Purification, Characterization and N-Terminal Sequence of Phosphoserine Aminotransferase from the Green Alga *Scenedesmus obliquus*, Mutant C-2 A′

M. Stolz and D. Dörnemann

Fachbereich Biologie/Botanik, Philipps-Universität, Karl-v.-Frisch-Straße
D-35032 Marburg, Bundesrepublik Deutschland

Z. Naturforsch. **49 c**, 63–69 (1994); received November 11/December 16, 1993

Scenedesmus obliquus, Phosphorylated Pathway, Phosphoserine Aminotransferase

Phosphoserine aminotransferase (EC 2.6.1.52), an enzyme of the "phosphorylated pathway" leading to the formation of serine, was purified from *Scenedesmus obliquus*, mutant C-2 A′. Purification started from the soluble supernatant of a crude cell homogenate and included different affinity and DEAE chromatographic techniques, as well as gel filtration. The purified phosphoserine aminotransferase was enriched 1537-fold and identified to be a homodimer with subunit molecular masses of 40 kDa, each. The absorption spectrum is consistent with the presence of pyridoxal-5-phosphate as cofactor. From the purified enzyme 18 amino acids of the N-terminus could be determined, showing at least 67% homology with the *ser*C gene encoding phosphoserine aminotransferases from bacterial organisms.

Introduction

L-serine serves as a precursor in a variety of important biosynthetic pathways including protein biosynthesis, gluconeogenesis and phospholipid synthesis. For the formation of serine from 3-phosphoglycerate, an intermediate of glycolysis, the "phosphorylated pathway" (Ichihara *et al.*, 1957) is responsible in animal, bacterial, as well as in plant tissues. The glycolic intermediate D-3-phosphoglycerate is oxidized by D-3-phosphoglycerate dehydrogenase to form phosphohydroxypyruvate, which, in turn, is substrate for transamination with glutamate to yield 3-phosphoserine. The latter step is catalyzed by phosphoserine transaminase (PSAT). In the final reaction dephosphorylation of phosphoserine to serine is performed by phosphoserine phosphatase (Ichihara *et al.*, 1957; Walsh *et al.*, 1966). In animals and bacteria serine is mainly synthesized *via* this pathway (Schmidt *et al.*, 1973; Snell, 1986).

In photosynthetic plant tissues the situation is more complex since serine can be synthesized by photorespiration *via* glycolate, glyoxylate and glycine (Tolbert, 1980). After chemical inhibition of the glycolate pathway 50% of the initial serine synthesis remained (Servaites *et al.*, 1977), suggesting that under these inhibitory conditions a second pathway is activated for serine formation. For this reason under normal conditions only minor PSAT-activities can be measured in leaves of spinach (Larsson *et al.*, 1979) and pea (Walton *et al.*, 1986), while in tissues associated with rapid cell proliferation, *e.g.* seed leaves and apical meristem of pea (Cheung *et al.*, 1968), as well as root tissues of soybean and lupin (Reynolds *et al.*, 1986) considerable amounts of PSAT could be determined.

To elucidate some of the characteristics and the primary structure of PSAT from a plant organism, *Scenedesmus obliquus*, mutant C-2 A′, was employed, as green algae are known to exhibit only low photorespiratory activity (Tolbert, 1980). Additionally, as the greening of the employed mutant is light-dependent, it is from its developmental state comparable to tissues with rapid cell proliferation. In the present paper we describe the purification, partial characterization and the N-terminal sequence of PSAT from *Scenedesmus*, an organism with oxygenic photosynthesis.

Materials and Methods

Chemicals and separation materials

Sephacryl S-300, Blue Sepharose CL-6 B, AH-Sepharose 4 B and the pre-packed FPLC column Mono Q HR 5/5 were obtained from Pharmacia LKB Biotechnology Inc. (Freiburg, F.R.G.). Matrex-gel A was obtained from Amicon Corp. (Dan-

Abbreviations: DTT, 1,4-dithiothreitol; EDTA, N,N,N′,N′-ethylendiaminetetraacetic acid; LDH, L-lactate: NAD^+ oxidoreductase; NADH, nicotinamide-adenine-dinucleotide (reduced form); PLP, pyridoxal-5-phosphate; PMP, pyridoxamine-5-phosphate; PSAT, O-Phospho-L-serine: 2-oxoglutarate aminotransferase.

