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Some structural properties of plant serine:glyoxylate aminotransferase*,*

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The structural properties of photorespiratory serine:glyoxylate aminotransferases (SGAT, EC 2.6.1.45) from maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) leaves were examined. By means of molecular sieving on Zorbax SE-250 column and filtration through centrifugal filters it was shown that dimers of wheat enzyme (molecular mass of about 90 kDa) dissociate into component monomers (molecular mass of about 45 kDa) upon decrease in pH value (from 9.1 or 7.0 to 6.5). At pH 9.1 a 50-fold decrease of ionic strength elicited a similar effect. Under the same conditions homodimers of the maize enzyme (molecular mass similar to that of the wheat enzyme) remained stable. Immunoblot analysis with polyclonal antiserum against wheat seedling SGAT on leaf homogenates or highly purified preparations of both enzymes showed that the immunogenic portions of the wheat enzyme are divergent from those of the maize enzyme. The sequence of 136 amino acids of the maize enzyme and 78 amino acids of the wheat enzyme was established by tandem mass spectrometry with time of flight analyzer. The two enzymes likely share similarity in tertiary and quaternary structures as well as high level of hydrophobicity on their molecular surfaces. They likely differ in the mechanism of transport from the site of biosynthesis to peroxisomes as well as in some aspects of secondary structure.

Keywords: maize, peroxisomes, photorespiration, serine:glyoxylate aminotransferase, structural properties, wheat

Recovery of carbon lost for photosynthesis due to the oxygenase activity of ribulose bisphosphate carboxylase/oxygenase (Rubisco) is a general role of photorespiration (Bauwe & Kolukisaoglu, 2003). Reactions that occur during photorespiration lead to transformation of two molecules of 2-phosphoglycolate (one of the two products of O_2 incorporation into ribulose 1,5-bisphosphate catalyzed by Rubisco) into 3-phosphoglycerate accompanied by release of CO_2 and NH_3 (Leegood *et al*., 1995). At the same time, photorespiration has been suggested to protect the plant against photoinhibition (Osmond *et al*., 1997). Taler *et al.* (2004) recently reported that two *Cucumis melo* genes encoding two peroxisomal photorespiratory glyoxylate aminotransferases confer resistance against an oomycete pathogen. At least six photorespiratory reactions occur in peroxisomes (Reumann, 2000), including two transaminations catalyzed by glutamate:glyoxylate (EC 2.6.1.4) and serine:glyoxylate (SGAT, EC 2.6.1.45) aminotransferases (Liepman & Olsen, 2001; 2003; Igarashi *et al*., 2003). We have investigated the biochemical characteristics of these two enzymes for several years in our laboratory (Orzechowski *et al*., 1999; Paszkowski, 1991; Paszkowski & Niedzielska, 1989; 1990; Truszkiewicz & Paszkowski, 2004). This report focuses on the relationship between the structure and function of SGAT from leaves of two plants from the grass family: maize and wheat. Chapple *et al.* (1990) hypothesized the ability of plant aminotransferases to dissociate into enzymatically active subunits as a general property, which could explain the contradictory reports on the oligomeric structure of plant SGAT molecule. Dimer (Paszkowski & Niedzielska, 1990; Liepman & Olsen, 2001), but also tetramer (Noguchi & Hayashi, 1980) were proposed as native SGAT forms. Only Hondred *et al.* (1985) observed dissociation of the serine:glyoxylate aminotransferase multimer into catalytically active subunits. The most thoroughly stud-

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*Accession numbers of protein sequences: Swiss-Prot P84187 and P84188.

