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Molecular biology of the plastidic phosphorylated serine biosynthetic pathway in *Arabidopsis thaliana*

Review Article

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Summary. Serine biosynthesis in plants proceeds by two pathways; the glycolate pathway which is associated with photorespiration and the pathway from 3-phosphoglycerate which is presumed to take place in the plastids. The 3-phosphoglycerate pathway (phosphorylated pathway) involves three enzymes catalyzing three sequential reactions: 3-phosphoglycerate dehydrogenase (PGDH), 3-phosphoserine aminotransferase (PSAT) and 3-phosphoserine phosphatase (PSP). cDNA and genomic clones encoding these three enzymes from spinach and *Arabidopsis thaliana* were isolated by means of heterologous probe screening, homologous EST clones and genetic complementation in an *Escherichia coli* mutant. The identity of the isolated cDNAs was confirmed by functional complementation of serine auxotrophy in *E. coli* mutants and/or the detection of catalytic activity in the recombinant enzymes produced in *E. coli*. Northern blot analyses indicated the most preferential expression of these three genes in light-grown roots. In contrast, the mRNAs of two proteins involved in the glycolate pathway (H-protein of glycine decarboxylase multienzyme complex and serine hydroxymethyltransferase) accumulated to high levels in light-grown shoots. Environmental stresses, such as high salinity, flooding and low temperature, induced changes in mRNA levels of enzymes in the plastidic phosphorylated serine biosynthetic pathway but not in that of the glycolate pathway. These results indicate that the plastidic 3-phosphoglycerate pathway plays an important role in supplying serine in non-photosynthetic tissues in plants and under environmental stresses.

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Abbreviations: EST = expressed sequence tag, GDC = glycine decarboxylase multienzyme complex, PGDH = 3-phosphoglycerate dehydrogenase, 3PHP = 3-phosphohydroxypyruvate, PSAT = 3-phosphoserine aminotransfearse, PSP = 3-phosphoserine phosphatase, SHMT = serine hydroxymethyltransferase

Introduction

Serine is a key intermediate in a number of important metabolic pathways including the photorespiratory metabolism of glycolate to 3-phosphoglycerate (3PGA) (Walton and Woolhouse, 1986). It is also important as a precursor in the generation of glycine, tryptophan, cysteine and in the interconversion of homocysteine and methionine (Walton and Woolhouse, 1986; Ireland and Hiltz, 1995). Besides that, serine is involved in the synthesis of phospholipids, porphyrins, purines and thymidine. It is the precursor of phosphatidylcholin and the source of one-carbon units as well.

Serine may have some significance in the biochemical responses of plants to various forms of environmental stresses. Accumulation of serine in plants grown at low temperature and elevated salinity has been reported (Stewart and Larcher, 1980). In addition, serine was found to be an important precursor of ethanolamine in certain halophytes. It is subsequently converted to glycine betaine which accumulates markedly in certain plants subjected to environmental stresses (Stewart and Larcher, 1980).

Biosynthesis of serine

In the yeast *Saccharomyces cerevisiae*, serine and glycine are mainly synthesized by two pathways. On fermentable carbon sources, serine is generated via the glycolytic pathway from 3-phosphoglycerate (3PGA), whereas, on nonfermentable carbon sources, the gluconeogenic pathway (Melcher et al., 1995), starting from glyoxylate is utilized.

In animals and bacteria, serine is mainly synthesized via the phosphorylated pathway (Ichihara and Greenberg, 1957), utilizing the 3PGA which was derived from glycolysis or from the oxidative or reductive pentose phosphate pathway.

The situation is more complicated in photosynthetic plant tissues since biosynthesis of serine can proceed via the glycolate pathway (Tolbert, 1980) (Fig. 1). The glycolate pathway, associated with photosynthesis and responsible for photorespiration is the most well studied pathway. The oxidative photosynthetic carbon cycle, constitutes the principal biochemical pathway responsible for the uptake of O_2 and release of CO_2 in photorespiration (Kleczkowski and Givan, 1989). Glycolate is the substrate utilized during photorespiration (Hatch, 1976). Glycine and serine are intermediates in the photorespiratory metabolism of glycolate to 3PGA (Fig. 1). Serine production

via this pathway has received considerable attention and has been well documented.

The glycine decarboxylase multienzyme complex (GDC), along with the enzyme serine hydroxymethyltransferase (SHMT), is responsible for the respiratory conversion of glycine to yield CO_2, serine and NADH in plant mitochondria (Neuberger et al., 1986; Srinivasan and Oliver, 1995). Serine is generated by reactions involving two molecules of glycine; one molecule is oxidatively decarboxylated and deaminated to produce an active hydroxymethyl group that becomes attached to the β-carbon of the second molecule. Most of the serine produced is returned to the Calvin cycle via hydroxypyruvate and glycerate. In C_3 leaves where photorespiration provides substantial amounts of glycine, flux through this pathway is rapid (Keys, 1980).