Reprint requests to Dr. D. Dörnemann.

Verlag der Zeitschrift für Naturforschung,
D-72072 Tübingen
0939–5075/94/0100–0063 $ 01.30/0

vers, M.A., U.S.A.), DEAE cellulose (DE 52) was purchased from Whatman (Maidstone, England), Fractogel TSK DEAE-650 (S) and Fractogel TSK HW-55 (S) were from Merck (Darmstadt, F.R.G.). PM 30 ultrafiltration membranes and concentrators were obtained from Filtron (Karlstein, F.R.G.). Protein determinations were performed with Bio-rad-reagent as described in the users manual (Bio-rad, München, F.R.G.). Tricine buffer was obtained from Roth (Karlsruhe, F.R.G.). All other applied chemicals were p.a. grade or of the highest available purity.

Plant material and growth conditions

For all experiments the X-ray-induced pigment mutant C-2 A′ (Bishop, 1971) of *Scenedesmus obliquus* was used. In contrast to the wild type the mutant synthesizes chlorophyll only in the light. After heterotrophic growth in darkness for 3–4 days (Bishop *et al.*, 1972) the algae were exposed to white light of an intensity of 20 $W \times m^{-2}$ for 6 h.

Buffers

Buffer A consisted of 0.1 M Tricine/NaOH (pH 9), 0.3 M glycerol with 25 mM $MgCl_2$ and 1 mM DTT. Buffer B was a 1:5 and buffer C a 1:2.5 dilution of buffer A. Buffer D was 20 mM in Tricine/NaOH (pH 9) with additionally 0.3 M glycerol, 25 mM $MgCl_2$ and 1 mM DTT. Buffer E was identical with buffer D, but contained in addition 0.5 M NaCl.

Purification procedure

Cells were harvested by centrifugation, resuspended in buffer A and broken with glass beads (0.7 mm diameter) in a Vibrogen cell mill (type Vi-2, Bühler, Tübingen, F.R.G.) as described before (Senger and Mell, 1977). After separation of the glass beads from the homogenate by suction on a sinter glass filter, the filtrate was centrifuged at 300 000 × *g* for 1 h. The soluble protein supernatant was subjected to further purification.

Pre-purification of phosphoserine aminotransferase (PSAT) was performed by a serial column system according to Wang *et al.*, 1981, consisting of Sephacryl S-300 (5.3 × 50 cm), Blue Sepharose Cl-6 B (2.8 × 15 cm), Matrex-gel red A (2.8 × 7 cm) and Chlorophyllin Sepharose (2.8 × 7 cm). Buffer A was used as equilibration and basic elution medium.

For the purification procedure the cell-free homogenate was applied to the Sephacryl S-300-column and the eluate monitored for absorption at 280 nm. The first eluting "yellow" fraction containing high molecular weight proteins and protein-aggregated carotenoids was discarded. The following fraction was passed through the above mentioned affinity chromatography system. After washing with buffer A, the non-binding protein fraction, containing PSAT-activity, was concentrated by ultrafiltration (Amicon-ultrafiltration cell; Amicon, Witten, F.R.G.) equipped with a Filtron-membrane (30 kDa exclusion limit).

This pre-purified protein fraction was adjusted to a Tricine concentration of 20 mM and applied to a DE 52-Cellulose column (2.8 × 8 cm), equilibrated before with buffer B (20 mM Tricine). The column was washed with buffer C (40 mM Tricine) prior to elution with a linear gradient from 40–100 mM Tricine (500 ml) at a flow rate of 1.9 ml/min. PSAT-active fractions eluting at about 60 mM Tricine were collected, adjusted to 100 mM Tricine and concentrated to a volume of 3 ml.