Abbreviations: CFA, complete Freund's adjuvant; IFA, incomplete FA; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; SGAT, serine:glyoxylate aminotransferase; TFA, trifluoroacetic acid.

ied of the higher plant aminotransferases — aspartate aminotransferase – is known to be a dimer with two active sites both formed by amino-acid residues of each subunit, therefore only the non-dissociated (complexed) form of the enzyme molecule (dimer) is catalytically active (Wilkie *et al.,* 1996). The conformation and stability of SGAT may be important in the so-called "metabolon" which assumes the occurrence of peroxisomal photorespiratory enzymes in the form of a multienzyme complex able to channel the metabolites (Reumann, 2000). It should be emphasized that this hypothesis arose solely on the basis of kinetic studies (Heupel & Heldt, 1994; Reumann *et al.,* 1994). The fact that the tested enzymatic proteins originated from two species of plants that rely on different types of photosynthesis (wheat C_3, maize C_4) and thus with different photorespiration levels, could potentially account for the differences. Earlier we demonstrated that differences in pH optimum, thermal stability and kinetic constants also contributed to the differences observed (Truszkiewicz & Paszkowski, 2004). Glycolate oxidase – an important photorespiratory enzyme from a C_4 plant (*Amaranthus hypochondriacus*)-exhibited distinct kinetic properties as compared to the same enzyme from a C_3 plant (*Pisum sativum*) (Devi *et al.,* 1996). Here we present the results of studies on the structure of SGAT from maize and wheat by means of molecular sieving on Zorbax SE-250 column and Millipore centrifugal filters. Results of sequencing of both proteins by mass spectrometry, as well as immunochemical experiments using anti-wheat SGAT antibodies are also presented and discussed.

MATERIALS AND METHODS

Plant material. Maize (*Zea mays* L. cv. Duet) and wheat (*Triticum aestivum* L. cv. Jasna) seedlings were grown and harvested as described previously (Truszkiewicz & Paszkowski, 2004).

Purification of serine:glyoxylate aminotransferase from maize and wheat seedlings. The enzymes were purified using procedures described previously (Truszkiewicz & Paszkowski, 2004) to obtain Sephadex G-150 or HPLC fractions of specific activities of 2 U/mg protein (maize) and 2.5–5 U/mg protein (wheat) or 33 U/mg protein (maize) and 59.2 U/mg protein (wheat), respectively.

Native PAGE and detection of enzymatic activity in gels. Polyacrylamide gel (7.5%) was prepared in 50 mM Tris/glycine buffer, pH 9.1, containing 10% glycerol. Electrophoresis was run in the same buffer but without glycerol. Gels were stained for serine:glyoxylate aminotransferase activity according to Hatch and Mau (1972) with modifications described previously (Truszkiewicz & Paszkowski, 2004).

SDS/PAGE. Polyacrylamide gels were prepared and run according to Laemmli (1970). Protein bands were silver stained according to the method of Blum *et al.* (1987). The gels were calibrated with Bio-Rad low molecular mass markers (14.4–97.4 kDa).

Preparation of antibody. The preparation of SGAT from wheat after Sephadex G-150 step (Truszkiewicz & Paszkowski, 2004) was further purified by SDS/PAGE by Kucharczyk T.E.Co. (Warsaw, Poland). After electrophoresis proteins were transferred on PVDF membrane, stained with 0.1% Ponceau S; the band corresponding to SGAT was excised and the protein eluted at high pH (9.5) according to the method of Szewczyk and Summers (1988). Antiserum was raised by Kucharczyk T.E.Co. (Warsaw, Poland) by subcutaneously injecting the purified protein with CFA (the first injection) and two subsequent boosters with IFA at day 14 and 21. Approx. 600 μg of protein was used to immunize each of two New Zealand white rabbits.

Western blotting. The applied procedure was adapted from Towbin *et al.* (1979). After SDS/PAGE or native PAGE proteins were electrophoretically transferred (20 V, overnight) onto a nitrocellulose membrane using 25 mM Tris/glycine buffer, pH 8.3, in a Mini Trans-Blot Cell from Bio-Rad (Hercules, CA, USA) and stained with 0.1% Ponceau S. After soaking blots for 1 h at room temp. on a rocking platform in a blocking solution of 3% skim milk in a buffer containing 0.5 M NaCl and 50 mM Tris/HCl (pH 7.5) specific binding of the antigen immobilized on the membrane was carried out using SGAT antiserum diluted 1:250 in the same buffer with constant rocking at room temp for 1 h. Following a rinse in the 3% skim milk solution, immunodetection was performed using alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (Sigma-Aldrich Co., St. Louis, MI, USA) diluted 1:30000 in the same buffer.