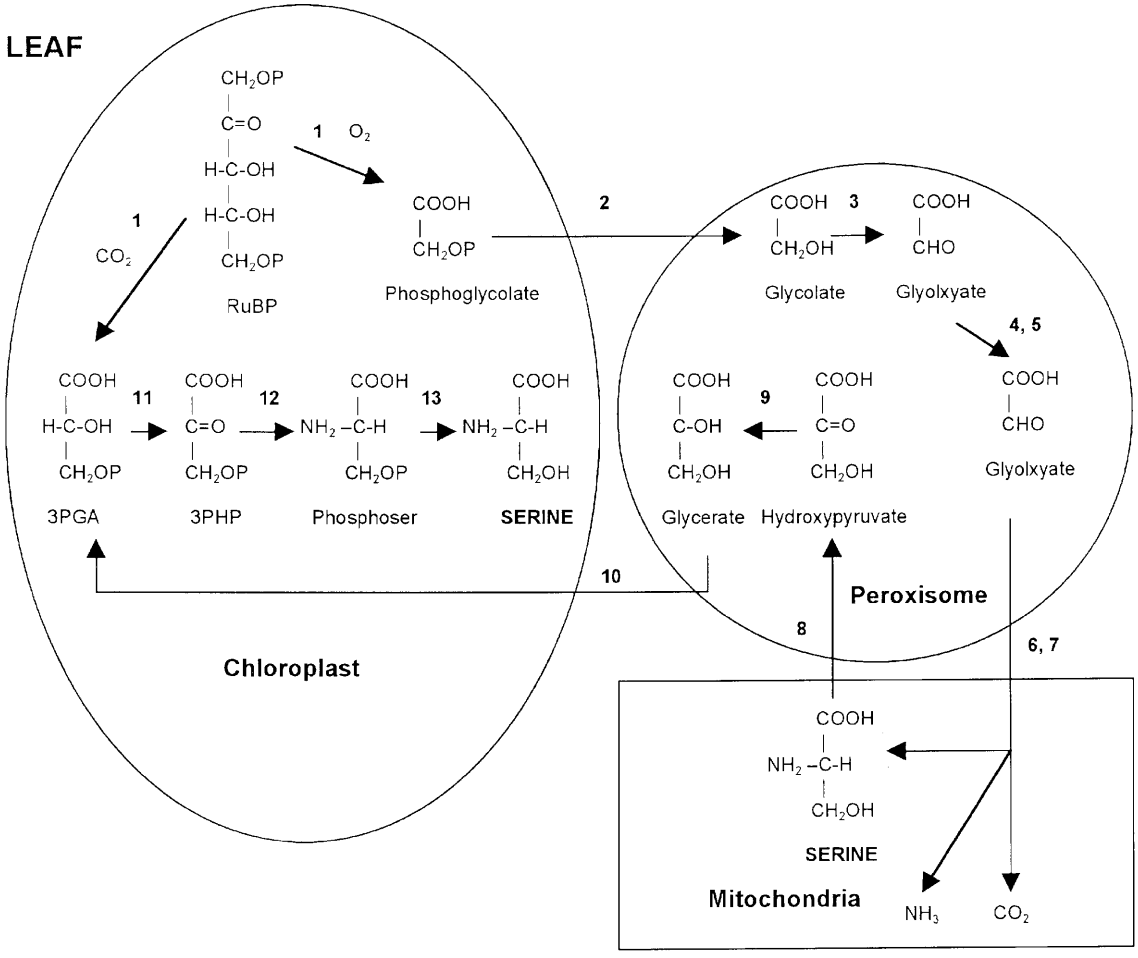
In the leaves of green plants, the mitochondria glycine cleavage reaction is part of the glycolate pathway which recycles two carbon molecules produced as a result of the oxidative reaction of ribulose 1,5-bisphosphate carboxylase/oxygenase. The four polypeptides of GDC and SHMT appeared to be light-induced (Day et al., 1985; Walker and Oliver, 1986). No glycine oxidation activity is present in mitochondria from non-green etiolated tissues (Oliver et al., 1990). The amount of enzyme within the matrix of mitochondria isolated from the leaves of C_3 plants is several fold higher than the levels measured in any other tissues.

SHMT accumulates to high concentration in mitochondria of green leaves. It is a reversible enzyme with the equilibrium slightly in favour of glycine production. In leaves, the reaction is driven in the direction of serine synthesis by the high concentration of glycine that is produced by photorespiration. Root SHMT synthesizes glycine instead of consuming it (Ireland and Hiltz, 1995).

In non-photosynthetic tissues (e.g. roots, developing and germinating seeds etc.), the glycolytic intermediate, 3PGA is oxidised by 3-phosphoglycerate dehydrogenase (PGDH) to form 3-phosphohydroxypyruvate (3PHP), which in turn is converted to 3-phosphoserine by 3-phosphoserine aminotransferase (PSAT). In the final step, dephosphorylation of 3-phosphoserine is performed by 3-phosphoserine phosphatase (PSP) (Stolz and Dörnemann, 1994) (Fig. 1).

