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Regulation of Triacylglucose Fatty Acid Composition[1]

Uridine Diphosphate Glucose:Fatty Acid Glucosyltransferases with Overlapping Chain-Length Specificity

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UDP-glucose (UDP-Glc):fatty acid glucosyltransferases catalyze the UDP-Glc-dependent activation of fatty acids as 1-*O*-acyl-β-glucoses. 1-*O*-Acyl-β-glucoses act as acyl donors in the biosynthesis of 2,3,4-tri-*O*-acylglucoses secreted by wild tomato (*Lycopersicon pennellii*) glandular trichomes. The acyl composition of *L. pennellii* 2,3,4-tri-*O*-acylglucoses is dominated by branched short-chain acids (4:0 and 5:0; approximately 65%) and straight and branched medium-chain-length fatty acids (10:0 and 12:0; approximately 35%). Two operationally soluble UDP-Glc:fatty acid glucosyltransferases (I and II) were separated and partially purified from *L. pennellii* (LA1376) leaves by polyethylene glycol precipitation followed by DEAE-Sepharose and Cibacron Blue 3GA-agarose chromatography. Whereas both transferases possessed similar affinity for UDP-Glc, glucosyltransferase I showed higher specificity toward short-chain fatty acids (4:0) and glucosyltransferase II showed higher specificity toward medium-chain fatty acids (8:0 and 12:0). The overlapping specificity of UDP-Glc:fatty acid glucosyltransferases for 4:0 to 12:0 fatty acid chain lengths suggests that the mechanism of 6:0 to 9:0 exclusion from acyl substituents of 2,3,4-tri-*O*-acylglucoses is unlikely to be controlled at the level of fatty acid activation. UDP-Glc:fatty acid glucosyltransferases are also present in cultivated tomato (*Lycopersicon esculentum*), and activities toward 4:0, 8:0, and 12:0 fatty acids do not appear to be primarily epidermal when assayed in interspecific periclinal chimeras.

Foliar glandular trichomes of wild tomato (*Lycopersicon pennellii*) secrete a complex mixture of polar lipids composed of 2,3,4-tri-*O*-acylglucose esters of short- and medium-chain-length branched- and straight-chain fatty acids (Fobes et al., 1985; Burke et al., 1987). These Glc esters are responsible for the broad-spectrum insect resistance of *L. pennellii* (Goffreda et al., 1989; Hawthorne et al., 1992; Liedl et al., 1995). The cultivated tomato (*Lycopersicon esculentum*) lacks the ability to accumulate sugar esters (Fobes et al., 1985), and transfer of this trait could significantly reduce the impact of insect pests on this and other crop species (Mutschler et al., 1993).

The degree to which sugar polyester biosynthesis dominates carbon flux in this species (secreted 2,3,4-tri-*O*-acylglucoses represent more than 20% of leaf dry weight) makes this an attractive system to study biosynthesis and metabolic control. Biosynthesis of 2,3,4-tri-*O*-acylglucoses in *L. pennellii* exhibits several novel qualities (Fig. 1). Rather than proceeding through thioester-activated intermediates, fatty acid activation proceeds via the UDP-Glc-dependent activation of fatty acids as 1-*O*-acyl-β-Glc esters (Ghangas and Steffens, 1993), and is catalyzed by UDP-Glc:fatty acid transglucosylases. The regiospecific transacylation of the activated 1-*O*-acyl moieties to other Glc and/or partially acylated Glc derivatives, resulting in polyacylated glucoses, occurs through repetitive steps of disproportionation (Ghangas and Steffens, 1993, 1995) and is catalyzed by an enzyme, the cDNA of which exhibits high sequence similarity to Ser carboxypeptidases (X. Li and J.C. Steffens, unpublished data).