Determination of molecular mass. This was determined on a Zorbax SE-250 (Dionex Co., Sunnyvale, CA, USA) column (9.4 × 250 mm) attached to an HPLC system (Waters, Milford, MA, USA) and equilibrated with 50 mM Mes buffer, pH 6.5, or 50 mM K-phosphate buffer, pH 7.0, both containing 0.3 M KCl. Fractions of 0.2 ml were collected at a rate of 0.5 ml/min. The column was calibrated with blue dextran 2000 (2000 kDa), bovine serum albumin dimer (134 kDa) and monomer (67 kDa), ovalbumin (43 kDa) and cytochrome *c* (12.2 kDa).

To evaluate approximately the molecular masses of the aminotransferases tested their preparations were exhaustively dialyzed against appropriate buffer and filtered through centrifugal filters NMWL 100000 purchased from Millipore Co. (Bedford, MA, USA).

Protein sequencing. SDS/PAGE (12% gel) of partially purified SGAT preparations from maize

and wheat (after Sephadex G-150 step) was run. The gel was stained with Coomassie Blue R-250 (Paszkowski & Niedzielska, 1989); the bands corresponding to SGAT were excised and put into destaining solution (10% acetic acid in 40% methanol). The protein present in one gel sample was reduced with 10 mM dithiothreitol for 30 min and alkylated with 55 mM iodoacetamide for 30 min at room temperature. Trypsin digestion was carried out for 12 h at 37°C using 12.5 ng/μl of trypsin. Chemical cleavage of peptide bonds was performed by applying CNBr in 70% formic acid for 12 h at room temperature in the dark. The peptides were washed out from the gel strips using 0.1% TFA with 2% acetonitrile. The MS/MS analysis with the use of ESI-Q-TOF (Micromass) was carried out in the Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Warsaw, Poland).

Determination of aminotransferase activities. Transaminations were performed at 30ºC in an incubation mixture containing in a volume of 0.65 ml 15.4 mM L-serine, 5 mM glyoxylate, 20 μM pyridoxal phosphate, 77 mM K-phosphate buffer, pH 8.0, and the enzymatic protein (1–150 μg). The enzyme was preincubated with the amino-acid substrate for 10 min. The reaction was started by addition of glyoxylate and stopped after 15 min by addition of 10% trichloroacetic acid. Hydroxypyruvate (product) was determined by a spectrophotometric method using NADH and lactate dehydrogenase (Paszkowski & Niedzielska, 1989).

Specific activity was expressed in μmol of product formed per minute at 30ºC (U) and per 1 mg of protein. Protein was determined according to Bradford (1976) with bovine serum albumin as a standard.

RESULTS

As shown in earlier studies (Truszkiewicz & Paszkowski, 2004), the molecular masses of serine:glyoxylate aminotransferases from maize and wheat determined by the SDS/PAGE appeared to be identical (45 kDa). The molecular mass of both enzymes under native conditions was determined on a Zorbax SE-250 column (Table 1). The behaviour of aminotransferase from wheat was clearly dependent on column buffer (Table 1). Chromatography course at pH 6.5 (Mes buffer) revealed the predominance of a monomeric form with a molecular mass of about 45 kDa, whereas at pH 7.0 (phosphate buffer) a dimer of about 90 kDa was detected (Table 1). This result suggested that the state of association–dissociation equilibrium of the enzyme was shifted towards association at the higher pH. Analogous experiments using the enzyme from maize unequivocally pointed to a lack of dependence on the buffer pH. The enzyme was eluted with almost the same elution volume from the Zorbax SE-250 column at pH 7.0 or pH 6.5. Similar molecular masses, comparable to those obtained for the wheat enzyme dimer were calculated at both these pH values (Table 1).