Servaites and Ogren (1977) demonstrated that 50% of the initial serine synthesis remained after chemical inhibition of the glycolate pathway, suggesting that a second pathway is activated for serine formation. Carbon atoms of serine produced by the glycolate pathway during photosynthesis from $^{14}CO_2$ should become uniformly labelled in C1 and C2. Serine made from [1-^{14}C] 3PGA would be predominantly carboxyl-labelled. The initial distributions of [^{14}C] in serine made by chloroplasts (Chang and Tolbert, 1965), leaves of C_4 plants (Rabson et al., 1962; Johnson and Hatch, 1969) and homogenates of pea epicotyles (Cheung et al., 1968) suggested some synthesis from 3PGA. Hess and Tolbert (1966) also demonstrated that serine synthesis from 3PGA occurred in tobacco leaves treated with α-hydroxy-2-pyridine methane sulfonate, an inhibitor of glycolate oxidase.

It has been proposed that the phosphorylated pathway is probably of minor significance compared to the glycolate pathway during daylight hours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H-C-OH
H-C-OH
CH2OP
RuBP
1
CO2
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Phosphoglycolate
2
COOH 3 COOH
CH2OH CHO
Glycolate Glyolxyate
4, 5
COOH
CHO
Glyolxyate
COOH 11 COOH 12 COOH 13 COOH
H-C-OH C=O NH2-C-H NH2-C-H
CH2OP CH2OP CH2OP CH2OH
3PGA 3PHP Phosphoser SERINE
COOH 9 COOH
C-OH C=O
CH2OH CH2OH
Glycerate Hydroxypyruvate
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CH2OH
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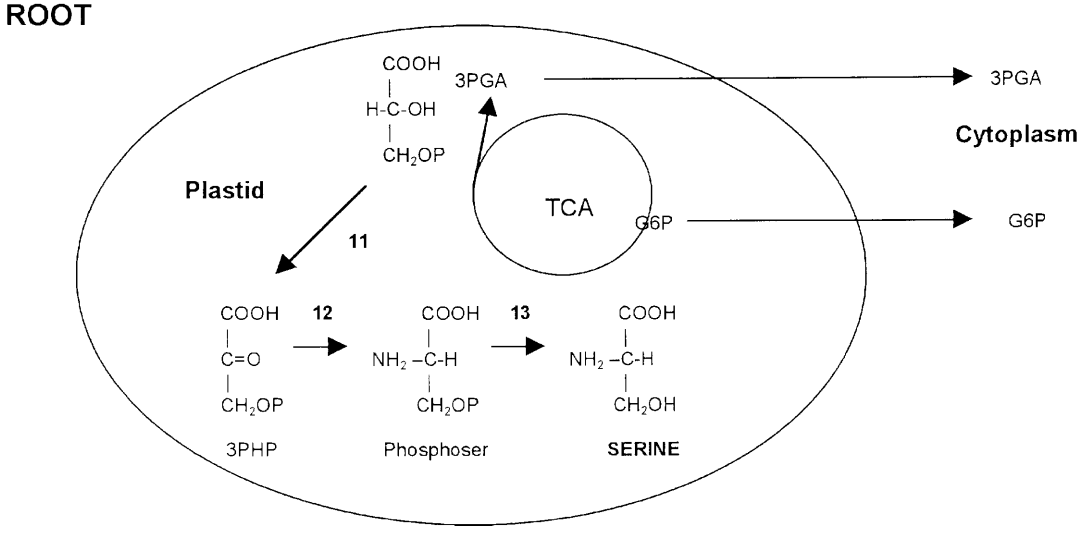
CO2


ROOT
COOH
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3PGA
3PGA
Cytoplasm
Plastid
TCA
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G6P
11
COOH 12 COOH 13 COOH
C=O NH2-C-H NH2-C-H
CH2OP CH2OP CH2OH
3PHP Phosphoser SERINE

when photorespiration takes place, but may be of more importance in the dark or early in leaf development (Ireland and Hiltz, 1995). However, there is some evidence for the phosphorylated pathway operating in green tissues during photosynthesis (Ireland and Hiltz, 1995; Keys, 1980). Exactly why these activities are present in leaves has not been adequately explained, but it seems likely that they may contribute significantly to serine synthesis in the dark when the pathway of carbon flux from glycolate to serine ceases to function. Its importance in providing serine to non-photosynthetic tissues could be another possibility for its coexistence in plants.

The glycerate pathway is initiated by dephosphorylation of 3PGA, and with glycerate and hydroxypyruvate as intermediates (Fig. 1). This pathway comprises of three or four enzymes, namely 3-phosphoglycerate phosphatase, glycerate dehydrogenase and one or two aminotransferases: alanine-hydroxypyruvate and possibly glycine-hydroxypyruvate aminotransferase.

Walsh and Sallach (1965) showed that both the phosphorylated and glycerate/non-phosphorylated pathway were operative in animal system. The availability of fructose may determine which pathway predominates in a given tissue. In some tissues, the major pathway involves non-phosphorylated intermediates, whereas in others, the formation of serine occurs essentially by the phosphorylated pathway. In pig liver and dog kidney, considerable serine biosynthesis occurs by both pathways. Snell (1986) argued that, the pathway involving non-phosphorylated intermediates uses 2-phosphoglycerate instead of 3PGA, therefore it is more likely involved in serine catabolism. This could be also the case in plant cells. It is not known to what extent glycerate can be further converted to serine.