A conspicuous structural characteristic of *L. pennellii* acylglucoses is the bimodal distribution of chain length in the fatty acyl substituents. The acyl substituents of these esters are composed of branched 4:0 and 5:0 (approximately 65%) and branched- and straight-chain 10:0 and 12:0 (approximately 35%) fatty acids (Burke et al., 1987). Stable isotope-labeling studies show that a structurally diverse series of substrates are incorporated into triacylglucose as short-chain acyl substituents or are elongated in two-carbon increments to 10:0 to 12:0 and then acylated (Walters and Steffens, 1990). In in vitro studies of fatty acid activation as 1-*O*-β-acylglucoses, *L. pennellii* leaf extracts exhibited UDP-Glc-dependent fatty acid glucosyltransferase activity toward 4:0, 12:0, and 16:0 fatty acids (Ghangas and Steffens, 1993). The objectives of this study were (a) to determine whether the UDP-Glc:fatty acid glucosyltransferase activation step of this pathway is controlled by a single enzyme of very low specificity or by multiple enzymes possessing differential specificity for fatty acid substrates, and (b) to assess whether specificity of UDP-Glc:fatty acid glucosyltransferases could exert control over 2,3,4-tri-*O*-acylglucose fatty acyl composition by regulating access of fatty acids to the pool of activated acyl donors. In this paper we describe the characterization and partial

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Abbreviations: GTI and II, fractions exhibiting enhanced activity toward isobutyrate and dodecanate, respectively; X:Y, a fatty acyl group containing X carbon atoms and Y *cis* double bonds.

