Cellular Sterol Ester Synthesis in Plants Is Performed by an Enzyme (Phospholipid:Sterol Acyltransferase) Different from the Yeast and Mammalian Acyl-CoA:Sterol Acyltransferases*

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A gene encoding a sterol ester-synthesizing enzyme was identified in Arabidopsis. The cDNA of the Arabidopsis gene At1g04010 (AtPSAT) was overexpressed in Arabidopsis behind the cauliflower mosaic virus 35S promoter. Microsomal membranes from the leaves of overexpressor lines catalyzed the transacylation of acyl groups from phosphatidylethanolamine to sterols. This activity correlated with the expression level of the AtPSAT gene, thus demonstrating that this gene encodes a phospholipid:sterol acyltransferase (PSAT). Properties of the AtPSAT were examined in microsomal fractions from the tissues of an overexpressor. The enzyme did not utilize neutral lipids, had the highest activity with phosphatidylethanolamine, had a 5-fold preference for the sn-2 position, and utilized both saturated and unsaturated fatty acids. Various sterols and sterol intermediates, including triterpenic precursors, were acylated by the PSAT, whereas other triterpenes were not. Sterol selectivity studies showed that the enzyme is activated by end product sterols and that sterol intermediates are preferentially acylated by the activated enzyme. This indicates that PSAT both regulates the pool of free sterols as well as limits the amount of free sterol intermediates in the membranes. Two T-DNA insertion mutants in the AtPSAT gene, with strongly reduced (but still measurable) levels of sterol esters in their tissues, had no detectable PSAT activity in the microsomal fractions, suggesting that Arabidopsis possesses other enzyme(s) capable of acylating sterols. The AtPSAT is the only intracellular enzyme found so far that catalyzes an acyl-CoA-independent sterol ester formation. Thus, PSAT has a similar physiological function in plant cells as the unrelated acyl-CoA:sterol acyltransferase has in animal cells.

Sterols are components of all eukaryotic membranes and are important regulators of membrane fluidity and thus membrane properties and functions (1, 2). Because acylated sterols or sterol esters (SE) cannot participate in the bilayer of the membranes, the acylation of sterols is believed to play a crucial role in maintaining free sterol homeostasis in the cell membranes (3–7). In other words, sterol esters are generally thought to constitute a storage pool of sterols when those are present in amounts greater than immediately required for the cells. For instance, accumulation of sterol esters has been described during seed matura-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY989885.

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2 The abbreviations used are: SE, sterol esters; PSAT, phospholipid:sterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; ACAT, acyl-CoA:sterol acyltransferase; PDAT, phospholipid:diacylglycerol acyltransferase; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PA, phosphatidic acid; RPHPTLC, reversed phase high performance TLC; AtPSAT, A. thaliana phospholipid sterol acyltransferase; Wt, wild type.
activity (20). We now report that a third of these LCAT-like genes in Arabidopsis encodes a PSAT. We have overexpressed the AtPSAT gene in Arabidopsis behind the 35S promoter, and we characterized the enzyme regarding substrate specificity and selectivity in microsomal membranes from the overexpressers. In addition we investigated the SE synthesizing activity in two T-DNA insertion mutants of the PSAT gene.

**EXPERIMENTAL PROCEDURES**

Plant Material—Arabidopsis thaliana (ecotype Columbia-0) plants were grown as described earlier (19). Plant materials for gene expression and enzymatic studies were obtained from liquid cultured plants (19). Arabidopsis insertion mutant lines (21) for the AtPSAT gene were identified in the Salk Institute T-DNA insertion library data base (signal.salk.edu/cgi-bin/tdnaexpress), and seeds were obtained from the Nottingham Arabidopsis Stock Centre (University of Nottingham, UK). Screening for putative mutants was done by PCR (19). Individual plants from each mutant line lacking a T-DNA insertion in the AtPSAT gene (null segregants) were also identified in the same PCR screening.

Isolation of AtPSAT cDNA—Primers designed from the genomic AtPSAT sequence N123456 (corresponds to protein A1tg04100) were used to isolate an AtPSAT cDNA fragment using total RNA from 2-week-old Arabidopsis seedlings. Poly(A)



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Plant Phospholipid:Sterol Acyltransferase

The sterols and related compounds used in experiments were either commercially available (cholesterol, campesterol, stigmasterol, sitosterol, and dihydrocholesterol) or extracted from Arabidopsis or tobacco tissues or yeast mutants, treated or not by sterol biosynthesis inhibitors. After purification (>90%), their structure was confirmed by gas chromatography-mass spectrometry.

