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Communication

A Mutant of *Nicotiana sylvestris* Lacking Serine:Glyoxylate Aminotransferase

SUBSTRATE SPECIFICITY OF THE ENZYME AND FATE OF [2-^{14}C]GLYCOLATE IN PLANTS WITH GENETICALLY ALTERED ENZYME LEVELS

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ABSTRACT

The photorespiratory mutant of *Nicotiana sylvestris*, NS 349, lacking serine:glyoxylate aminotransferase (SGAT) grows in 1% CO_2 but not in normal air (NA McHale, EA Havir, I Zelitch 1988 Theor Appl Genet. In press). Alanine:hydroxypyruvate and asparagine:hydroxypyruvate aminotransferase activities were also lacking in the mutant, and plants heterozygous with respect to SGAT which grow in normal air had 50% of the activities present in homozygous plants. Therefore, all these activities are associated with the same enzyme. On feeding [2-^{14}C]glycolate to leaf discs in the light, NS 349 showed reduced incorporation of radioactivity into the neutral and organic acid fractions and increased incorporation into the amino acid fraction, principally into serine. The effect of reducing SGAT by 50% in heterozygous plants produced little change in the metabolism of [2-^{14}C]glycolate, showing there is a large excess of this enzyme in wild-type plants.

The isolation and preliminary characterization of a photorespiratory mutant of *Nicotiana sylvestris*, NS 349, incapable of surviving in air and deficient in SGAT[1] has been described (8). The deficiency was due to a single recessive mutation. Callus cultures of the mutant were regenerated into plantlets which did not contain SGAT activity and could not survive in air. Mutants lacking SGAT activity have also been described in *Arabidopsis* (15) and barley (9), both of which accumulate serine under photorespiratory conditions. The inability of these mutants to survive in air has been attributed to the block in recycling of carbon to carbohydrate and starch (9, 15). Although attempts to employ the *Arabidopsis* mutant as a vehicle for whole-plant selection of low photorespiration mutants were unsuccessful (15), the SGAT mutant of tobacco offers the first opportunity to apply the techniques of somatic cell genetics in schemes designed to recover chloroplast mutations affecting ribulose bisphosphate carboxylase/oxygenase (8).

In addition, SGAT mutants of tobacco are potentially useful in two other ways. First, the substrate specificities of the aminotransferases are complex and a survey of activities in the mutant allows a conclusion to be reached about the activities associated with a single protein. These deductions would supplement those derived from studies of purified proteins (5, 11) and other methods (10). Second, mutations producing specific biochemical lesions permit direct examination of the interaction of the photorespiratory cycle with other metabolic pathways. The availability in this study of plant material which is heterozygous for the mutation (sat^+/sat^-) (8) provides an opportunity to study the effect on metabolism of reducing the enzyme level by 50%.

[1] Abbreviations: SGAT, serine:glyoxylate aminotransferase; AT, aminotransferase; GGAT, glutamate:glyoxylate aminotransferase.

MATERIALS AND METHODS

Plant Material. The techniques of mutagenesis and screening have been described (8). Mutant plants (NS 349) of *Nicotiana sylvestris* and wild-type controls (not exposed to ethyl methane sulfonate) were grown and maintained under an atmosphere of 1% CO_2/21% O_2 for 3 to 4 weeks. Normal M_2 siblings of the mutant were grown to maturity in the greenhouse and identified as homozygous (sat^+/sat^+) or heterozygous (sat^+/sat^-) by analysis of selfed M_3 progeny (8).

Enzyme Assays. Leaf tissue (0.75 g) was ground in a glass homogenizer with 5 ml cold 0.05 M K-phosphate (pH 7.4), containing 1.5 mg ml^{-1} DTT. After centrifuging the suspension for 10 min at 27,000*g*, the clear supernatant was decanted and passed through a column (2.5 × 8.0 cm) of G-25 Sephadex which had been equilibrated with 0.05 M K-phosphate (pH 7.4). The enzyme was eluted from the column with the same buffer. Aspartate:α-ketoglutarate AT was assayed spectrophotometrically (13). All other ATs were assayed by an HPLC method (4) by incubating 10 μmol amino donor, 1.5 μg pyridoxal 5-P, 0.10 ml G-25 eluate, and 2.5 μmol α-keto acid (total volume 0.25 ml) for 30 to 60 min. The reaction was stopped by heating the mixture in a boiling water bath for 2 min. Determination of the α-keto acid produced enzymically (*i.e.* hydroxypyruvate, α-ketoglutarate, or pyruvate) was by HPLC under conditions described previously (2). The amino acid produced in the reaction (*i.e.* glycine, alanine, or serine) was derivatized according to the general procedures of Jones *et al.* (7). A portion (15 μl) of the reaction mixture (after deproteinization) was reacted with 15 μl *o*-phthaldialdehyde reagent solution (Sigma Chemical Co.) for 1 min, and then 20 μl of the derivatized sample was separated by HPLC on a Spherisorb column (LDC/Milton Roy). The elution buffer was 80% solvent A (1% tetrahydrofuran, 19% methanol, 80% 0.05 M Na acetate, pH 5.9) and 20% solvent B (80% methanol, 20% 0.05 M Na acetate, pH 5.9). Peak areas of the derivatized amino acids were proportional to concentration. Controls with boiled enzyme were used to correct for nonen-