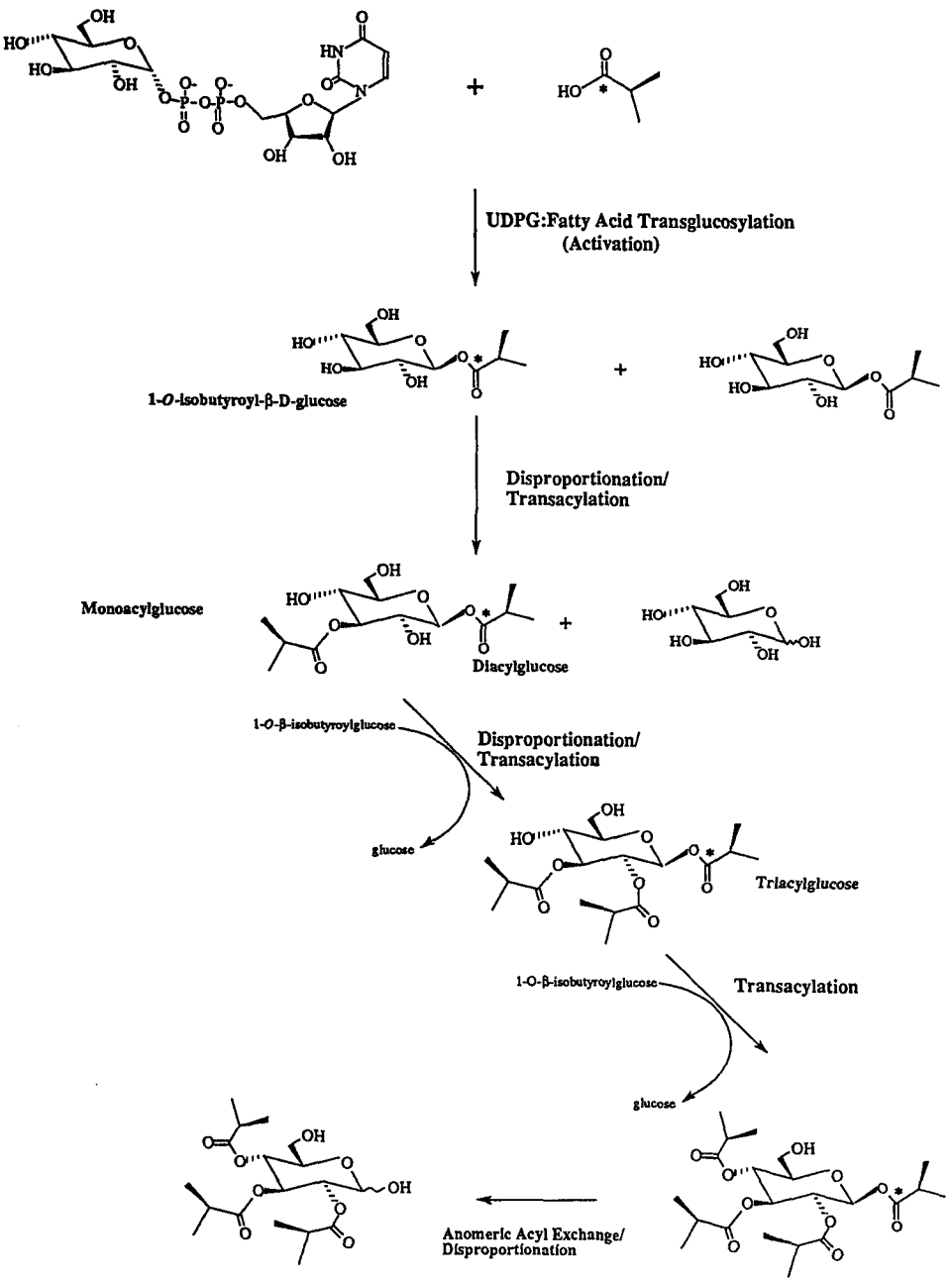

Figure 1. Biosynthesis of 2,3,4-tri-*O*-acylglucoses by *L. pennellii*. Activation of fatty acids proceeds via UDP-Glc (UDPG) transglucosylation; iterative disproportionation of activated 1-*O*-acyl-β-Glc donors leads to formation of Glc polyesters (Ghangas and Steffens, 1993, 1995).

purification of two forms of UDP-Glc:fatty acid glucosyltransferase with differential specificity toward short- and medium-chain-length fatty acids.

MATERIALS AND METHODS

Wild tomato (*Lycopersicon pennellii*; LA1376 and LA716) seeds were obtained from the Tomato Genetic Resources Center (Davis, CA). Periclinal chimeras constructed from *L. pennellii* LA716 and cultivated tomato (*Lycopersicon esculentum*; Goffreda et al., 1990b) were kindly provided by Dr. M.A. Mutschler (Department of Plant Breeding, Cornell University, Ithaca, NY). All plants were grown in greenhouses supplemented with metal-halide lighting on a 16-h photoperiod.

Reagents

UDP-Glc, BSA, Cibacron Blue 3GA agarose, UDP agarose, UDP-glucuronic acid agarose, concanavalin A-Sepharose, DEAE-Sepharose, DTT, diethyldithiocarbamic acid (sodium salt), PVPP, Hepes, PEG, Suc, and PMSF were

purchased from Sigma. Glycerol was purchased from Fisher Scientific. ^{14}C-Fatty acids were purchased from American Radiolabeled Chemicals (St. Louis, MO). The reagent for the Bradford protein assay was purchased from Pierce. Desalting columns (Econo-PacI0DG) were purchased from Bio-Rad. Chromatofocusing gel and Polybuffer were purchased from Pharmacia.

Purification of UDP-Glc:Fatty Acid Glucosyltransferases

All procedures were carried out at 4°C. *L. pennellii* LA1376 leaves (0.5 kg) were homogenized with 750 mL of extraction buffer A containing 75 mM Hepes, pH 7.5, 0.25 M Suc, 10 mM DTT, 1 mg/mL diethyldithiocarbamic acid, and 1% (w/v) acid-washed PVPP, the homogenate was filtered through four layers of cheesecloth, and the filtrate was centrifuged at 15,000*g* for 20 min. PEG 3350 was added to the supernatant at 0.22 g/mL. After the PEG was completely dissolved, the protein extract was centrifuged at 15,000*g* for 15 min and pellet was discarded. PEG was then added to a final concentration of 0.37 g/mL, and the resulting solution was centrifuged again at 15,000*g* for 15 min to pellet the protein. The pellet was resuspended in about 80 mL of buffer B containing 50 mM Hepes, pH 7.5, 20% glycerol (v/v), 0.2 mM PMSF, and 10 mM DTT, and the suspension was clarified by centrifugation at 30,000*g* for 5 min. The pellet was washed once with buffer B to recover residual protein, and the two supernatants were combined and loaded onto a DEAE-Sepharose column (1 × 20 cm) pre-equilibrated with buffer B. After the sample was extensively washed with buffer B (about 150 mL), bound protein was eluted with a 100-mL linear gradient of 0 to 0.3 M NaCl in buffer B.