For further purification the concentrated DEAE cellulose preparation was fractionated on a Fractogel HW-55 column (3.3 × 46 cm) which was equilibrated before with buffer A. Chromatography was performed at a flow rate of 0.55 ml/min. The active fractions were collected and then applied to a Fractogel DEAE column (2.4 × 8 cm). Chromatography was carried out analogous to the DEAE cellulose separation with a linear gradient of 40–100 mM Tricine (300 ml) at a flow rate of 1.5 ml/min. Active fractions were pooled again.

As the final purification step FPLC on Mono Q was performed. Four separate runs employing the collected Fractogel DEAE-preparation were carried out. Fractions of 10 ml were applied to a Mono Q HR 5/5-column which was equilibrated with buffer D. After washing off the non-binding proteins with the equilibration buffer bound proteins were eluted at a flow rate of 1 ml/min with a linear gradient (110 ml) from 0 to 0.5 M NaCl (buffer E). The total purification procedure was carried out at 4 °C.

Electrophoretical separations

Electrophoresis was performed in denaturing polyacrylamide gels (18 × 15 × 0.15 cm) as described by Laemmli, 1970. The separation gel contained

10% polyacrylamide, 0.38 M Tris/HCl, pH 8.0, with additionally 0.1% (w/v) SDS, 0.03% (w/v) ammonium persulfate (APS) and 0.025% (v/v) tetramethylethylendiamine (TEMED) and 4.5% sucrose. The stacking gel was 5% in polyacrylamide with 0.13 M Tris/HCl, pH 6.8, 0.1% (w/v) SDS, 0.12 (w/v) APS and 0.07% (v/v) TEMED. The electrophoresis buffer consisted of 0.25 M Tris and 0.2 M glycine, pH 8.3, and 0.1% SDS. Electrophoresis was carried out overnight (14 h) at a constant current of 15 mA. The proteins were visualized by silver staining (Blum, 1987). For the determination of the molecular mass the following protein markers (Pharmacia, Freiburg) were used: Phosphorylase b (93 kDa), Bovine serum albumine (BSA 67 kDa), ovalbumine (43 kDa), carboanhydrase (30 kDa), Trypsine inhibitor (20.1 kDa).

Determination of the native molecular mass

The determination of the molecular mass of PSAT was performed with Fractogel HW 55 as described above, except that the applied volume of the protein solution was only 1 ml. Proteins for the calibration of the column were Cyt *c* (12.3 kDa), myoglobin (17.2 kDa), carboanhydrase (30.0 kDa), ovalbumin (45 kDa), ovotransferrin (76.0–78.0 kDa), LDH (145.0 kDa).

Enzyme assay

Enzyme activity was determined spectrophotometrically applying a modification of the coupled assay procedure according to Hurst *et al.*, 1981. For standard assays, the reaction mixture contained 50 mM Tris/HCl, pH 7.6, 100 mM KCl, 17.5 mM sodium 2-oxoglutarate, 1 mM EDTA, 0.1 mM pyridoxalphosphate, 1 mM DTT, 2.5 mM L-phosphoserine, 0.12 mM NADH, 3 mU L-lactate dehydrogenase (LDH) and 0.5 mU D-3-phosphoglycerate dehydrogenase in a final volume of 1 ml. The reaction mixture was preincubated at 30 °C for 15 min to remove traces of an unidentified LDH-reactive contaminant. The reaction was initiated by the addition of a PSAT protein sample.

One unit of enzyme activity is defined to be equivalent to the utilization of 1 mol of L-phosphoserine per minute at 30 °C, based on a molar extinction coefficient for NADH of $6.22 \times 10^6 \times cm^2 \times m^{-1}$ at 340 nm.