Table I. *Aminotransferase Activities in NS 349 and Its Heterozygous (sat⁺/sat⁻) Siblings*

Activity in wild-type plantlets grown in 1% CO_2 (0.79, 0.19, 0.11, 0.21, 0.82, 0.43 μmol min^{-1} g^{-1} fresh weight for the activities listed in order below) served as control for NS 349. Activity in homozygous plants (mature leaves, greenhouse grown) (1.6, 0.45, 0.18, 0.42, 1.2, 0.82 μmol min^{-1} g^{-1} fresh weight for the activities listed in order below) served as control for heterozygous (sat^+/sat^-) plants. Results are the averages of 3 to 5 determinations.

Aminotransferase Activity	NS 349	sat^+/sat^-
	% of control	
Serine:glyoxylate	0	57
Alanine:hydroxypyruvate	0	40
Asparagine:hydroxypyruvate	0	55
Alanine:glyoxylate	95	85
Glutamate:glyoxylate	105	80
Aspartate:α-ketoglutarate	120	92

zymic reactions. In no instance was the correction greater than 10% of the measured activity.

[2-^{14}C]Glycolate Feeding Experiments. The exposure of six leaf discs (1.2 cm) of NS 349, wild-type, or (sat^+/sat^+) and (sat^+/sat^-) plants to 10 mM [2-^{14}C]glycolate (ICN Radiochemicals, Irvine, CA) (4.13 × 10^5 dpm μmol^{-1}) was carried out in air in the light at 350 μE m^{-2} s^{-1} for 60 min at 30°C as previously described (8). After homogenization of each sample in 20% ethanol the samples were separated into neutral, organic acid, amino acid, and strong acid fractions by the procedure of Redgwell (12). The neutral compounds and organic acids were analyzed by HPLC (2, 3), and amino acids were determined as described above.

RESULTS AND DISCUSSION

Aminotransferase Activities in Wild-Type *N. sylvestris*. The most active ATs in both mature greenhouse-grown leaves and plantlets grown in 1% CO_2 are SGAT and GGAT. Values for these activities (μmol min^{-1} g^{-1} fresh weight) were 1.1 to 2.1 and 0.6 to 1.6, respectively, for the normal homozygous (sat^+/sat^+) plants, and 0.68 to 0.86 and 0.68 to 0.97, respectively, for wild-type plantlets grown in 1% CO_2. Levels of AT activities for wild type were similar whether plants were grown in 1% CO_2 or air. Other activities measured as percentages of the average SGAT values (for both plantlets and mature plants) were asparate:α-ketoglutarate AT, 51 to 55; alanine:hydroxypyruvate AT and alanine:glyoxylate AT, 23 to 30; asparagine:hydroxypyruvate AT, 10 to 15. In the tobacco extracts, serine:pyruvate AT was not detected although its activity is at least 30% of SGAT in barley leaf extracts (9) and 8% in pea leaves (6). Asparagine:glyoxylate AT also was not detected although its activity is 38% of SGAT activity in pea leaf preparations (6).

Aminotransferase Activities Associated with SGAT. We have shown that SGAT is absent in NS 349, and plants heterozygous for the mutation possess half the normal activity of the enzyme, although four other peroxisomal enzymes are present at normal levels in the mutant (8). Other workers have concluded that activities associated with SGAT in other species are serine:pyruvate (14), asparagine:glyoxylate or pyruvate (6), alanine:hydroxypyruvate (1), and alanine:glyoxylate (5, 11) ATs. In Table I the change in activities of a number of ATs are summarized for NS 349 and (sat^+/sat^-) plants. Those activities associated with SGAT are asparagine:hydroxypyruvate and alanine:hydroxypyruvate ATs since both are absent in NS 349 and are decreased about 50% in (sat^+/sat^-) plants. Preparations of SGAT purified to electrophoretic homogeneity from spinach leaves (11) and cucumber cotyledons (5) catalyzed an alanine:glyoxylate AT reaction, leading to the conclusion that both activities reside on the same protein. However, mutants of barley (9), *Arabidopsis* (15), and tobacco (Table I) lacking SGAT have normal activities of alanine:glyoxylate AT. Identifical results associated with SGAT mutations recovered independently in three plant species constitute strong evidence that these enzyme activities are coded by separate genes.