Two-milliliter fractions were collected and assayed for UDP-Glc:fatty acid glucosyltransferase activity using [^{14}C]isobutyrate (4:0) and [^{14}C]dodecanoic acid (12:0). Fractions were organized into two pools: GTI and GTII. These pooled activities were desalted separately on Econo-PacI0DG desalting columns, diluted 2-fold, and then loaded to Cibacron Blue 3GA-agarose columns (1 × 13 cm) pre-equilibrated with buffer C (50 mM Hepes, pH 7.5, 20% glycerol [v/v], 5 mM DTT, and 0.2 mM PMSF), respectively. The two columns were washed with 5 bed volumes of buffer B. The glucosyltransferase activities were then eluted with 2 mM UDP-Glc in buffer C. The active fractions were pooled, concentrated by dialyzing against solid PEG 20,000, and dialyzed extensively against buffer C. Glycerol was added to the samples up to 30% (v/v) and the enzymes were then stored at −20°C.

Glucosyltransferase Assay

The standard assay mixture contained 2.5 μmol Bis-Tris, pH 6.8, 75 nmol UDP-Glc, 0.067% (v/v) Triton X-100, 5 × 10^5 dpm ^{14}C-fatty acid (about 55 mCi/mmol), and 5 μL of enzyme in a total volume of 15 μL in 0.65-mL polypropylene tubes. ^{14}C-Fatty acid or mixtures with varying amounts of unlabeled fatty acid were dried in the tube, 10 μL of reaction mixture (no enzyme) was added, tubes were vortexed briefly and sonicated in a water bath until turbidity disappeared, and then enzyme was added. Mixtures were incubated at 37°C for 2 h. Five microliters of each reaction was analyzed by silica gel TLC (chloroform:methanol:H_2O, 75:22:3 [v/v/]; Ghangas and Steffens, 1993). For kinetic studies, the incubation was carried out at 37°C for 45 min and the reaction was terminated by boiling. One unit of enzyme was defined as the amount of enzyme producing 1 nmol of 1-*O*-acyl-β-Glc in 1 h. K_m and V_{max} were determined from Lineweaver-Burk reciprocal plots; K_m for fatty acids was determined at 5 mM UDP-Glc; K_m for UDP-Glc was determined at 5 mM 4:0 and 610 μM 12:0 for GT I and GT II, respectively.

RESULTS

Separation and Partial Purification of Two UDP-Glc:Fatty Acid Glucosyltransferases

PEG fractionation (0.22–0.37 g PEG/mL) resulted in a 13-fold enrichment of UDP-Glc:4:0 glucosyltransferase activity, with more than 90% of total protein removed and 76% recovery of activity (Table I). Two peaks of UDP-Glc: 4:0 glucosyltransferase activities were resolved by DEAE-Sepharose chromatography, with one major sharp peak (GTI) of 4:0-specific glucosyltransferase activity followed by one minor peak (GTII; Fig. 2). However, when [^{14}C]12:0 was used as the substrate, the GTII peak exhibited much higher activity than GTI (Fig. 2). These results suggest the presence of at least two UDP-Glc glucosyltransferases with differential fatty acid specificity.

GTI and GTII fractions from DEAE chromatography were pooled separately and each was further purified by Cibacron Blue 3GA-agarose chromatography on the basis of activity toward 4:0 or 12:0, respectively. In both cases,

Table I. *Purification of GTI from L. pennellii leaves*[a]

Step	Total Protein	Total Activity	Specific Activity	Purification	Yield
	mg	*units*	*units/mg*	*-fold*	*%*
Crude extract	3075	1071	0.348		
PEG (0.22–0.37 g/mL) pellet	183	815	4.45	12.8	76.0
DEAE-Sepharose	31.5	564	17.9	51.4	52.7
Cibacron Blue 3GA/agarose	0.222	261	1175	3376	24.4

[a] The reaction mixture contained 5 × 10^5 dpm [^{14}C]4:0 (56 mCi/mmol), 2.5 μmol Bis-Tris, pH 6.8, 75 nmol UDP-Glc, 0.067% (v/v) Triton X-100, and 5 μL of enzyme in a total volume of 15 μL. The incubations were carried out at 37°C for 2 h.