N-terminal sequencing

The purified protein was concentrated and washed twice with bidistilled water on Filtron concentrators (30 kDa exclusion limit; Filtron, Karlstein, F.R.G.). For sequencing 0.5 mg of protein were dissolved in 30% acetonitrile containing 0.1% trifluoroacetic acid. Sequencing was kindly performed by Dr. D. Linder, Universität Gießen, F.R.G. with an automatic gas-phase sequenator (Rospert *et al.*, 1990).

Results

Purification of PSAT to apparent homogeneity

The purification of PSAT was started with the isolation of a $300\,000 \times g$ cell-free supernatant, which was applied to a Sephacryl S-300 column to detach high molecular weight protein aggregates, lipids and attached carotenoids. By the subsequent serial affinity column system nucleotide-dependent enzymes, which interfere with the employed combined assay for PSAT, are removed by their binding to Blue Sepharose and Red Matrex gel A. The *t*RNA-fraction and other contaminants bind to the Chlorophyllin Sepharose column (Wang *et al.*, 1981). The non-binding protein fraction (run-off column (ROC)-fraction) contains, besides other proteins, the PSAT activity. By the affinity columns the initial protein content of the $300\,000 \times g$ supernatant was reduced to about 36%. The crude cell-free supernatant itself converted NADH in high amounts, revealing the presence of considerable amounts of NADH-dependent enzymes. After passing the affinity columns a conversion of NADH by the ROC-fraction was not detectable, indicating that NADH-consuming enzymes were fully separated. From this fact it may be derived that the total PSAT-activity of the crude extract (0.009 μmol) possibly is overestimated in comparison to the ROC-fraction (0.014 μmol).

By further purification on DEAE cellulose remaining carotenoids and further proteins were separated, reducing the total protein to about 3.6% of the starting material. PSAT activity was enriched 12-fold. The following gel filtration step on Fractogel HW-55 decreased total proteins to 2.3%, yielding a 15-fold enrichment of the active enzyme. In the next purification step on Fractogel DEAE a gradient identical to the one described for DEAE cellulose was employed. Due to the applied lower pro-

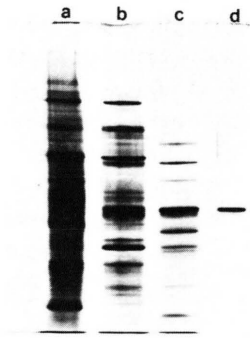

Fig. 1. Analysis of the protein fractions of different purification steps by SDS-polyacrylamide gel electrophoresis. a: ROC fraction; b: DE 52-Cellulose fraction; c: Fractogel-DEAE fraction; d: Mono Q fraction.

tein amount and a higher resolution of this matrix a further strong reduction of the total protein amount to 0.06% was achieved (Fig. 1). PSAT activity was enriched 118-fold by this procedure. The final purification step was FPLC-fractionation on Mono Q. Two major protein peaks were detected showing PSAT activity only in the second peak eluting at a salt concentration of 0.4 M NaCl. A total amount of 0.45 mg of purified enzyme was collected and an overall enrichment of 1538-fold concerning PSAT activity was achieved. A compilation of the purification steps is given in Table I.

Determination of the molecular mass of PSAT

Both, the apparent molecular mass (*M*r) of the denatured protein and the *M*r of the native PSAT, were determined. For the determination of the apparent molecular mass of the native enzyme gel filtration on Fractogel HW-55 was employed using 1 ml of the DEAE cellulose fraction (Fig. 2 B). A molecular mass of 80 ± 5 kDa was determined. SDS-