Microsomal Membrane Preparations and Enzyme Assays—Microsomal fractions from leaves and roots of Arabidopsis plants grown in liquid culture were prepared as described previously (27). In order to remove most of the non-polar lipids from the membranes, the microsomal preparations for some enzyme assays were pretreated with acetone as follows. 1 ml of microsomal membranes (corresponding to 0.5–1.5 μmol of microsomal PC) in 100 mM potassium phosphate buffer, pH 7.2, was added to 50 ml of cold (−80 °C) acetone under stirring, and the solution was left in a cold room for 10 min with slow stirring. The membranes were collected by centrifugation (5000 × g for 10 min), and the remaining acetone was removed under vacuum for 3 h. The acetone-treated membranes were then resuspended in 1 ml of water and stored in aliquots at −80 °C before being used in enzyme assays.

Aliquots of crude or acetone-treated microsomal fractions (ranging from 5 to 24 nmol of microsomal PC, equivalent to 20–100 μg of protein) were lyophilized overnight. Various radioactive and non-radioactive substrates, added as indicated in the tables and figures, were dissolved in 15–30 μl of benzene and then added to the dried microsomes. The benzene was immediately evaporated under a stream of N₂ at 35 °C, leaving the lipids in direct contact with the membranes, after which 0.1 ml of 50 mM potassium phosphate, pH 7.2, was added. The suspension was thoroughly mixed and incubated at 30 °C. It should be noted that prolonged exposure of the microsomes to benzene severely affected sterol ester synthesis. The incubations were terminated, after periods of times indicated in the tables and figures, by extracting the lipids into chloroform (28).

All enzyme assays were done at least in duplicate, and the amounts of the synthesized radioactive lipids never deviated more than 10% between duplicate samples from the same microsomal preparations. Data shown are standard means of replicates.

Lipid Analysis—Lipids in the chloroform fractions obtained from the assays were separated by TLC with hexane/diethyl ether/acetic acid (70:30:1 by volume) using straight phase Silica Gel 60 plates (Merck). Wax esters and SE co-migrated in the solvent front. To further resolve these lipids from each other as well as to separate SE with different acyl or sterol moieties, the lipids were eluted from the gel by methanol/chloroform (2:1), extracted into chloroform (28), and rechromatographed on reversed phase high performance TLC (RPHPTLC; Merck) in acetonitrile/tetrahydrofuran (40:60 by volume). The radioactive lipids were visualized and quantified on the plates by using electronic autoradiography (Instant Imager, Packard Instrument Co.). This system separated 16:0/18:1, 18:0, 18:2, and 18:3 cholesterol esters from each other. Also, the dominating wax esters formed by the membranes from 14C-acyl substrates were clearly separated from the [14C]acyl-cholesterol by the RPHPTLC system. Furthermore, RPHPTLC separated some of the sterol esters with the same acyl group from each other, making it possible to do acyl acceptor competition experiments with the PSAT. Radioactive wax esters were synthesized in assays with all the different 14C-acyl substrates, but the amount, relative to the radioactive sterol esters formed, was not significant in the membranes from the PSAT overexpresser when cholesterol was used as an acyl acceptor. However, when some sterols that were poor acceptors were used or when assays were done with membranes from Wt or PSAT mutants, co-migration of wax esters and sterol esters was still a problem, even after separation on RPHPTLC. Also, the presence of endogenous sterols in the membranes gave a high “background” of sterol ester synthesizing activity in assays in the absence of added sterols. The formation of radioactive sterol esters and wax esters from 14C-acyl substrates in the absence of added sterols or fatty alcohols had been reduced by 90% after acetone treatment of the microsomal membranes, indicating that most of endogenous sterols and fatty alcohols had been removed by the acetone. Furthermore, the specific activity of PSAT (based on amount of microsomal PC) was increased about 5-fold in the acetone-treated compared with untreated membranes. Quantification of the PC content in the microsomal fractions was done as described earlier (19).