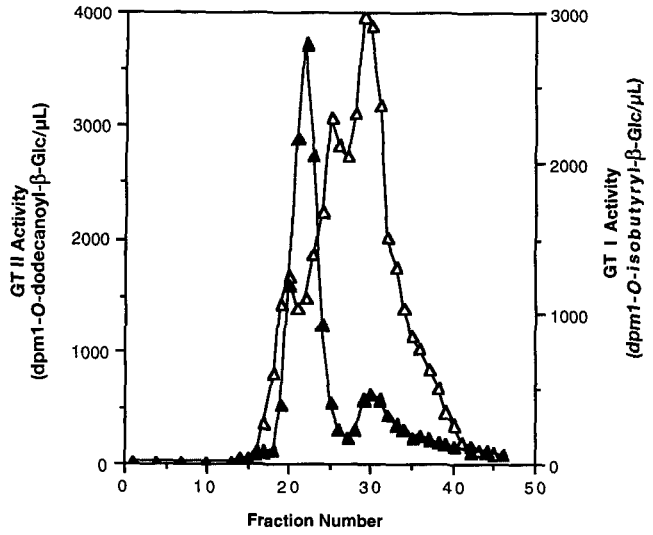

Figure 2. Separation of UDP-Glc:fatty acid transglucosylation activities GTI and GTII by DEAE-Sepharose column chromatography. ▲, Glucosyltransferase activity assayed with 4:0 (GTI); △, glucosyltransferase activity assayed with 12:0 (GTII).

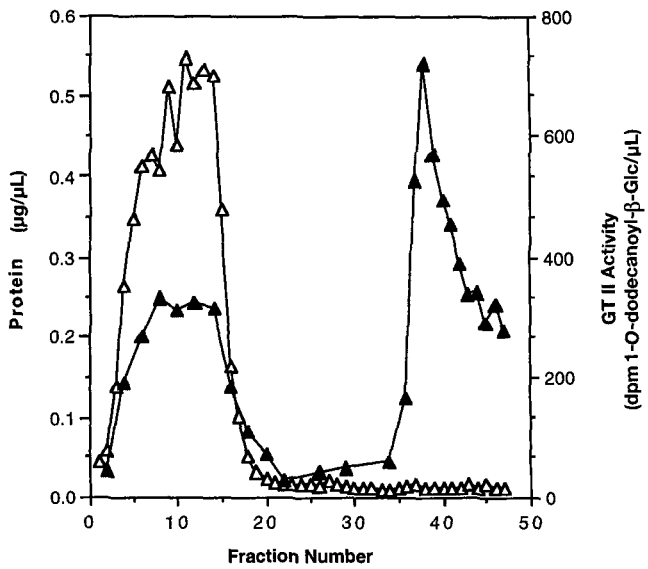

Figure 4. Purification of GTII by Cibacron Blue 3GA-agarose chromatography. ▲, GT II activity; △, protein.

about one-half of the protein loaded was bound to the dye column. For GTI, almost all of the activity was bound to the dye column and was eluted with UDP-Glc (Fig. 3), resulting in 66-fold purification and a 46% yield at this step. For GTII, some of the activity came out of the column in the flow-through, and the bound enzyme was eluted by UDP-Glc, although the resulting activity peak was not as narrow as that of GTI (Fig. 4). GTII was purified nearly 18-fold with a 9% yield at this step. Overall, the combination of PEG precipitation and DEAE-Sepharose and Cibacron Blue 3GA-agarose chromatographies resulted in 3376-fold purification of GTI with a 24.4% yield and 300-fold purification of GTII with a 2.1% yield (Tables I and II). The enzyme