Keys (1980), Kleczkowski and Givan (1989) and Bourguignon et al. (1998) have published three extensive literature reviews on the various pathways of serine biosynthesis, up to the end of the 1970s, 1980s and 1990s, respectively. The present review will only focus on the recent progress achieved by molecular cloning and characterization of the three enzymes: PGDH, PSAT and PSP that are involved in the phosphorylated pathway of serine biosynthesis in *Arabidopsis thaliana*.

The phosphorylated pathway of serine biosynthesis

The phosphorylated pathway involves three enzymes catalyzing three sequential reactions: 3-phosphoglycerate dehydrogenase (PGDH), 3-phosphoserine aminotransferase (PSAT) and 3-phosphoserine phosphatase (PSP).

Fig. 1. Serine biosynthetic pathways in plant. *RuBP* ribulose 1,5-bisphosphate; *3PHP* 3-phosphohydroxypyruvate; *Phosphoser* 3-phosphoserine; *G6P* glucose 6-phosphate. Step *1* ribulose 1,5-bisphosphate carboxylase/oxygenase; step *2* phophoglycolate phosphatase; step *3* glycolate oxidase; step *4* glutamate:glyoxylateaminotransferase; step *5* serine:glyoxylate aminotransferase; step *6* GDC; step *7* SHMT; step *8* serine:glyoxylate aminotransferase; step *9* hydroxypyruvate reductase; step *10* glycerate kinase; step *11* PGDH; step *12* PSAT; step *13* PSP

3-Phosphoglycerate dehydrogenase (PGDH)

3-Phosphoglycerate dehydrogenase (PGDH) (EC 1.1.1.95), the first enzyme in the serine biosynthetic pathway from 3PGA, catalyzes the oxidation of 3PGA to form 3PHP by utilizing NAD^+ as a cofactor.

The molecular cloning and biochemical characterization of PGDH have been reported for a variety of bacterial (Tobey and Grant, 1986; Sorokin et al., 1993; Fleischmann et al., 1995) and animal (Walsh and Sallach, 1966; Achouri et al., 1997) sources. The *ser*A gene which encodes PGDH has been cloned and characterized in *E. coli* (Grant and Zapp, 1981; Tobey and Grant, 1986; Schuller et al., 1989).

In higher plants, the biochemical characterization of PGDH has been carried out in pea (Slaughter and Davies, 1968) and spinach (Larsson and Albertsson, 1979). The first molecular cloning of plant PGDH (Ho et al., 1999b) was done in our laboratory by screening an *Arabidopsis* whole plant cDNA library using a synthetic 50-mer oligonucleotide probe, based on the sequence of *A. thaliana* EST (expressed sequenced tag) clone which shows high homology to PGDH from *B. subtilis* (Table 1). The deduced protein with a molecular mass of 66,453 Da, is composed of three distinct domains: a nucleotide-binding domain, a substrate-binding domain and a regulatory do-

Table 1. Summary of the molecular cloning and characterization of the three plastidic serine biosynthetic enzymes

	PGDH	PSAT	PSP
Cloning strategy	λgt11 *Arabidopsis* cDNA library screening	λgt11 Arabidopsis cDNA library screening	Complementation of *E. coli* mutant
Accesion nos.			
– cDNA	AB003280	D88541	AB018408
– Genomic	AB010407	AB010408	AB018409
Genomic organization	3 exons – 2 introns	1 exon – 0 intron	7 exons – 6 introns
ORF (Size of protein)	624 amino acids (66 kDa)	430 amino acids (47 kDa)	295 amino acids (32 kDa)
Gene localization	Chromosome 1	Chromosome 4	Chromosome 1
Gene copy	Single-copy	Single-copy	Single-copy
Organelle localization	Plastid	Plastid	Plastid
Functional complementation of *E. coli* mutant	*ser*A$^-$ χ536	*ser*C$^-$ KL282	*ser*B$^-$ SK472
Serine inhibition	No inhibition	No inhibition	10 mM
mRNA expression	Root/Light	Root/Light (meristem, non-photosynthetic cells)	Root/Light
Protein accumulation		Stele/Light	
Reference	Ho et al. (1999b)	Ho et al. (1998)	Ho et al. (1999a)

main or a serine binding domain in each subunit of the tetrameric protein of PGDH. The main contact points between the subunits are at the level of the coenzyme-binding domains and the regulatory domains, indicating the importance of these zones for tetramerization.

It also contains three of the most conserved regions in the NAD-dependent-2-hydroxyacid dehydrogenase family. The first pattern is based on a Gly-rich region that probably corresponds to the NAD-binding domain, Gly-X-Gly-X_2-Gly-X_{17}-Asp (Weirenga et al., 1986). Two other patterns contain a number of conserved charged residues, some of which may play a role in the catalytic mechanism.

The deduced amino acid sequence has high similarity to eukaryotes (human and rat), but not yeast. Surprisingly, the nucleotide- and substrate-binding domains of *B. subtilis* PGDH exhibit more similarity with the eukaryotic enzymes than to other bacterial PGDH enzymes (*E. coli* and *Haemophilus influenza*), whereas the yeast enzyme is closer to the latter. This suggests that there are two different types of PGDH that may have evolved before diverging to eukaryotes and prokaryotes.

