminoalcohol phosphotransferase motif reside within this amphipathic helix. Hydrophilic residues are in bold characters.

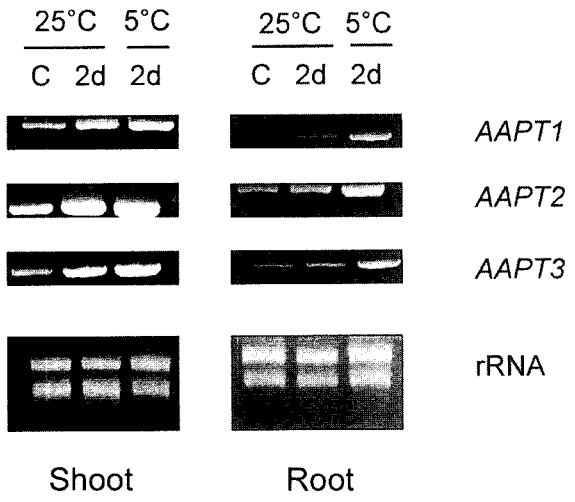

Fig. 4. Chinese cabbage seeds were germinated and grown for 5 d at 25°C (C), and then either remained or transferred to a growth chamber of 5°C for cold treatment and further grown for 2 d. Total RNA was extracted from shoots and roots, and subjected to RT-PCR for the amplification of 1,219 bp (*AAPT1*), 858 bp (*AAPT2*) and 1,351 bp (*AAPT3*) fragments.

conserved in plants (Fig. 1). The homology between organisms is greater than 60%, and the hydropathy analysis revealed the same profiles with seven membrane-spanning domains in the structure. All aminoalcohol-phosphotransferase isoforms contain a conserved motif of phospholipid synthesizing enzymes catalyzing CDP-alcohol phosphotransferase reactions, Asp Gly X_2 Ala Arg X_8 Gly X_3 Asp X_3 Asp, within CDP-aminoalcohol binding domain.

The results from RT-PCR showing that Chinese cabbage *AAPT1, AAPT2* and *AAPT3* are well expressed in shoots and roots were expected, since rapidly expanding cells are actively involved in the synthesis of membranes and hence in the synthesis of phospholipids (Fig. 4). Soybean *AAPT1* exhibited basically the same expression pattern, accumulating in all organs: roots, leaves, stems and developing seeds (Dewey et al., 1994).

In our previous experiments Chinese cabbage *AAPT2* exhibited an increase in the level of the transcript in response to the cold treatments, and was suggested to play a role in resistance to low temperature (Choi et al., 2000). The idea was supported by a recent discovery that *Arabidopsis AAPT* transcription is also induced by low temperature treatments (Qi et al., 2003). In our current study, the expression of Chinese cabbage *AAPT1* and *AAPT3* was also up-regulated by low temperature. A number of studies suggested that exposure of plants to low temperature leads to an increase in the amount of phosphatidylcholine and/or in the activity of phosphatidylcholine synthesizing enzymes (Horvath et al., 1981; Kinney et al., 1987; Cheesbrough, 1989; Cho and Cheesbrough, 1990).

The presence of aminoalcoholphosphotransferase isoforms in plants now raises questions about their roles. Recent studies on *Arabidopsis thaliana* revealed that while the level of *AtCCT2* increased by about 6-fold during chilling treatment, no significant change was observed in the level of *AtCCT1* transcripts (Inatsugi et al., 2002). Both *AtCCT1* and *AtCCT2* encode a CTP:phosphocholine cytidylyltransferase, which catalyzes the synthesis of CDP-choline, the substrate of aminoalcoholphosphotransferase. In our current study, we could not find such differential roles of AAPT isoforms, all of which were up-regulated by low temperature. In soybean, it was suggested that *AAPT1* and *AAPT2* are responsible for the synthesis of storage oil in developing seeds and for the production of membrane lipids in young tissues, respectively (Dewey et al., 1994). It would be interesting to see whether Chinese cabbage aminoalcoholphosphotransferase isoenzymes utilize different molecular species of diacylglycerol, the other substrate of the enzyme reaction, in order to provide the cellular membranes with phosphatidylcholine or phosphatidylethanolamine with modified molecular species composition in response to various environmental changes.

Acknowledgments

This research was supported by a grant from the Basic Research Program of the Korea Science and Engineering Foundation (R02-2000-00164).

References

Belyavsky AT, Vinogradova T, and Rajewsky K (1989) PCR-based cDNA libraries at the level of a few cells. *Nucl Acids Res* 17: 2919-2932.

Cheesbrough TM (1989) Changes in the enzymes for fatty acid synthesis and desaturation during acclimation of developing soybean seeds to altered growth temperature. *Plant Physiol* 90: 760-764.

Cho SH and Cheesbrough TM (1990) Warm growth temperatures decrease soybean cholinephosphotransferase activity. *Plant Physiol* 93: 72-76.

Choi YH, Lee JK, Lee C-H, and Cho SH (2000) cDNA Cloning and expression of an aminoalcoholphosphotransferase isoform in Chinese cabbage. *Plant Cell Physiol* 41: 1080-1084.

Chou PY and Fasman GD (1978) Empirical predictions of protein conformation. *Annu Rev Biochem* 47: 251-276.

Dewey RE, Wilson RF, Novitzky WP, and Goode JH (1994) The *AAPT1* gene of a soybean complements: a cholinephosphotransferase-deficient mutant of a yeast. *Plant Cell* 6: 1495-1507.