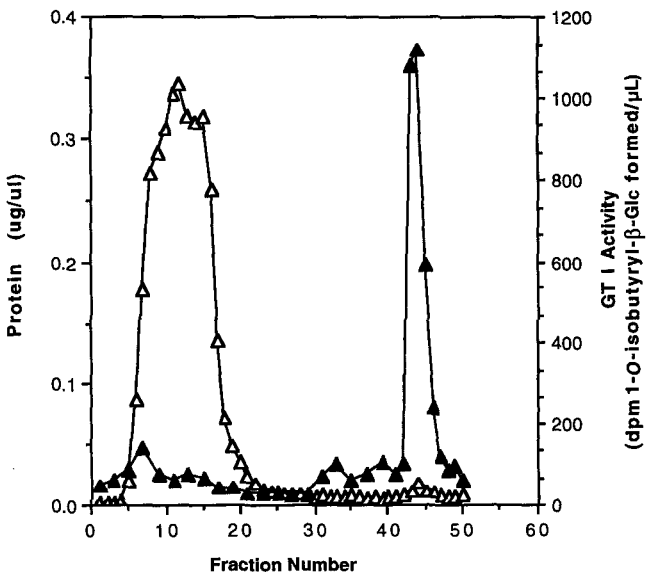

Figure 3. Purification of GTI by Cibacron Blue 3GA-agarose chromatography. ▲, GTI activity; △, protein.

preparations were then concentrated, dialyzed, and used for subsequent kinetic studies.

Other Chromatographic Techniques for GTI

Several chromatographic media were tested for optimal GTI purification. Unlike other UDP-Glc-dependent glucosyltransferases (Latchinian et al., 1987; Leznicki and Bandurski, 1988; McIntosh et al., 1990), GTI did not bind strongly to either UDP-agarose or UDP-glucuronic acid agarose. When a GTI sample obtained following DEAE-Sepharose chromatography was submitted to chromatofocusing (Polybuffer Exchanger, Pharmacia, pH 4–6) or IEF (Bio-Rad Rotofor, pH 4–6), much of the activity was lost; however, the pI of GTI was determined by chromatofocusing to be about 5.0. When a partially purified GTI preparation (PEG precipitation, followed by DEAE-Sepharose and Cibacron Blue 3GA-agarose chromatography) was chromatographed on a Mono-Q HPLC column (Pharmacia), no activity was recovered. GTI did not bind to octyl-Sepharose. Neither GTI nor GTII bound to concanavalin A-Sepharose. Both GTI and GTII possessed a M_r of 47,000 when chromatographed on Sephacryl S-200 and exhibited similar M_rs when analyzed by SDS-PAGE, indicating the monomeric structure of these enzymes. Activity was doubled in the presence of 10 mM Mn^{2+} and increased approximately 50% by the same concentration of Mg^{2+} (data not shown). The GTI activity of a 0.22- to 0.37-g PEG/mL pellet is stable at −20°C for at least 2 months. Fractions obtained from DEAE-Sepharose chromatography were usually stored at −20°C without significant loss of either GTI or GTII activity. Both GTI and GTII purified from Cibacron Blue 3GA-agarose columns retained nearly full activity when stored in 30% glycerol at −20°C for 2 months. The pH optimum for both enzymes in Hepes was 6.8.

Table II. *Purification of GTII from L. pennellii leaves*[a]

Step	Total Protein	Total Activity	Specific Activity	Purification	Yield
	mg	*units*	*units/mg*	*-fold*	*%*
Crude extract	3075	4536	1.48		
PEG (0.22–0.37 g/mL) pellet	183	1452	7.93	5.36	32.0
DEAE-Sepharose	41.4	1034	25.0	16.9	22.8
Cibacron Blue 3GA/agarose	0.211	94	445	300	2.1

[a] The reaction mixture contained 5×10^5 dpm [^{14}C]dodecanoate (12:0) (55 mCi/mmol), 2.5 μmol Bis-Tris, pH 6.8, 75 nmol UDP-Glc, 0.067% (v/v) Triton X-100, and 5 μL of enzyme in a total volume of 15 μL. Incubations were carried out at 37°C for 2 h.