The identity of the isolated cDNA was confirmed by functional complementation of serine auxotrophy in *E. coli* mutants and detection of catalytic activity in the recombinant enzymes produced in *E. coli*. The recombinant protein exhibited PGDH activity of $0.14 \pm 0.01\,\mu$mole/min/mg of protein in the physiological direction, oxidizing 3PGA to 3PHP; and $10.95 \pm 1.36\,\mu$mole/min/mg of protein in the opposite direction, reducing 3PHP to 3PGA. Lower enzyme activities of PGDH in the physiological direction compared with the non-physiological direction were also reported by Slaughter and Davies (1968) under the indicated condition. The equilibrium constant of bacterial PGDH does not favor serine biosynthesis (Walsh and Sallach, 1965; Merill et al., 1981), suggesting that 3PHP formed must be removed as soon as possible to drive the reaction towards that 3PHP production and subsequently serine synthesis.

Double reciprocal plots of the initial rates data demonstrated K_m values of 0.35 and 0.12 mM for 3PHP and NADH, respectively, at pH 7.1. The activity was inhibited by 3PHP (ca. $90\,\mu$M) as reported for the rat enzyme (Achouri et al., 1997). This inhibition could be released by 100–400 mM KCl. K_m values for 3PGA and NAD^+ were 1.19 mM and 0.01 mM, respectively, at pH 9.0. Ser, Thr, Val, Gly, Trp, O-acetyl-L-Ser and Cys (in the range of 5 to 50 mM) had no effect on the rate of reactions.

Slaughter and Davies (1968) reported that pea PGDH was inhibited by serine but became progressively less sensitive as the purification progressed. However, Larsson and Albertsson (1979) could not find serine or 3-phosphoserine inhibition of the enzyme in spinach chloroplast extracts. Our results concur with those of Larsson and Albertsson (1979) i.e. serine does not show any inhibition effect on the enzyme activities. Hence, serine levels are unlikely to modulate the metabolite flux of the pathway from 3PGA to serine through enzyme activities.

Analysis of genomic and cDNA sequences, revealed the presence of two introns. The first and second introns are positioned at 660–2517 and 3007–

3096, respectively. We showed that *Arabidopsis* PGDH is a single-copy gene and it was mapped to the upper arm of chromosome 1, between the markers g3786 and g3829.

3-Phosphoserine aminotransferase (PSAT)

3-Phosphoserine aminotransferase (PSAT) (EC. 2.6.1.52), the second enzyme in the phosphorylated pathway of serine biosynthesis, catalyzes the transamination from glutamate to α-ketoglutarate yielding 3-phosphoserine from 3PHP (Fig. 1).

The biochemistry of this enzyme has been studied in bacteria (Duncan and Coggins, 1986), yeast (Belhumeur et al., 1994; Melcher et al., 1995); animals (Merill et al., 1981; Lund et al., 1987), microalgae (Stolz and Dörnemann, 1994) and higher plants (Cheung et al., 1968; Larsson and Albertsson, 1979; Reynolds and Blevins, 1986; Reynolds et al., 1988; Saito et al., 1997; Ho et al., 1998). Stolz and Dörnemann (1994) have sequenced and characterized 18 N-terminal amino acids from of the PSAT protein of the green alga, *Scenedesmus obliquus*.

We isolated the first plant cDNA encoding PSAT from spinach by screening the cDNA libraries using a heterologous probe (Saito et al., 1997). An *Arabidopsis* EST clone FAFL52, which shows 55–67% homology to bacterial and yeast PSAT proteins, was identified and used to screen a whole plant *Arabidopsis* cDNA library for the *Arabidopsis* PSAT (Ho et al., 1998) (Table 1). Both the signature sequence of PSAT proteins (Belhumeur et al., 1994) and the conserved sequence motif involving the pyridoxal-phosphate binding lysine site found in Class V aminotransferases (Ouzounis and Sander, 1993) are conserved in *Arabidopsis* and spinach PSATs. *Arabidopsis* and spinach PSATs form a family distinct from bacterial and animal PSATs.

The identity of the isolated cDNAs were confirmed by functional complementation of serine auxotrophy in *E. coli* mutants and detection of catalytic activity in the recombinant enzymes produced in *E. coli*.

We determined the K_m values for glutamate and 3PHP by using the overexpressed recombinant proteins of PSAT from *Arabidopsis* and spinach in *E. coli*. Double reciprocal plots of the initial rates data demonstrated K_m values for the *Arabidopsis* PSAT of 70 μM and 5 mM for glutamate and 3PHP, respectively; and for the spinach PSAT of 150 μM and 3 mM for glutamate and 3PHP, respectively.

Serine failed to inhibit PSAT from both *Arabidopsis* and spinach. These findings agree with the observations by Reynolds et al. (1988) using pea PSAT but were in contrast to the report of Larsson and Albertsson (1979) that PSAT from spinach was strongly inhibited by serine.