The results from the Zorbax SE-250 column were verified by filtration of enzyme solutions through centrifugal filters Millipore NMWL 100 000 permeable to proteins with molecular masses lower than 100 kDa. Such a filter would block or at least hinder the passage of an SGAT dimer, but allow a protein with molecular mass of about 45 kDa (approximately equal to the subunit mass of the enzyme) to freely diffuse through it. The SGAT protein passes through the filter, based on measuring the serine:glyoxylate enzyme activity in the concentrated solution and in the filtrate. Thus the filtration results confirmed the observations obtained by molecular sieving on a Zorbax SE-250 column. SGAT from wheat in solutions of a higher pH value (pH 9.1 – 50 mM Tris/glycine buffer) exhibited a behaviour consistent with a molecular mass of about 90–100 kDa (Table 2). This enzyme likely dissociated into two subunits at lower pH (6.5) (5 mM Mes buffer, Table 2). Upon a 50-fold reduction of the Tris/glycine buffer concentration (pH 9.1) from 50 mM to 1 mM (Table 2), the percentage of the enzyme activity found in the filtrate increased considerably. In the chromatography or filtration experiments (Tables 1 and 2) SGAT from maize always occurred in the form of a dimer, regardless of the pH applied (6.5, 7.0 or 9.1). The filtration of either enzyme was not associated with any loss of activity. Rabbit polyclonal antibodies against SGAT protein from wheat seedlings were prepared using SGAT protein band cut out from the gel after SDS/PAGE (Fig. 1) for immunization. Immunoblot analysis after SDS/PAGE was used to determine the immunological similarity between the SGAT proteins from wheat and maize leaves (Fig. 2). Because of reports on serine:glyoxylate activity in non-photosynthesizing plant organs (Roberts & Lees, 1997; Liepman & Olsen, 2001), root tissues of both plants were also examined (Fig. 2). A band corresponding to SGAT was observed in wheat

Table 1. Molecular sieving of partially purified serine: glyoxylate aminotransferase preparations from maize and wheat seedlings on Zorbax SE-250 column (9.4 × 250 mm).

Enzyme preparations after purification on Sephadex G-150 column were used. The column was calibrated as described in Materials and Methods.

Source of enzyme preparation	Column buffer	Molecular mass (kDa)
Maize	K-phosphate pH 7.0	105 ± 14
	Mes pH 6.5	79 ± 18
Wheat	K-phosphate pH 7.0	88 ± 10
	Mes pH 6.5	45 ± 8

Table 2. Filtration of partially purified serine:glyoxylate aminotransferase preparations from maize and wheat seedlings through NMWL 100 000 centrifugal filters.

Enzyme preparations after purification on Sephadex G-150 column were filtered.

Source of enzyme preparation	Filtration buffer	Activity (%)	
		Concentrated enzyme solution	Filtrate
Maize	5 mM Mes pH 6.5	100	0
	1 or 5 mM Tris/glycine pH 9.1	100	0
Wheat	5 mM Mes pH 6.5	50	50
	1 mM Tris/glycine pH 9.1	60	40
	50 mM Tris/glycine pH 9.1	87	13

leaves but not in roots (Fig. 2). Maize leaves or roots did not give such a band (Fig. 2).

The immunological similarities between the two leaf enzymes were also evaluated by immunoblot analysis of highly purified preparations of both aminotransferases (Truszkiewicz & Paszkowski, 2004) on native PAGE at pH 9.1 (Fig. 3). One part of the gel served for blotting with anti-SGAT (wheat) antiserum (Fig. 3A); the other half was stained for serine:glyoxylate activity (Fig. 3B) as described in Materials and Methods. A single band was visible at the level corresponding to the active enzyme from wheat (Fig. 3). In contrast to the immunoblot analysis on SDS/PAGE gels (Fig. 2), the band corresponding of the active maize enzyme appeared, however, indistinct and diffuse (Fig. 3).

The next stage of studies consisted in determination of the amino-acid sequence for both SGAT proteins. The primary structure of SGAT from leaves of both wheat and maize is unknown. Fragments of the sequence of these two enzymes were established by relating the results of mass spectrometric analysis with the known amino-acid sequence of SGAT from leaves of *Arabidopsis thaliana* (NCBI protein accession No. BAB20811). This provided an additional confirmation of the identity of the tested enzymatic proteins. A tandem mass spectrometer with a time of flight analyzer was used. About 3 μg of SGAT protein cut out from the gel after SDS/PAGE of a partially purified preparation (Fig. 1) was subjected to sequencing twice, first following cleavage with trypsin and also following cyanogen bromide cleavage. The results are presented as an alignment with *A. thaliana* SGAT (Fig. 4). For the maize enzyme, the sequence of 136 amino acids (Swiss-Prot accession No. P84187, and for wheat of 78 amino acids, P84188) were established, which can be estimated to constitute about 34% and 19%, respectively, of the entire sequences for these proteins (Fig. 4). The percentage of identity with the corresponding fragments of the known SGAT sequence from *A. thaliana* leaves