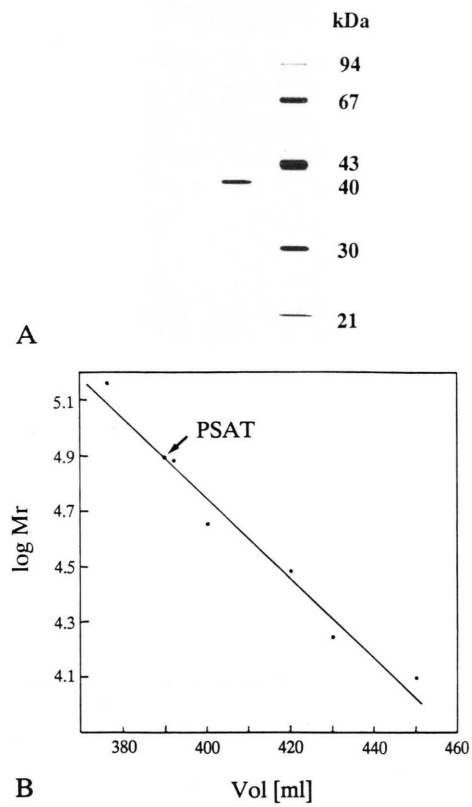

Fig. 2. A. Determination of the *M*r of purified phosphoserine aminotransferase from *Scenedesmus* by SDS-polyacrylamide gel electrophoresis. B. Determination of the native *M*r of PSAT by gel filtration on Fractogel-HW 55.

polyacrylamide gel electrophoresis of the purified enzyme revealed an *M*r of 40 kDa for the denatured protein (Fig. 2 A). From these results we conclude that PSAT is a homodimer with identical subunits of an *M*r of 40 kDa.

Table I. Procedure for the purification of phosphoserine aminotransferase. Activity and specific activity are indicated in units (U = μmol × min^{-1}).

	Total volume [ml]	Total protein [mg]	Total activity [U]	Specific activity [U × mg^{-1}]	Enrichment factor	Recovery [%]
Cell-free fraction	125.0	4091.00	36.98	0.009	1.0	100
ROC fraction	45.0	1485.00	21.22	0.014	1.6	57
DEAE cellulose	3.3	149.00	15.81	0.106	11.8	43
Fractogel-HW 55	19.0	94.00	12.46	0.133	14.8	34
Fractogel-DEAE	41.0	4.60	4.88	1.061	117.9	13
Mono Q	14.8	0.45	6.23	13.844	1537.7	17

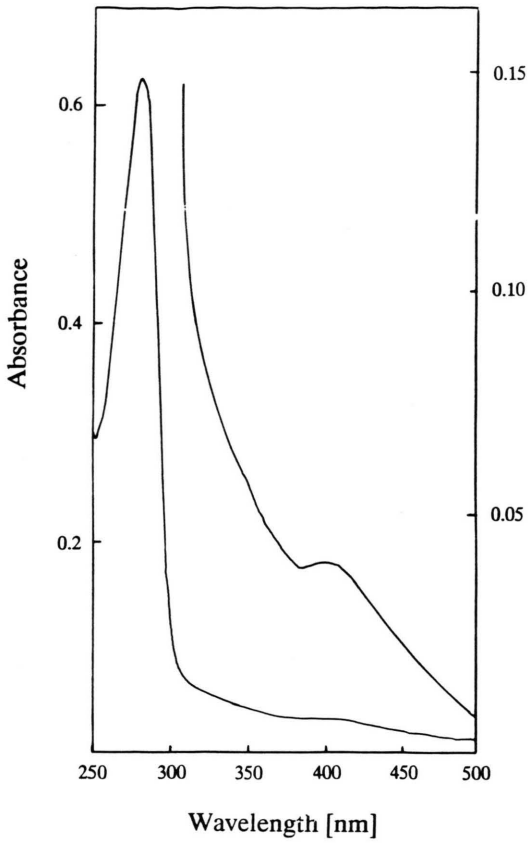

Fig. 3. Absorption spectrum of purified phosphoserine aminotransferase (0.8 mg/ml), demonstrating the presence of a pyridoxal-phosphate cofactor (400 nm).

Cofactor requirements

As transaminases usually require pyridoxal-/pyridoxamine-5-phosphate as cofactor (Christen *et al.*, 1985) the requirement of the purified PSAT for these cosubstrates was studied. Enzyme assays were performed with or without a preincubation period of 1 h with 25 μM PLP or 25 μM PMP, respectively. In both series of experiments only a very slight stimulation by the cofactors was observed. To possibly visualize the bound cofactor, absorption spectra of the purified enzyme were recorded (Fig. 3). The absorption maximum in the region of 390–410 nm indicates the presence of PLP in the native PSAT.