A genomic clone of *Arabidopsis* PSAT was isolated and sequenced. No intro is present in the coding region of PSAT gene. The Southern blot analysis suggested the presence of a single gene corresponding to the isolated cDNA, but there is a possibility of related sequences encoding

other isoforms of PSAT in *Arabidopsis* since several weakly hybridizing bands were also observed in addition to the expected bands based on the genomic DNA sequence. This concurs with the previous observation of Larsson and Albertsson (1979) of a multisubcellular distribution of PSAT.

3-Phosphoserine phosphatase

In the final step of the phosphorylated pathway of serine biosynthesis, dephosphorylation of 3-phosphoserine to serine is performed by 3-posphoserine phosphatase (PSP) (EC. 3.1.3.3).

The first molecular cloning of PSP from the plant kingdom was successfully achieved in the present study from *Arabidopsis*, by functional complementation of *E. coli ser*B mutant SK472 (Ho et al., 1999a) (Table 1).

Arabidopsis PSP is closely related to human PSP. In the deduced amino acid sequence, two highly conserved motifs containing aspartate residues in the human PSP, DXDST and GDGXXD, are found in *Arabidopsis* PSP. Both of these domains have high similarity with the consensus phosphorylation site of ATPases of the Na^+/K^+ ATPases family, suggesting that there may be functional homology between PSP and ATPases (Collet et al., 1997). Replacement of the first aspartate of the DXDST motif by asparagine or glutamate resulted in complete inactivation of the human PSP enzyme suggesting the first aspartate of this motif is the phosphorylated residue in human PSP (Collet et al., 1998). PSP belongs to a new phosphotransferase family with an amino-terminal DXDX(T/V) motif that serves as an intermediate phosphoryl acceptor (Collet et al., 1998).

Arabidopsis PSP is a single-copy gene located at chromosome 1, approximately 2.2 cM below marker g3786 and at 0.1 cM above the gene for PGDH. Analysis of genomic and cDNA sequences revealed the presence of six short introns, splitting the open reading frame into seven exons.

The K_m value of PSP in the chloroplast extract of spinach was reported to be 1.1 mM for 3-phosphoserine and the activity was Mg^+ dependent at optimum pH 7 (Larsson and Albertsson, 1979). The *Arabidopsis* recombinant PSP exhibited a higher K_m value of 3.5 mM for 3-phosphoserine.

In *E. coli*, PSP was inhibited by serine (Pizer, 1963). The inhibition is of neither the competitive nor the non-competitive type with respect to serine. The equilibrium positions of PSAT and PGDH will favor 3PGA when PSP is inhibited, thereby ensuring that the pathway as a whole is effectively controlled. The phosphorylated pathway is controlled primarily by the demand for serine rather than the supply of the precursor, 3PGA (Pizer, 1963). Approximately 60% of *Arabidopsis* PSP activity is inhibited at 10 mM serine. This finding is consistent with Larsson and Albertsson's report (1979), that the spinach PSP activity was reduced rather than eliminated completely at high serine concentration. Analysis of the control of mammalian serine biosynthesis also revealed product inhibition of PSP activity, the final step of the pathway (Fell and Snell, 1988).

Glycine can also inhibit *E. coli* PSP activities to a limited extent. High intracellular concentrations of glycine might inhibit serine synthesis (Pizer, 1964). However, our finding showed that glycine does not reduce or inhibit the *Arabidopsis* PSP activity.

Subcellular localization of the phosphorylated serine biosynthetic enzymes

PGDH has been detected in the proplastids of soybean (Larsson and Albertsson, 1979; Bolland et al., 1982), and there may also be an extra-chloroplastic form (Larsson and Albertsson, 1979). However, Southern blot analysis and RFLP results confirmed that *Arabidopsis* PGDH is a single-copy gene.

Reynolds and Blevins (1986) reported that PSAT was localized in the proplastid, a conclusion based on enzyme isolation from the plastidic fraction. However, Larsson and Albertsson (1979) detected 90% of PSAT activity outside the chloroplasts in leaves in contrast to PSP, which was of exclusively chloroplastic origin. Thus, Larsson and Albertsson (1979) proposed that PHP formed by PGDH was exported from chloroplasts to form 3-phosphoserine which was then imported into the chloroplasts to serve as a substrate for PSP.

We have provided evidence for the visualization of the targeting of the fusion proteins of the N-terminal transit peptides and green fluorescence protein to leaf chloroplasts, thus demonstrating that all three enzymes (PGDH, PSAT and PSP) of the phosphorylated pathway are plastidic proteins (Ho et al., 1998, 1999a, 1999b). The export of 3PHP from chloroplasts into cytoplasm for the reaction of PSAT to take place, and then followed by the import of 3-phosphoserine into chloroplasts for further convertion to serine is unlikely to happen since plastids can provide their own 3-phosphoserine for serine biosynthesis.

Gene expression of the plastidic serine biosynthesis

The mRNA expression level of PGDH, PSAT and PSP was examined in leaf and root tissues of both light-grown and dark-treated *A. thaliana* (Ho et al., 1998, 1999a, 1999b). Northern analyses were also carried out with two other enzymes, H-protein (a subunit of GDC) (Srinivasan and Oliver, 1995), and SHMT (Turner et al., 1993) in the glycolate pathway for comparison with the three plastidic pathway enzymes.