Metabolism of [2-^{14}C]Glycolate. In Table II the distribution of radioactivity in leaf discs of NS 349, wild-type, homozygous (sat^+/sat^+), and heterozygous (sat^+/sat^-) siblings after exposure to [2-^{14}C]glycolate is shown. The reduction of SGAT by 50% in the heterozygous plants or its absence in NS 349 had no effect on the uptake or metabolism of glycolate compared to wild type or homozygous controls. However, the absence of SGAT activity in NS 349 altered the distribution of radioactivity considerably. There was a decrease in radioactivity into the neutral fraction from 33% (wild type) to 2% (NS 349). Radioactivity in organic acids also decreased in the mutant to 9%, whereas it was 30% of the total in wild type. Glyoxylate was the only organic acid labeled in leaf discs of NS 349, but malate and glycerate contained about 4 times as much radioactivity as glyoxylate in wild type. In tobacco leaf discs in light, malate is quickly labeled from [^{14}C]oxalate and $^{14}CO_2$ (3), and [1-^{14}C]- and [2-^{14}C]glyoxylate (our unpublished results). The lack of [^{14}C]malate formation and the reduction of label in the neutral fraction indicate that carbon from glycolate is not readily recycled into other metabolic pathways in this mutant. The increase in radioactivity into the amino acid fraction in NS 349 was primarily caused by an increase in serine labeling. At the same time the mutant showed an eightfold increase in the pool size of serine compared to the wild type (7.7 *versus* 0.98 μmol g^{-1} fresh weight) when both were grown in 1% CO_2. Leaf discs from the heterozygous plant had a fourfold increase in serine over the homozygous plants (2.2 *versus* 0.5 μmol g^{-1} fresh weight), but it was not matched by a larger proportion of the incorporated radioactivity. There were no significant differences in the pool size of glycine in any of these

Table II. *Metabolism and Distribution of Radioactivity from [2-^{14}C]Glycolate Supplied to Leaf Discs of Wild-Type, NS 349, and Its Heterozygous (sat⁺/sat⁻) and Homozygous Normal (sat⁺/sat⁺) Siblings*

Wild-type and NS 349 plantlets were grown in 1% CO_2; homozygous (sat^+/sat^+) and heterozygous (sat^+/sat^-) plants were grown in the greenhouse. Exposure to 1.2 ml [2-^{14}C]glycolate (4.13 × 10^5 dpm μmol^{-1}) was for 1 h in the light (350 μE m^{-2} s^{-1}) in air. Total radioactivity incorporated by wild-type, NS 349, sat^+/sat^+, and sat^+/sat^- leaf discs was 1.04, 1.02, 1.18, 1.00 × 10^5 dpm, respectively. Results shown are the average of two experiments.

Plant Material	[2-^{14}C]Glycolate Metabolized	Neutral Fraction			Organic Acids			Amino Acids		Strong Acids
		Sucrose	Glucose	Fructose	Glyoxylate	Malate	Glycerate	Glycine	Serine	
	µmol/g fresh wt	*% of total radioactivity incorporated*								
Wild type	0.251	24.0	4.8	4.1	6.3	13.8	9.9	7.7	16.3	12.0
NS 349	0.247	1.4	0.4	0.3	9.1	0	0.1	9.1	73.9	5.4
sat^+/sat^+	0.285	26.9	3.4	2.7	8.4	16.7	5.9	5.5	8.0	15.0
sat^+/sat^-	0.242	23.4	3.0	2.4	10.7	17.4	8.9	2.9	11.3	12.0

plants (~0.3 μmol g^{-1} fresh weight for the mutant and wild-type plantlets grown in 1% CO_2 and ~0.2 μmol g^{-1} fresh weight for greenhouse-grown and heterozygous plants).

The results presented here for tobacco leaf discs supplied [2-^{14}C]glycolate are similar to those obtained with a barley mutant lacking SGAT which was supplied [2-^{14}C]gloxylate (9). In barley there was also a decrease in labeling of the neutral fraction and an increase in the amino acid fraction. However, the barley mutant displayed increased incorporation of radioactivity into the organic acid fraction and specifically into a compound which was tentatively identified as citric or malic acid. As discussed above, malate was readily labeled in leaf discs of wild-type tobacco but not in NS 349.

In general, there were only minor differences in the distribution of radioactivity from [2-^{14}C]glycolate in the homozygous and heterozygous plants. The similarity in metabolism of [2-^{14}C] glycolate by wild-type and heterozygous plants which have 50% of the normal SGAT activity, as well as the ability of the heterozygous plants to grow in normal air, demonstrates that there is a large excess of SGAT activity in wild-type plants.

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