Determination of the N-terminal amino acid sequence

Partial N-terminal amino acid sequencing was performed using an automatic gasphase sequenator. By this method the first 18 amino acids could be determined. The N-terminal sequence of PSAT from *Scenedesmus obliquus* is shown in Fig. 4. Comparison of this sequence with the aligned amino acid sequence of the *ser*C gene encoding PSAT revealed a 73% identity with the *ser*C gene of *Salmonella* (Griffin, 1990) and *Yersinia* (O'Gaora *et al.*, 1990). The identity with the *ser*C gene of *Escherichia coli* (Duncan *et al.*, 1986) and rabbit (Van der Zel *et al.*, 1989) was 67% and 39%, respectively. From these and the other results we conclude that the partially sequenced protein is a PSAT.

Discussion

PSAT activity was discovered in dark-grown cells of the yellow, light-dependent greening mutant C-2 A′ of *Scenedesmus obliquus* (9 nmol × min^{-1} × mg^{-1} protein) after illumination with white light for 6 h. In higher plants serine formation in considerable amounts takes place *via* photorespiration and subsequently *via* the glycolate pathway (Tolbert, 1980). In consequence, only minor enzyme activities for PSAT were observed in pea (leaves: 0.13, germinated cotyledons: not

```
EC   M A - - - Q I F N F S S G P A M L P A E V L K Q A Q Q E L D W
SG   M A - - - Q V F N F S S G P A M L P A E V L K L A Q Q E L R D
YE   M T - - - Q V Y N F S A G P A M L P V E V L R R A E Q E L R N
OC   M D S P R Q I V N F G P G P A K L P H S V L L E I Q K E L L D
SO       A C G R V F N F E A G P A M L P L E
```

Fig. 4. Comparison of the N-terminal amino acid sequence of phosphoserine aminotransferase from *Sceredesmus obliquus* (SO) with the aligned amino acid sequence for the translated *ser*C genes from *Escherichia coli* (EC), *Salmonella gallinarum* (SG), *Yersinia enterocolitica* (YE) and *Oryctolagus cuniculus* (rabbit, OC). Bold letters denote the positions where the observed amino acid is present in all of the sequences.

detectable, root apices: 0.12 nmol × min^{-1} × mg^{-1} protein, respectively; Walton *et al.,* 1986) and spinach (leaf extract: 1.7/3.7, chloroplast extract: 0.22/0.23 nmol × min^{-1} × mg^{-1} protein, respectively; Larsson *et al.,* 1979). In comparison to the above described values, *Scenedesmus* PSAT-activity (9 nmol × min^{-1} × mg^{-1} protein) was more similar to those of plant tissues associated with rapid cell proliferation (Pea: 11 d seed leaf: 4.7, 11 d apical meristem: 11.1 nmol × min^{-1} × mg^{-1} protein, respectively; Cheung *et al.,* 1968), as well as to those for legume nodules (18 d cytosol: 11.7, 18 d bacteroid: 8 nmol × min^{-1} × mg^{-1} protein, respectively; Reynolds *et al.,* 1986). This may be due to the lack of "photorespiration" of algae under normal CO_2 conditions (Tolbert, 1973).

The enzyme from *Scenedesmus* was purified to apparent homogeneity and enriched 1538-fold compared to the initial cell-free homogenate with a total recovery of 17%. The final specific activity of 13.84 μmol × min^{-1} × mg^{-1} protein is comparable to those from soybean root nodules (7.00 μmol × min^{-1} × mg^{-1} protein; Reynolds, 1988), from bovine liver (13.2 μmol × min^{-1} × mg^{-1} protein; Lund *et al.,* 1987) and sheep brain (1.3 μmol × min^{-1} × mg^{-1} protein; Hirsch *et al.,* 1967). Cited values are adjusted to the unit definition used in this paper to make activities comparable.