The highest level of PGDH mRNA expression was observed in light-grown root tissues (Fig. 2A). It was approximately two- to three-fold higher than the mRNA expression in dark-grown root and leaf tissues. A minor amount of mRNA expression (ca. 1/15 of root in light) was detected in the light-grown leaf tissue.

Similar to PGDH, the highest level of PSAT (Fig. 2B) and PSP (Fig. 2C) mRNA expression was observed in light-grown root tissues, followed by a significant amount of mRNA expression in light-grown shoots. A lower level

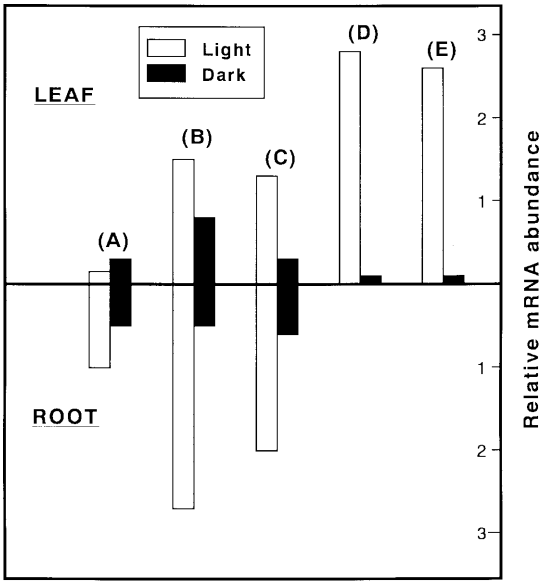

Fig. 2. Gene expression of serine biosynthetic genes from *Arabidopsis*. *A*, PGDH; *B*, PSAT; *C*, PSP; *D*, H-protein of GDC; *E*, SHMT (Adopted from Ho et al., 1999a)

of expression was also detected in dark-grown shoots and roots. However, the PSP mRNA expression level in dark-grown roots was still higher than dark-grown shoots.

In situ hybridization results suggested that mRNA of PSAT was expressed at some level in all types of cells in leaf, stem and root tissues (Ho et al., 1998). However, a strong signal was detected in the meristem tissue of root tips indicating significant higher level of expression in fast proliferating tissues. The root tissues surrounding the vascular bundle and root epidermis were also suggested as sites of preferential expression.

The preferential expression of these three genes in root tissues of light-grown plants was in contrast to the expression pattern exhibited by H-protein and SHMT, which accumulated primarily in the light-growth leaf tissues (Fig. 2, D and E). Our results indicated that the phosphorylated pathway may play an important role in supplying serine to root or other non-photosynthetic tissues in which the photorespiration rates are low.

In the absence of photorespiration, and particularly, in rapidly proliferating tissues of low photosynthetic activity (Cheung et al., 1968; Reynolds and Blevins, 1986), the phosphorylated pathway may play the primary role in the total cellular supply of serine. This is probably because the glycolate pathway does not operate efficiently in the dark (Keys, 1980). Therefore, photosynthetic tissues, e.g. leaves under illumination, may have a greater capacity for utilizing glycine as a serine precursor; whereas in non-photosynthetic tissues, e.g. roots, dark-treated leaves and etiolated leaves etc., the phosphorylated pathway may become the main supply of serine in plants (Reynolds et al., 1988; Ireland and Hiltz, 1995; Saito et al., 1997; Ho et al., 1998, 1999a, 1999b).

From the previous literature, PGDH is primarily associated with seeds, apical meristem (Cheung et al., 1968) and etiolation. Under normal conditions, only minor PSAT activities were detected in the leaves of spinach (Larsson and Albertsson, 1979) and pea (Walton and Woolhouse, 1986), while in tissues associated with rapid cell proliferation, e.g. seed leaves and apical meristems of pea (Cheung et al., 1968) as well as root tissues of soybean and lupin (Reynolds and Blevins, 1986), considerable amounts of PSAT were found. Stolz and Dörnemann (1994) also detected PSAT activity in a *Scenedesmus obliquus* mutant with low photorespiratory activity under normal CO_2 condition which was comparable to tissues with rapid cell proliferation. The phosphorylated pathway enzymes are important in proliferating plant and animal tissues where there is a high serine requirement. Snell (1985) reported that the rat neoplastic tissues have higher SHMT and PSAT activities, perhaps reflecting the increased demand for one-carbon fragments ultimately required for DNA synthesis.

Accumulation of PSAT protein

Abundance of PSAT protein was detected in the total proteins in both shoots and roots of light-grown seedling (Ho et al., 1998). Immunolocalization of PSAT protein provided further information that PSAT protein is mainly detected in cells in and around the vascular bundle, and is preferentially associated with xylem in leaf, stem and root tissues. Since these cells do not take part in photosynthesis, the phosphorylated pathway may therefore play an important role in supplying serine requirement to these tissues. It is very likely that the increase in protein synthesis is partly regulated at the mRNA level.