Kinetic Studies

Both GTI and GTII showed similar affinity toward UDP-Glc. The K_m of GTI and GTII for UDP-Glc were 108 and 126 μM, respectively (Table III), which is typical of UDP-Glc-dependent glucosyltransferases (Shimizu and Kojima, 1984; Latchinian et al., 1987). However, GTI and GTII exhibited very different specificities toward fatty acids of varying chain lengths. The K_m of GTI for 4:0 was 230 μM, whereas K_m for 8:0 and 12:0 were 2 and 3 times higher, respectively. In addition, the V_{max} of GTI for 4:0 was 5 times higher than that for 8:0 and 12:0. The V_{max}/K_m ratio was 10 times higher for 4:0 than for 8:0 and 12:0, indicating that 4:0 was a much better substrate. Only low glucosyltransferase activity was detected with GT I when long-chain fatty acids (16:0, 18:0, 18:1) were used as substrates (data not shown).

In contrast to GTI, GTII favored medium-chain fatty acids. The K_ms of GTII for 8:0 and 12:0 were 87.9 and 196 μM (Table III), respectively, significantly lower than that for *i*4:0 (789 μM). Also, V_{max} was 2 times higher for 8:0 and 12:0 than for 4:0. GTII showed higher activity toward long-chain fatty acids (16:0, 18:1, 18:2) than did GT I (data not shown), although K_m and V_{max} values indicated that these acids were substantially poorer substrates than 8:0 and 12:0.

Table III. *Kinetic parameters for GTI and GTII*[a]

Substrate	K_m	V_{max}	V_{max}/K_m
	μM	*units/mg*	$\times 10^{-3}$
GTI			
UDP-Glc	108[b]		
*i*4:0	230	2.389	10.4
8:0	538	0.543	1.01
12:0	660	0.540	0.82
GTII			
UDP-Glc	126[c]		
*i*4:0	789	0.215	0.27
8:0	87.9	0.595	6.77
12:0	196	0.509	2.60

[a] All K_m determinations for fatty acids used were 5 mM UDP-Glc. [b] Determined at 5 mM 4:0. [c] Determined at 610 μM 12:0.

Comparison of UDP-Glc:Fatty Acid Glucosyltransferase Activities of *L. esculentum*, *L. pennellii*, and Two Interspecific Periclinal Chimeras

To assess the tissue localization of UDP-Glc:fatty acid glucosyltransferases, activities toward *i*4:0, 8:0, and 12:0 acids were compared in *L. pennellii*, *L. esculentum*, and in periclinal chimeras constructed from *L. pennellii* and *L. esculentum* (Table IV). In contrast to our previous report indicating that *L. esculentum* and *L. pennellii* possess similar levels of UDP-Glc:fatty acid glucosyltransferase activities (Ghangas and Steffens, 1993), under the conditions used in this study, the glucosyltransferase activity of *L. esculentum* was significantly higher than that of *L. pennellii*, particularly when the medium-chain fatty acids (8:0 and 12:0) were assayed (Table IV).

Interspecific periclinal chimeras have been used to demonstrate that the ability to accumulate acylglucoses is an epidermis-autonomous trait (Goffreda et al., 1990b): the PEE periclinal chimera contributes only layer 1 (epidermis) of *L. pennellii* to layers 2 and 3 (mesophyll and vascular system, respectively) of *L. esculentum*, secretes acylglucoses at the same level, and possesses the same fatty acid substituents as *L. pennellii* itself (Clayberg, 1975; Goffreda et al., 1990b). Hence, layer 1, or the epidermis, of *L. pennellii* is sufficient to confer upon *L. esculentum* the ability to secrete and/or synthesize triacylglucoses at approximately 20% of leaf dry weight. Furthermore, when additional chimeras were examined, for example, one consisting of *L. pennellii* layers 1, 2, and 3 derived from *L. esculentum* (PPE), no changes in the levels or structural characteristics of triacylglucoses were observed. Therefore, the distinct tissue lineages do not interact in their control of triacylglucose synthesis and/or secretion, and the regulation of triacylglucose synthesis and/or secretion is described as layer-1 autonomous.