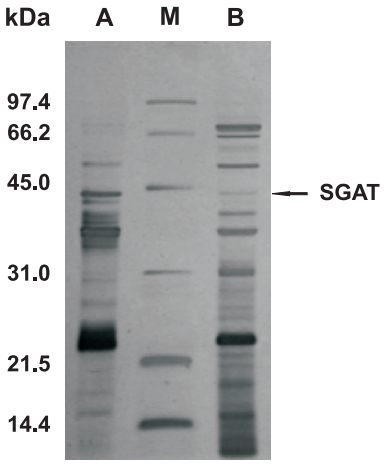

Figure 1. SDS-PAGE of partially purified serine:glyoxylate aminotransferase preparations from wheat and maize seedlings.

Lanes A and B, wheat (5 μg of protein loaded) and maize (7 μg of protein loaded) preparations, respectively, each after purification on Sephadex G-150 column; M, molecular mass (kDa) markers.

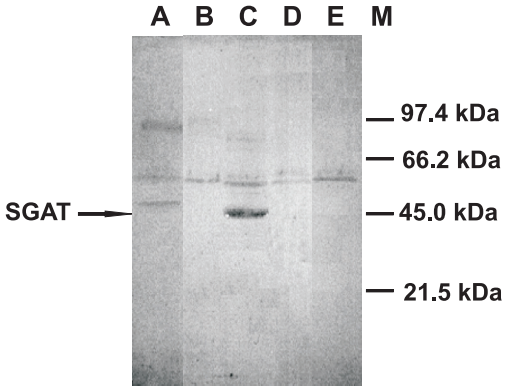

Figure 2. Western blot analysis after SDS/PAGE of leaf and root homogenates from wheat and maize.

Polyclonal rabbit antibodies raised against serine:glyoxylate aminotransferase from wheat leaves were used. Lanes: A, homogenate of wheat seedlings; B, homogenate of maize seedlings; C, partially purified serine:glyoxylate preparation (after chromatography on Sephadex G-150 column) from wheat used as a positive control; D, homogenate of wheat roots; E, homogenate of maize roots each lane was loaded with 5 μg of protein; M, molecular mass (kDa) markers.

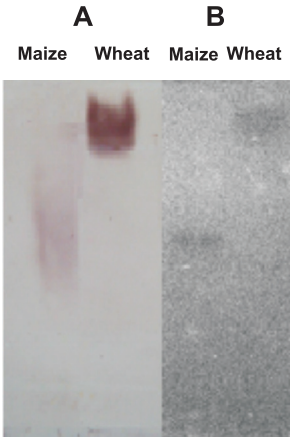

Figure 3. Western blot analysis after native PAGE at pH 9.1 of highly purified serine:glyoxylate aminotransferase from maize and wheat seedlings.

Preparations from maize (3 μg and 0.9 μg of protein) and wheat (3 μg and 0.5 μg of protein) after purification on HPLC column were loaded. Half of the gel was used for Western blottings (A with more protein loaded), the other was stained for serine:glyoxylate activity (B) as described under Materials and Methods. Polyclonal rabbit antibodies raised against serine:glyoxylate aminotransferase from wheat leaves were used.

(BAB20811 and 8 others from *Arabidopsis*, all with 100% of sequence identity) was evaluated for the established sequence fragments of SGAT from maize as well as wheat. The sequence fragments of SGAT from maize showed 89% sequence identity and the enzyme fragments from wheat 81% identity.