Determination of Sterol Ester Content in Planta—Sterol ester content of the two T-DNA insertion mutants and their null segregants were determined from plants grown in liquid medium. Lipids were extracted from freeze-dried material (100–200 mg), and sterol esters were purified by TLC and analyzed by gas-liquid chromatography and gas chromatography-mass spectrometry as described previously (29).

RESULTS

Cloning of AtPSAT—In the search for the function of the six PDAT/LCAT-like genes found in the Arabidopsis genome data bases, we have identified previously the catalytic function of At5g13640 as a PDAT (19) and At3g03310 as a phospholipase A₁ (20). In the present paper we report the identification of the At1g04010 gene as a phospholipid:sterol acyltransferase (AtPSAT). Cloning and sequencing of AtPSAT (GenBank™ accession number AY989985) revealed a gene sequence of 4826 bases containing 15 exons and an open reading frame that encodes a protein of 633 amino acids (Fig. 1A). This protein shows 28% identity with human LCAT. It is, like the AtPDAT but unlike the soluble LCAT, predicted to have one membrane spanning region at the N-terminal end according to TMHMM2.0 (30). It should be noted that the now published amino acid sequence for AtPSAT differs in its 3’ end from the existing predicted sequence in the GenBank™.

Sequence Comparison between PSAT and Other LCAT-like Proteins—EST clones from Medicago truncatula (GenBank™ accession numbers BE321377 and BI267156) and Citrus sinensis (GenBank™ accession number CK993714) presenting strong homologies with AtPSAT were identified in data bases. After complete sequencing, these cDNAs were shown to encode proteins of 364 amino acids (MtPSAT) and 641 amino acids (CsPSAT) having 76 and 75% identity, respectively, with AtPSAT. In the frame of the rice (Oryza sativa) genome sequencing, a gene encoding a protein showing 73% identity with AtPSAT was also found.

Alignments were performed for deduced amino acid sequences of the following cDNAs (Fig. 1B): a first group of plant PDATs, a second group containing AtPSAT and the three orthologues named MtPSAT, CsPSAT, and OsPSAT, genuine mammalian and avian lecithin cholesterol acyltransferases (LCAT), and various LCAT homologues. Isolation and characterization of plant cDNAs have been described elsewhere (20). Six characteristic conserved regions are shown in Fig. 1B. From these alignments, it is clear that the catalytic triad (Ser-Asp-His) already identified previously in PSAT is present in all LCAT-like proteins. It is, like the AtPDAT but unlike the soluble LCAT, predicted to have one membrane spanning region at the N-terminal end according to TMHMM2.0 (30). It should be noted that the now published amino acid sequence for AtPSAT differs in its 3’ end from the existing predicted sequence in the GenBank™.

An important conserved hydrophobic domain (domain 1 from left to right in Fig. 1B) is found in all LCAT-like proteins. Yet its function is

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FIGURE 1. Gene structure of PSAT and amino acid sequence alignment with other LCAT-like proteins. A, AtPSAT gene (GenBankTM accession number AY989885). Gene structure, amino acid sequence, and T-DNA insertion sites for Arabidopsis mutation lines SALK_117091 and SALK_037289. Gray boxes denote exons and block bars introns. B, amino acid sequence alignment of AtPSAT and other LCAT-like proteins. From the top to the bottom: AtPDA1 (A. thaliana phospholipid diacylglycerol acyltransferase, GenBankTM accession number AF493159), CsPSAT (C. sinensis, GenBank accession number AY957608), AP3AAT (A. thaliana phospholipid sterol acyltransferase, GenBank accession number AY989885), AtPSAT accession number AF493159, AtPSAT (C. sinensis, GenBank accession number AY957608), AP3AAT (A. thaliana phospholipid sterol acyltransferase, GenBank accession number AY989885), AtPSAT (D. sativa, GenBank accession number XP468371), AtLCAT (A. thaliana, GenBank accession number NP_001220), OcLCAT (Oryctolagus cuniculus, GenBank accession number NP_001220), AtPSAT (M. truncatula, GenBank accession number P53760), MmLCAT (Mus musculus LCAT, GenBank accession number NP_001220), GgLCAT (Gallus gallus LCAT, GenBank accession number P53760), AtPLA1 (A. thaliana phospholipase A1, GenBank accession number P53760), AtPSAT (A. thaliana, GenBank accession number AY421146), AtPSAT (A. thaliana, GenBank accession 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synthesis (13–17), we investigated if these lipids were more immediate acyl donors than PE for the SE-synthesizing enzyme. SE and wax esters could be separated from each other on RP-HPTLC (see “Experimental Procedures”). From these separations it was evident that no cholesterol esters were formed from the radioactive neutral lipids, but a radioactive acyl acceptor for the AtPSAT protein. We here suggest the name phospholipid:sterol acyltransferase or PSAT for this plant enzyme.