By investigations on the molecular mass (*M*r) of PSAT, 80 ± 5 kDa for the native protein and 40 kDa for one protein subunit were determined, revealing the typical homodimer structure which is quite common for aminotransferases (Christen, 1985). Similar values were reported for the native enzyme of the plant fraction from soybean nodules (85 ± 5 kDa; Reynolds *et al.,* 1988), for the calculated *M*r of the *Escherichia coli* enzyme (SU = 39.8 kDa; from the derived amino acid sequence of the *ser*C gene; Kallen *et al.,* 1987) and 84 ± 4 kDa for the dimeric *Escherichia coli* enzyme (Kallen *et al.,* 1987). However, compared to the plant enzyme, the *M*r of the mammalian enzymes (bovine liver: SU = 43 kDa, dimer = 89 – 92.4 kDa, Lund *et al.,* 1987; sheepbrain: native *M*r = 96 kDa, Hirsch *et al.,* 1967) is considerably higher.

For the *Scenedesmus* enzyme an absorption maximum of the purified PSAT around 400 nm was determined at pH 7.6, possibly indicating the presence of PLP in the native phosphoserine aminotransferase. This is in agreement with the purified enzyme from a PSAT-overexpressing *Escherichia coli* strain (Duncan *et al.,* 1986) showing an absorption maximum at 408 nm (above pH 7.5) and at 340 nm (above pH 8.4; Kallen *et al.,* 1987). By N-terminal sequencing we could demonstrate that the N-terminus of the purified PSAT is identical to conserved regions of the first aligned amino acids from the *ser*C gene encoding PSAT from bacterial organisms (Duncan *et al.,* 1986; Griffin, 1990; O'Gaora *et al.,* 1991; Griffin *et al.,* 1991) as well as from rabbit (Van der Zel *et al.,* 1989).

Forthcoming work will focus on kinetic studies, inhibitory experiments and the primary structure of PSAT from *Scenedesmus obliquus.*

Acknowledgements

The authors are indebted to the Deutsche Forschungsgemeinschaft for financial support. Furthermore, we acknowledge the help and the intensive discussion by Prof. Dr. H. Senger, head of our laboratory. For the engaged performance of protein sequencing we thank Dr. D. Linder, Universität Gießen, F.R.G.

Bishop, N. I. (1971). Preparation and properties of mutants: *Scenedesmus.* In: Methods in Enzymology (S. Colowick and N. Kaplan, eds.) Vol. *23,* part A, p. 130–143, Acad. Press, New York.

Bishop, N. I. and Senger, H. (1972). The development of structure and function in chloroplasts of greening mutants of *Scenedesmus.* II. Development of the photosynthetic apparatus. Plant Physiol. *13,* 937–953.

Blum, H., Beier, H., and Gross, H. J. (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis *8,* 93–99.

Cheung, G. P., Rosenblum, I. Y., and Sallach, H. J. (1968). Comparative studies of enzymes related to serine metabolism in higher plants. Plant Physiol. *43,* 1813–1820.

Christen, P. and Metzler, D. E. (1985). Transaminases (Wiley Publishers) Chichester/New York.

Duncan, K. and Coggins, J. R. (1986). The *ser*C–*aro*A operon of *Escherichia coli.* Biochem. J. *234,* 49–57.

Griffin, H. G. and Griffin, A. M. (1991). Cloning and DNA sequence analysis of the *ser*C–*aro*A operon from *Salmonella gallinarum;* evolution between the prokaryotic and eukaryotic *aro*A-encoded enzymes. J. Gen. Mol. *137,* 113–121.

Griffin, H. G. (1990). Nucleotide sequence of the *Salmonella ser*C gene. Nuc. Ac. Res. *18,* 4260.

Hirsch, H. and Greenberg, D. M (1967). Studies on phosphoserine aminotransferase of sheep brain. J. Biol. Chem. *242,* 2283–2287.