DISCUSSION

The results from chromatography and filtration suggest that the dimer of the wheat enzyme dissociates after lowering the pH from 9.1 or 7.0 to 6.5. Dissociation of the native multimer into subunits as a result of a similar pH change was reported for plant aspartate (Hart & Langston, 1977) and methionine:glyoxylate (Chapple *et al.*, 1990) aminotransferases. Our evidence further suggests that serine:glyoxylate aminotransferase from wheat is active in both dimeric and dissociated forms. We do not exclude the possibility of dimer dissociation at a lower pH (6.5) and subunit association at pH 8.0 during the activity assay. The literature data on animal (Kochkina, 2000) and plant aspartate aminotransferase (Wilkie *et al.*, 1996) as well as on the human ana-

	10	20	30	40	50	60		
A. thaliana	MDYMYGPGRH	HLFVPGPVNI	PEPVIRAMNR	NNEDYRSPAI	PALTKTLLED	VKKIFKTTSG	TP	62
maize	LDYVYGPGR		RAMN	SPAV	PALTKVLLED	VKK		
wheat		HLFVPGPVNI	PDQVLR		TLLED	VKK		
A. thaliana	FLFPTTGTGA	WESALTNTLS	PGDRIVSFLI	GQFSLLWIDQ	QKRLNFNVDV	VESDWGQGAN	L	123
maize		ALTNTLS	PGDR					
wheat								
A. thaliana	QVLASKLSQD	ENHTIKAICI	VHNETATGVT	NDISAVRTLL	DHYKHPALLL	VDGVSSICAL	DF	185
maize					LLL	VD		
wheat	LASRLRSD	SQHTIK		LL	DAYR			
A. thaliana	RMDEWGVDVA	LTGSQKALSL	PTGLGIVCAS	PKALEATKTS	KSLKVFFDWN	DYLKFYKLGT		245
maize	MDEWGVDVA	LTGSQKALSF	PTGMGLVCAS	PR	VFFDWK	DYLR T		
wheat					VFFDWK	DYLK		
A. thaliana	YWPYTPSIQL	LYGLRAALDL	IFEEGLENII	ARHARLGKAT	RLAVEAWGLK	NCTQKEEWIS	NT	307
maize	YWHYD	QALDL	E		LAVEAWGLS	N		
wheat								
A. thaliana	VTAVMVPPHI	DGSEIVRRAW	QRYNLSLGLG	LNKVAG KVF	RIGHLGNVNE	LQLLGCLAGV		366
maize			RYNLSLGLG	LNKVAGGKVF	R			
wheat				KVF	R NVNT	L		
A. thaliana	EMILKDVG YP	VVMGSGVAAA	STYLQHHIPL	IPSRI				401
maize	DVG YP	VK						
wheat	LKDLG YP	VK	PL	IPSR				

Figure 4. Sequence analysis of serine:glyoxylate aminotransferases from maize and wheat seedlings.

The determined fragments of maize and wheat enzyme sequence (Swiss-Prot accession Nos: P84187 and P84188, respectively) were aligned with the amino-acid sequence of serine:glyoxylate aminotransferase from leaves of *A. thaliana* (NCBI protein accession No. BAB20811).

logue of SGAT — alanine aminotransferase (Zhang *et al*., 2003) suggest that reassociation underlies the activity and that the monomeric enzyme is inactive. In the case of SGAT from maize, the results of molecular sieving and experiments applying Millipore filters did not depend on the pH value within the range tested. It was earlier found by us (Truszkiewicz & Paszkowski, 2004) that under denaturing conditions (SDS/PAGE) the molecular mass of the enzyme was about 45 kDa, thus a stable homodimer of about 90 kDa is suggested to be the native form of SGAT from maize. The differences in stability between SGAT dimers from maize and wheat leaves may result from the different ways of transport of those proteins from the biosynthesis site to the peroxisomal matrix. Subramani (2002) described three different mechanisms of oligomeric protein transport through biological membranes. Only one of them requires the presence of pores in a membrane determining the limitations to the size of the transported protein molecule (Subramani, 2002). Wilkie *et al*. (1996) suggested that the N-terminal transit peptide might inhibit chloroplastic aspartate aminotransferase dimerization until the enzyme is translocated into chloroplasts. The easier diffusion through a Millipore filter of the enzyme from wheat as a result of buffer concentration decrease may be the effect of conformation changes under such conditions leading, to the dissociation of dimers. Low and Somero (1975a; 1975b) reported that the presence of salt disrupts the dense hydration spheres around the exposed protein surface groups, which leads to either an increase or decrease of the enzyme volume. Teipel and Koshland (1971) examined the kinetic aspects of conformation changes in six oligomeric enzyme proteins. They concluded that the change in the ionic strength of the medium, among other factors (the presence of substrate or cofactor, protein concentration), influenced the regaining of the native conformation, however, the direction and magnitude of the effect varied from enzyme to enzyme.