Gene expression under environmental stress

Serine may have some significance in the biochemical responses of plants to various environmental stresses (Stewart and Larher, 1980). Elevated accumulation of serine was reported in plants grown at low temperatures (Draper, 1972), under anaerobic condition (Guinn and Brinkerhoff, 1970) and in elevated salinity (Stewart and Larher, 1980). It is hardly surprising as serine was found to be an important precursor of ethanolamine, which is subsequently converted to glycine betaine which accumulates markedly in certain plants subjected to environmental stresses (Rhodes et al., 1998).

We studied the mRNA expression level of the four serine biosynthetic enzymes, PGDH, PSAT, PSP and the H-protein to investigate the possible role of serine in response to various forms of environmental stress.

Our preliminary results showed that environmental stresses, such as high salinity, flooding and low temperature, induced changes in mRNA levels of enzymes in the plastidic phosphorylated serine biosynthetic pathway but not in that of the mitochondrial pathway (Ho et al., unpublished). The mRNA expression of PGDH increased under high salinity condition, whereas, flood-

ing condition increased PSAT mRNA expression level. Slight increase of PGDH mRNA in plants under cold treatment was also observed.

Regulation of the plastidic serine biosynthesis

There are two pools of serine synthesized *de novo* in photosynthetic cells (Fig. 1), one in the mitochondria where serine is synthesized via the glycolate pathway; and the other one in the chloroplasts, where the phosphorylated pathway is present.

Serine production in the mitochondria, catalyzed by the reactions of GDC and SHMT, is thought to be the major source of serine supply in light-grown plants. However, significant gene expression of the plastidic serine synthesizing enzymes in the leaf tissues of light-grown plants indicated the coexistence of a source of serine in the chloroplasts. It is not surprising since 3PGA, the precursor of this pathway is generated in large amount in the chloroplasts via the carboxylation of ribulose 1,5-bisphosphate.

In the recent review of Bourguignon et al. (1998), the authors argued that the higher the photorespiration rate, the less photorespiratory glycine and serine are available for cell metabolism, therefore, photorespiration does not necessarily play an important role in net serine biosynthesis in plant leaves under light condition. The glycolate pathway together with the non-phosphorylated/glycerate pathway of serine biosynthesis form a catabolic route in recycling the photorespiratory carbon into the Benson-Calvin cycle (Bourguignon et al., 1998).

Cases of metabolic differentiation in which the enzymes associated with a particular pathway are synthesized or preferentially synthesized only in some tissues or cells of a plant would represent another possibility for the existence of multiple pathways. The preferential mRNA expression of PGDH, PSAT and PSP, the three genes which are responsible for the phosphorylated pathway of serine biosynthesis, are found in the root tissues of light-grown plants (Fig. 2). 3PGA generated through the oxidative pentose phosphate pathway may serve as the precursor of serine biosynthesis in the plastids of root tissues. The amount of PGDH and PSP mRNA detected in the roots was significantly higher than the leaves from dark-treated plants, revealing a root-preferential expression of these genes. Furthermore, immunolocalization experiments also demonstrated accumulation of PSAT protein in non-photosynthetic cells, particularly in the cells associated with xylems in the stele.

Coexisting multiple pathways may be preferentially used depending upon the physiological state of the cell at a given time. The phosphorylated pathway of serine biosynthesis is proposed to be an important serine supply in the dark when the photorespiratory rate is low. The above assumption is supported by the fact that both GDC and SHMT were hardly detected in both leaf and root tissues of the dark-treated seedlings.

Searching the transcriptional factor binding site profile database using TFSEARCH Ver. 1.3 (Kyoto Univ., Japan) showed that the motifs present in the PGDH and PSAT genes exhibit similarities with SBF-1 factor (Lawton et

al., 1991) binding site, suggesting the possibility that these genes may be light-regulated. Various analyses suggested that SBF-1 is identical to or closely related to GT-1 (Lawton et al., 1991). GT-1 and its corresponding cis-element have been implicated in the light-dependent expression of *rbcS-3A* in green tissues (Lam and Chua, 1990). The closely related transacting factor, SBF-1, is also found to be light-regulated in *CHS* and *PAL* genes. However, all these genes displayed diverse organ-specific expression.

GT-1 binding sites have also been found in the promoter of a photoreceptor phytochrome which is down-regulated by light (Kay et al., 1989). There are implications that GT-1 and SBF-1 cis-elements, and their corresponding factors are highly dependent on individual promoters in which the cis-elements reside and the particular combination of factors present in the nuclei of different cell types. Interactions with several different transcrip-tional factors may provide a flexible and diverse expression pattern as suggested by Lawton et al. (1991) so that a relatively small number of cis-elements and cognate-factors allow an array of different gene expression patterns.

High enzyme activities of PSAT in fast proliferating tissues (e.g. root meristems and apical tissues etc.) (Reynolds and Blevins, 1986) and preferential PSAT mRNA expression in root meristem of *Arabidopsis* (as shown by *in situ* hybridisation experiments), where there is high serine requirement, were confirmed. Therefore, the high expression level of this gene in the root tissues of light-grown plants is needed for high demand of serine or its related products. This pathway may also function under environmental stresses condition.

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