Acyl Specificity of the AtPSAT—The acyl specificity of the AtPSAT was tested with PE with 14C-acyl groups (16:0, 18:0, 18:1, 18:2, and 18:3) at the sn-2 position and with 18:1 at the sn-1 position. The activity was about the same for 16:0, 18:0, and 18:1, 2.5 times higher for 18:2, and 3 times higher for 18:3 (Fig. 3A). In order to study the effect of the type of the acyl groups at position sn-1 on sn-2 AtPSAT activity, assays were also done with 16:0 at the sn-1 position and 14C:18:2 at the sn-2 position of PE (Fig. 3A). The activity with the sn-1–16:0 substrate was slightly less efficient (92% of the activity) than the corresponding sn-1–18:1–16:0 substrate.

Lipid and Positional Specificity of the PSAT—The AtPSAT activity in microsomal fractions from the AtPSAT overexpresser was examined with three different phospholipids, PC, PE, and PA. The activity toward PE was 5.5-fold higher than PC and over 30 times higher than PA (TABLE ONE). We also compared the activity toward the different sn positions with 14C:18:1–PC labeled either in the sn-1 or sn-2 position and with unlabeled 18:1 in the other position. The AtPSAT showed a 5-fold preference for the sn-2 position (TABLE ONE). When corresponding incubations were done with microsomal preparations from Wt, radioactive SE was below the detection limit in any of the assays with 14C:PC as the substrate (TABLE ONE).

Sterol Specificity of the AtPSAT—The specificity of the AtPSAT toward different sterols and sterol intermediates was tested in microsomal preparations from the AtPSAT overexpresser. The experiments were first performed with crude microsomal fractions, and a number of the sterols were shown to have an inhibitory effect in the production of SE compared with assays without the addition of sterols (data not shown). In order to remove as much as possible of the endogenous sterols in the membranes, we pretreated the microsomal preparations with acetone. When AtPSAT was assayed in the acetone-treated microsomes without addition of sterols, the activity was decreased to 10–20% that of assays with the untreated microsomes. Moreover, the amount of wax esters produced in the acetone-treated microsomes was reduced by over 90% compared with the untreated microsomes (data not shown). Assays in order to determine acyl acceptor specificity were performed with acetone-treated microsomal preparations from Wt, radioactively labeled SE with [14C]18:1–PC in the sn-1 or sn-2 position, and with unlabeled 18:1 in the other position. The AtPSAT showed a 5-fold preference for the sn-2 position (TABLE ONE). When corresponding incubations were done with microsomal preparations from Wt, radioactive SE was below the detection limit in any of the assays with 14C:PC as the substrate (TABLE ONE).
obtusifoliol, and 24-ethylidene lophenol are intermediates in plant sterol biosynthesis, leading to the main end products campesterol, sitosterol, and stigmasterol, whereas cholesterol is a minor (<5%) end product sterol in Arabidopsis and in most plants. Lanosterol, zymosterol, and ergosterol are yeast sterols. Lupeol and β-amyrin appeared not to serve as substrates for the AtPSAT. Among the 14 tested sterols and sterol intermediates added, it was only the cholesta-5,7-dienol that did not significantly increase the activity over background. The activity toward the two major sterols in Arabidopsis, campesterol and sitosterol (33), was about half that of dihydrocholesterol, zymosterol, and cholesterol, the three best substrates. Although the relative activity for different sterols showed the same trend in both root and leaf membranes, there was a clear difference for certain sterols. The experiment was repeated once with different batches of microsomal preparations with essentially the same result. This indicates that the observed specificity was not entirely due to an inherent property of the enzyme per se but that it was also influenced by the nature of the membrane to which it was attached.