Frohman MA, Dush MK, and Martin GR (1998) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc Natl Acad Sci USA* 85: 8998-9002.

Goode JH and Dewey RE (1999) Characterization of aminoalcoholphosphotransferases from *Arabidopsis thaliana* and soybean. *Plant Physiol Biochem* 37: 445-457.

Harwood JL (1979) The synthesis of acyl lipids in plant tissues. *Prog Lipid Res* 18: 55-86.

Harwood JL (1998) Environmental effects on plant lipid biochemistry. In: Harwood JL (ed), Plant Lipid Biosynthesis, Cambridge University Press, Cambridge, pp 305-347.

Harwood JL, Jones AL, Perry HJ, Rutter AJ, Smith KL, and Williams M (1994) Changes in plant lipids during temperature adaptation. In: Cossins AR (ed), Temperature Adaptation of Biological Membranes, Portland Press, London, pp 107-118.

Hjelmstad RH and Bell RM (1991) *sn*-1,2-diacylglycerol choline- and ethanolaminephosphotransferase in *Saccharomyces cerevisiae*: mixed micellar analysis of the *CPT1* and *EPT1* gene product. *J Biol Chem* 266: 4357-4365.

Horvath I, Vigh L, and Farkas T (1981) The manipulation of polar head group composition of phospholipids in the wheat Miranovskaja 808 affects frost tolerance. *Planta* 151: 103-108.

Inatsugi R, Nakamura M, and Nishida I (2002) Phosphatidylcholine biosynthesis at low temperature: differential expression of CTP: phosphorylcholine cytidylyltransferase isogenes in *Arabidopsis thaliana*. *Plant Cell Physiol* 43: 1342-1350.

Justin AM, Demandre C, Tremolieres A, and Mazliak P (1985) No discrimination by choline- and ethanolaminephosphotransferase from potato tuber microsomes in molecular species of endogenous diacylglycerols. *Biochim Biophys Acta* 836: 1-7.

Kennedy EP and Weiss SB (1956) The function of cytidine coenzymes in the biosynthesis of phospholipids. *J Biol Chem* 222: 193-214.

Kinney AJ (1993) Phospholipid head groups. In: Moore TS (ed), Lipid Metabolism in Plants, CRC, Boca Raton, pp 259-284.

Kinney AJ, Clarkson DT, and Loughman BC (1987) The regulation of phosphatidylcholine biosynthesis in rye roots: stimulation of the nucleotide pathway by low temperature. *Biochem J* 242: 755-759.

Kyte J and Doolittle RF (1982) A simple method displaying the hydropathic character of a protein. *J Mol Biol* 157: 105-132.

Lee JK, Choi YH, Bae YG, Lee WS, and Cho SH (2001) Cloning and expression of a cDNA encoding aminoalcoholphosphotransferase from *Pimpinella brachycarpa*. *J Plant Biol* 44: 27-32.

Lord JM (1975) Evidence that phosphatidylcholine and phosphatidylethanolamine are synthesized by a single enzyme present in the endoplasmic reticulum of castorbean endosperm. *Biochem J* 151: 451-453.

Macher BA and Mudd JB (1974) Biosynthesis of phosphatidylethanolamine by enzyme preparations from plant tissues. *Plant Physiol* 53: 171-175.

Mancini A, Del Rosso F, Roberti R, Orvietani P, Coletti L, and Binaglia L (1999) Purification of ethanolaminephosphotransferase from bovine liver microsomes. *Biochim Biophys Acta* 1437: 80-92.

McMaster CR and Bell RM (1997) CDP-choline: 1,2-diacylglycerol cholinephosphotransferase. *Biochim Biophys Acta* 1348: 100-110.

Min KM, Bae YG, Lee JS, Choi YH, Cha YR, and Cho SH (1997) Cloning of an aminoalcoholphosphotransferase cDNA from Chinese cabbage roots. *J Plant Biol* 40: 234-239.

Moore TS (1982) Phospholipid biosynthesis. *Annu Rev Plant Physiol* 33: 235-259.

Qi Q, Huang YF, Cutler AJ, Abrams SR, and Taylor DC (2003) Molecular and biochemical characterization of an aminoalcoholphosphotransferase (*AAPT1*) from *Brassica napus*: effects of low temperature and abscisic acid treatments on *AAPT* expression in *Arabidopsis* plants and effects of over-expression of *BnAAPT1* in transgenic *Arabidopsis*. *Planta* 217: 547-558.

Sparace SA, Wagner LK, and Moore TS (1981) Phosphatidylethanolamine synthesis in castor bean endosperm. *Plant Physiol* 67: 922-925.

Vance DE (1989) CTP: cholinephosphate cytidylyltransferase. In: Vance DE (ed), Phosphatidylcholine Metabolism, CRC, Boca Raton, pp 34-45.

Weiss SB, Smith SW, and Kennedy EP (1958) The enzymatic formation of lecithin from cytidine diphosphate choline and D-1,2-diglyceride. *J Biol Chem* 231: 53-64.

Williams JG and McMaster CR (1998) Scanning alanine mutagenesis of the CDP-alcohol phosphotransferase motif of *Saccharomyces cerevisiae* cholinephosphotransferase. *J Biol Chem* 273: 13482-13487.

Williams M and Harwood JL (1997) Effects of carbon dioxide concentration and temperature on lipid synthesis by young wheat leaves. *Phytochemistry* 45: 243-250.

[Received February 16, 2004; accepted March 29, 2004]