Some information on the immunogenic properties of both aminotransferases was supplied by the immunological studies. After SDS/PAGE, the wheat enzyme partially renatured during the transfer onto the PVDF membrane (Szewczyk & Summers, 1988) was used for rabbit immunization. It is generally assumed that a secondary structures are reconstituted in such a preparation (Szewczyk & Summers, 1988). The lack of an immunological response towards SGAT in maize homogenate subjected to SDS/PAGE points out that the linear epitops dependent on the preservation of the secondary structure of the protein are different in both enzymes. Immunological detection of native, purified maize SGAT suggests similarities between these proteins. Most probably the smeared band on nondenaturing PAGE resulted from nonspecific background. We reported earlier (Truszkiewicz & Paszkowski, 2004), that a highly purified maize enzyme preparation contained about 9% unrelated proteins.

The antibodies against SGAT from wheat leaves were also used to test the maize and wheat root tissues towards the presence of related proteins. Plant aminotransferase tissue or subcellular localization — specific isoenzymes are common (Ireland & Lea, 1999). Furthermore, Roberts and Lees (1997), as well as Liepman and Olsen (2001) reported that SGAT activity can be detected in non-photosynthesizing tissues. Our results (not shown) pointed out to the presence of low serine : glyoxylate activity in wheat root homogenate. The lack of an immunological response for wheat root homogenate confirms our earlier thesis on a strict relation of SGAT to photorespiration (Truszkiewicz & Paszkowski, 2004).

That average homology level for full sequences of putative SGAT from three higher plants: *Cucumis melo*, *Fritillaria agrestis* and *Oryza sativa* leaves (NCBI protein accession Nos: AAL47679; AAQ56192; AAQ56195; AAQ56194; AAQ56193; AAL62332; AAB95218; XP507283; XP483211; BAD09269; BAD08917) with the full sequence of the same enzyme from *A. thaliana* leaves (BAB20811) was 87% (average result of BLAST analysis), makes our calculation of 89% homology for the maize enzyme and 81% for its wheat homologue, based only on fragments of the sequence, more credible. According to Hrmova and Fincher (2001), even 25–30% homology between plant protein sequences containing 100 or more amino-acid residues is sufficient for accepting the thesis that they have the same activity and similar stuctures. It is a well known rule that a protein's function often has a great impact on its conformation (Voet & Voet, 1995). According to John (1995), a large group of catalytic proteins (α-family) distinguished among enzymes cooperating with pyridoxal phosphate, in which most of transaminases (also SGAT) are included, use similar catalysis mechanisms and, despite low homology of amino-acid sequences, show great similarities of three-dimensional structure.

The high hydrophobicity of the protein molecule, particularly of its surface, favours the formation of complexes with other proteins. The mean hydropathy index (GRAVY score) of the amino-acid sequence, calculated according to the method of Kyte and Doolittle (1982), may be an approximate measure of such hydrophobicity. For SGAT from *A. thaliana* leaves localized in peroxisomes (Liepman & Olsen, 2001) and most probably showing high homology with the enzymes we studied, the index (0.023) appeared to be distinctly above the average value (–0.4) calculated for 84 fully sequenced soluble enzymes (Kyte & Doolittle, 1982). The exceptional stability of SGAT both from maize and wheat leaves in 60% acetone solution found by us earlier (Trusz-

kiewicz & Paszkowski, 2004) suggests high hydrophobicity of their surfaces. Those two facts support the hypothesis on the occurrence of photorespiratory peroxisomal enzymes in the form of a multienzyme complex (Reumann, 2000).

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