It should be noted that the SE synthesis was below the detection limit for all sterols in assays with acetone-treated membranes from Wt plants (data not shown).

**Sterol Selectivity of the AtPSAT**—Some of the sterols, particularly the intermediates in the biosynthesis of the end product sterols, were quite poor acceptors for the AtPSAT (see above), and yet they are known to accumulate as SE in some plant tissues such as *A. graveolens* cell suspension culture, *Avena sativa* roots, *Gossypium hirsutum* buds and anthers, and a sterol over producer tobacco mutant (4). We therefore performed a sterol selectivity study of the AtPSAT. Some of the acylated sterol intermediates could be separated from the acylated end product sterols by RPHPTLC. For example, the intermediates 18:2-cycloartenol, 18:2-obtusifoliol, and 18:2–24-ethylidene lophenol clearly separated from 18:2-sitosterol. When the intermediates were presented as single substrates, the PSAT activity for these substrates was less than half that for sitosterol. However, when presented as equimolar mixtures with sitosterol, the acylation of sitosterol was decreased by up to 80%, and the activity toward the intermediates increased up to 3-fold (Fig. 4). The total amount of sterol acylated was relatively unchanged compared with the assays with only sitosterol. A series of incubations with sitosterol and cycloartenol, where one compound was held at constant concentration and the amount of the other was increased, are shown in Fig. 5, A and B. Although addition of increasing amounts of cycloartenol to sitosterol decreased overall sterol ester formation (Fig. 5A), the addition of increasing amounts of sitosterol to cycloartenol increased overall sterol ester formation as well as acylation of cycloartenol (Fig. 5B).

**AtPSAT Activity in T-DNA Insertion Mutants**—Two putative AtPSAT insertion mutant lines were obtained from the SALK collection. One contained a nonsense mutation in exon (SALK_117091) and in the first intron (SALK_037289) (Fig. 1A). Both mutant plants showed a similar and strong reduction in their total SE content (about 70%) when compared with that of Wt (TABLE TWO), indicating that AtPSAT is the major SE-synthesizing enzyme in Arabidopsis. However, the residual SE content indicates leaky mutants or additional enzymes existing able to acylate sterols. Experiments were therefore performed in order to biochemically characterize the remaining SE synthesizing activity in Arabidopsis microsomal preparations. Because SE synthesizing activity was just on the limit of detection in Wt in our previous experiments, we now used substrates with about 10-fold higher specific radioactivity. Because the RPHPTLC separated cholesterol esters with 18:0, 16:0/18:1, 18:2, and 18:3 from each other, we could study the acyl selectivity of the SE-synthesizing enzymes from endogenous acyl donors in the membranes from both mutants and Wt, using radioactive cholesterol as acyl acceptor. The SE synthesizing activity in microsomal preparations from the mutants, the Wt, and the overexpresser showed the same pattern of acylation of cholesterol with mainly 18:3 and 18:2 incorporated and minor amounts of 18:0 and with the mutants accumulating about half the amounts of SE compared with the Wt (Fig. 6A). Because the microsomal preparations synthesized a range of wax esters, some of which co-migrated with the SE on RPHPTLC plates, it was not possible to determine the SE synthesis from radioactive PE as a donor in crude microsomal fractions from Wt and mutants. However, acetone-treated microsomes accumulated much less wax ester.

**TABLE ONE**

**Lipid and positional specificity of the AtPSAT**

In incubations were performed with microsomal preparations (equivalent to 24 nmol of microsomal PC) from leaves of Arabidopsis overexpressing PSAT (line D28-1-5-8) or Wt, cholesterol (10 nmol), and [14C]-substrates (10 nmol) as indicated in the table. Incubation time was 2 h. SE were separated out on RPHPTLC before quantification of radioactivity as described under “Experimental Procedures.”

| Substrate | [14C]SE formed |
|-----------|----------------|
|           | pmol           |
| **D28-1-5-8** | **Wt** |
| sn-1–18:1-sn-2-[14C]18:2-PE | 420 | 10 |
| sn-1–18:1-sn-2-[14C]18:2-PA | 12.7 | ND* |
| sn-1–18:1-sn-2-[14C]18:1-PE | 97.1 | 2.1 |
| sn-1–18:1-sn-2-[14C]18:1-PC | 17.6 | ND* |
| sn-1-[14C]18:1-sn-2–18:1-PC | 3.6 | ND* |

* ND indicates not detected.