Hurst, R. D., Lund, K., and Guynn, R. W. (1981). Enzymatic assays of L-serine in biological material. Anal. Biochem. *117,* 339–345.

Ichihara, A. and Greenberg, D. M. (1957). Further studies on the pathway of serine formation from carbohydrates. J. Biol. Chem. *224,* 331–340.

Kallen, J., Kania, M. Markovic-Housley, Z., Vincent, M. G., and Jansonius, J. N. (1987). Crystallographic and solution studies on phosphoserine aminotransferase (PSAT) from *E. coli,* in: Biochemistry of Vitamin B_6, p. 157–160, Birkhäuser Verlag, Basel.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of *bacteriophage T4.* Nature *227,* 680–685.

Larsson, C. and Albertson, E. (1979). Enzymes related to serine synthesis in spinach chloroplasts. Physiol. Plant. *45,* 7–10.

Lund, K., Merrill, D. K., and Guynn, R. W. (1987). Purification and properties of phosphoserine aminotransferase from bovine liver. Arch. Biochem. Biophys. *254,* 319–328.

O'Gaora, P., Maskell, D., Coleman, D., Cafferkey, M., and Dougan, G. (1989). Cloning and characterization of the *ser*C and *aro*A genes of *Yersinia enterocolitica,* and construction of an *aro*A mutant. Gene *84,* 23–30.

Reynolds, P. H. S. and Blevins, D. G. (1986). Phosphoserine aminotransferase in soybean root nodules. Plant Physiol. *81,* 293–296.

Reynolds, P. H. S., Hine, A., and Rodber, K. (1988). Serine metabolism in legume nodules: Purification and properties of phosphoserine aminotransferase. Physiol. Plant. *74,* 194–199.

Rospert, S., Linder, D., Ellermann, J., and Thauer, R. K. (1990). Isolation and characterization of polyferredoxin from *Methanobacterium thermoautothrophicum.* Eur. J. Biochem. *194,* 871–877.

Schmidt, L. S. and Sojka, G. A. (1973). Enzymes of serine biosynthesis in *Rhodopseudomonas capsulata.* Arch. Biochem. Biophys. *159,* 475–482.

Senger, H. and Mell, V. (1977). Preparation of photosynthetically active particles from synchronized cultures of unicellular green algae. In: Methods in Cell Biology *XV,* p. 201–219 (D. M. Prescott, ed.), Acad. Press, New York and London.

Servaites, J. C. and Ogren, W. L (1977). Chemical inhibition of the glycolate pathway in soybean leaf cells. Plant Physiol. *60,* 461–466.

Snell, K. (1986). The duality of pathways for serine biosynthesis is a fallacy. Trends Biochem. Sci. *11,* 241–243.

Tolbert, N. E. (1980). Photorespiration. In: The Biochemistry of Plants, Vol. *2,* p. 487–523, Academic press, Inc.

Van der Zel, A., Lam, H.-M., and Winkler, M. E. (1989). Extensive homology between the *Escherichia coli* K-12 *Ser*C(PdxF) aminotransferase and a protein encoded by a progesterone-induced mRNA in rabbit and human endometria. Nuc. Ac. Res. *17,* No. 20, 8379.

Walsh, D. A. and Sallach, H. J. (1966). Comparative studies on the pathway for serine biosynthesis in animal tissues. J. Biol. Chem. *241,* No. 17, 4068–4076.

Walton, N. J. and Woolhouse, H. W. (1986). Enzymes of serine and glycine metabolism in leaves and non-photosynthetic tissues of *Pisum sativum* L. Planta *167,* 119–128.

Wang, W.-Y., Gough, S. P., and Kannangara, C. G. (1981). Biosynthesis of δ-aminolevulinate in greening barley leaves. IV. Isolation of three soluble enzymes required for the conversion of glutamate to δ-aminolevulinate. Carlsberg Res. Comm. *46,* 243–257.