**FIGURE 4. Sterol selectivity of AtPSAT.** Sitosterol, 24-ethylidenelophenol, obtusifoliol, and cycloartenol were presented either as single substrate (5 nmol) or in mixtures as indicated in the figure. Assays were performed with acetone-treated microsomal preparations (20 μg of microsomal protein) from roots of AtPSAT overexpresser incubated with sterols and sn-1–18:1-sn-2-[14C]18:2-PE (5 nmol) for 2 h. Striped bars, sitosterol ester.
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from Arabidopsis. We show that the enzyme catalyzes the transacylation of acyl groups from phospholipids to a variety of different sterols, and we suggest the name phospholipid:sterol acyltransferase (PSAT) for this type of enzyme. For several reasons, we prefer not to call this enzyme LCAT, despite its catalytic similarities with this animal enzyme. First, lecithin is an old name for PC, and the plant enzyme prefers PE over PC as an acyl donor. Furthermore, cholesterol is by far not the only acyl acceptor for the plant enzyme. It should also be noted that the animal enzyme is an extracellular enzyme and plays another physiological role than PSAT.

Relatively little information on SE biosynthesis in plants is available in the literature. Published work indicates that the synthesis involves transacylation and that both phospholipids and neutral lipids, in particular diacylglycerols and triacylglycerols, could serve as acyl donors (13–17). We could not detect any activity toward neutral lipids by the PSAT enzyme. However, we found that our microosomal preparations rapidly hydrolyzed the neutral lipids to free fatty acids that were acylated to endogenous fatty alcohols to produce wax esters. These wax esters co-migrate with SE on silica gel chromatography. It is therefore possible that some of the previous work in sterol ester biosynthesis, using enzyme assays with radioactive neutral lipids as acyl donors, did not discriminate wax ester synthesis from sterol ester synthesis.

The PSAT had a strong preference for PE over PC, hardly utilized PA as substrate, and did not use neutral lipids as acyl donor. This suggests that the enzyme has quite strict requirements for the type of polar head group of the phospholipids. However, it does not necessarily mean that the binding site for the phospholipid substrate is highly specific. It could also be that the enzyme has easier access to the bilayer “disturbing” PE molecules than the bilayer forming phospholipids. However, it is relevant here to compare the PSAT with a structurally related enzyme, the PDAT, which is forming triacylglycerols from phospholipids and diacylglycerols (18, 19). The Arabidopsis PDAT has only 1.7-fold specificity for PE over PC (compared with 5-fold for the PSAT) and utilizes also PA at significant rates (19). Moreover, it was inferred that the AtPDAT, to a lesser extent, could also utilize diacylglycerol as an acyl donor (19). Therefore, it appears that PSAT has inherent higher acyl donor specificity than the PDAT.

All common acyl groups present in plant membranes were utilized well by the PSAT enzyme, although specificity for polyunsaturated fatty acids was seen. The enzyme was highly specific for position sn-2, which in plant lipids is essentially devoid of saturated acyl groups. This suggests that PSAT enzyme would play a minor role in the synthesis of SE with saturated fatty acids.

The sterol specificity of PSAT is broad, and both intermediates and end product sterols were acylated. However, no activity could be seen with the triterpenes lupeol and β-amyrin, which suggests that another enzyme is responsible for the formation of esters of these compounds. This is also consistent with the observation that the ester amount of these triterpenes was not reduced in the T-DNA insertion mutants (34). Among the various plant end product sterols, the rate of acylation was in the order cholesterol ≥ campesterol ≥ sitosterol > stigmasterol. This is in good agreement with the composition of the SE pool of a number of species where higher and lower, respectively, than that of the free sterol pool, whereas the proportions of campesterol and sitosterol are similar in both pools (4).

Some of the intermediates in sterol biosynthesis were poor substrates when presented as single substrates but were utilized preferentially and at a high rate when sitosterol, the major sterol in Arabidopsis, was present. The remarkable stimulation of acylation of sterol intermediates in the presence of an end product sterol suggests that the PSAT could be

In this work we have identified the first plant gene encoding an SE-synthesizing enzyme and further characterized the encoded enzyme...
an allosteric enzyme, positively regulated by end product sterols but not by the intermediates. It should be noted that the catalytic activity for the intermediates and end product sterols was similar, provided that the enzyme was activated by end product sterols.

It can be hypothesized that such an unusual regulation of enzyme activity could work in vivo as a modulator of the flux in the sterol biosynthetic pathway as follows. When end product sterols reach a threshold level in the membranes, the PSAT enzyme will be fully activated at its allosteric binding site and will efficiently esterify intermediates in SE biosynthesis and thereby slow down the further accumulation of end products. When the level of end product sterols drops to a point where PSAT is no more activated, the flux through the pathway will be resumed. Such an hypothesis finds some support from results obtained in a sterol overproducing tobacco mutant. The pool of free sterols was quantitatively and qualitatively unchanged in the mutant compared with Wt, but the pool of SE was increased 10-fold and contained both intermediates and end product sterols (35). A similar sterol profile was observed in transgenic Arabidopsis overexpressing the early enzyme in the sterol biosynthesis pathway, the hydroxyethylglutaryl-CoA reductase (29). Indeed, hydroxyethylglutaryl-CoA reductase is likely to be responsible for the regulation of the total flux in the biosynthesis of sterols (36), whereas PSAT contributes to maintain homeostasis of free sterols in the membrane as well as to limit the amount of free intermediates in sterol biosynthesis, which otherwise could be deleterious for membrane functions (4).

The two T-DNA insertion mutants studied had no detectable activity with PE as acyl donor when measured with acetone-treated microsomes, suggesting a lack of active PSAT enzyme in these plants. Yet these mutants were still able to synthesize SE, although the levels were strongly reduced (with about 70%) in intact plants. This indicates that PSAT is the main SE-synthesizing enzyme in Arabidopsis. The ubiquitous expression of the PSAT gene (see www.plantbiology.msu.edu/lipids/gensurvey/) reinforces this assumption. We could show that the acyl preference, using radioactive cholesterol and endogenous acyl donors present in the membranes, was similar in microsomal preparations from Wt and mutant and also similar to that of overexpressed PSAT. Because SE synthesizing activities in microsomal membranes from Wt and mutants were extremely low and formation of wax esters interfered with the detection of SE using radioactive acyl donors, a more detailed biochemical characterization of the remaining SE synthesizing activity in the mutants was not possible. It should be noted that the specific activity of the PSAT enzyme in the wild type Arabidopsis (measured as pmol × min⁻¹ × mg protein⁻¹ in microsomal fractions) was less than one-fifth that of the Arabidopsis PDAT enzyme (19).

The PSAT enzyme is a member of the PDAT/LCAT family. Indeed, the alignment of the amino acid sequences of the SE-synthesizing enzymes, the animal LCATs, AtPSAT, and other putative plant PSATs, with the yeast and plant triacylglycerol-synthesizing PDAT enzymes, reveals that important sequence similarities exist among these proteins. As discussed above, some of these domains might be involved in substrate binding and/or catalytic mechanism. A directed mutagenesis study should clarify this point.

In summary, the first plant gene encoding an SE-synthesizing enzyme, PSAT, has been identified and shown to belong to the LCAT/ PDAT gene family. This is the first identified intracellular enzyme catalyzing the synthesis of SE by an acyl-CoA-independent reaction. Thus, the physiological function of this enzyme is totally different from the evolutionarily related animal LCAT. PSAT is likely to serve similar cellular functions as the unrelated ACAT enzymes, found in animals and fungi. The PSAT enzyme utilizes a wide range of sterols and steroid intermediates. Esterification of steroid intermediates is greatly stimulated by presence of end product sterols, suggesting a role for PSAT in regulating both the amount and the quality of the free sterols in the membrane. The in vivo effects of overexpressed PSAT and mutations in the PSAT gene are currently under investigation. Taken together, although our results suggest the presence of an additional enzyme(s) able to acylate sterols, they clearly show that PSAT is the major SE-synthesizing enzyme in Arabidopsis.

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Cellular Sterol Ester Synthesis in Plants Is Performed by an Enzyme (Phospholipid:Sterol Acyltransferase) Different from the Yeast and Mammalian Acyl-CoA:Sterol Acyltransferases

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