Here we used periclinal chimeras to determine whether UDP-Glc:fatty acid glucosyltransferase activities are similarly epidermis-autonomous characters. Table IV shows, in agreement with previous studies, that chimeras possessing the epidermis of *L. pennellii* (PPE and PEE) exhibit levels of 2,3,4-tri-O-acylglucoses similar to that accumulated by *L. pennellii*, whereas *L. esculentum* (EEE) produces very low amounts of epicuticular sugar esters. However, UDP-Glc: fatty acid transglucosylase activities specific for *i*4:0, 8:0, and 12:0 are significantly higher in *L. esculentum* (EEE)

Table IV. *Comparison of UDP-Glc:fatty acid glucosyltransferase activities[a] in L. pennellii, L. esculentum, and interspecific periclinal chimeras*

Plant Source[b]	2,3,4-Tri-*O*-acylglucose	Isobutyrate (4:0)	Octanoate (8:0)	Dodecanoate (12:0)
	μg Glc eq/cm²[c]	*nmol mg⁻¹ protein h⁻¹*		
PPP	164.9 ± 23.4	0.324 ± 0.042[d]	1.954 ± 0.284	7.076 ± 0.207
PPE	143.5 ± 18.6	0.918 ± 0.053	6.598 ± 0.090	14.79 ± 0.727
PEE	159.6 ± 31.1	0.246 ± 0.036	7.155 ± 0.565	13.73 ± 2.768
EEE	11.74 ± 1.08	1.086 ± 0.064	16.53 ± 0.057	31.33 ± 1.584

[a] The reaction mixture was the same as described in "Materials and Methods," except that 10^6 cpm fatty acid and PEG pellets (0.15–0.37 g/mL) were used in the enzyme assay. [b] PPP, *L. pennellii*; PPE, a chimera composed of *L. pennellii* layers 1 and 2 (epidermis and mesophyll, respectively), and *L. esculentum* layer 3 (vascular tissue); PEE, a chimera composed of *L. pennellii* layer 1 (epidermis) and *L. esculentum* layers 2 and 3 (mesophyll and vascular tissue, respectively); and EEE, *L. esculentum*. [c] 2,3,4-Tri-*O*-acylglucose was determined according to the method described by Goffreda et al. (1990a). The values are the means ± SD of four replicates. [d] Enzyme activities expressed as nmol 1-*O*-β-acylglucose formed mg^{-1} protein h^{-1}. The values are the means ± SD of three replicates.

relative to *L. pennellii*. Both UDP-Glc:12:0 and UDP-Glc:8:0 glucosyltransferase activities appear to be controlled in a layer-autonomous manner, with epidermal layers of *L. esculentum* contributing approximately one-half of the total leaf UDP-Glc:fatty acid glucosyltransferase activity. The *L. esculentum* mesophyll contributes relatively little to total leaf UDP-Glc:fatty acid glucosyltransferase activity toward the 8:0 and 12:0 substrates. However, a significant portion of the leaf UDP-Glc:fatty acid glucosyltransferase activities is contributed by the vascular tissue layer of *L. esculentum*.

The control of UDP-Glc:4:0 glucosyltransferase activity in periclinal chimeras is more complex. Again, activity toward this substrate is significantly higher in *L. esculentum* leaf extracts than in *L. pennellii*. However, comparison of the PPE chimera and *L. esculentum* (EEE) indicates that most of this difference is contributed by the presence of the *L. esculentum* L3 (vascular system). Evidence for nonlayer-autonomous control of UDP-Glc:4:0 glucosyltransferase activity is apparent in the observation that the *L. esculentum* genotype in layers 2 and 3 in the PEE chimera results in an overall reduction of leaf UDP-Glc:4:0 glucosyltransferase activity to a level similar to that of *L. pennellii*. Our understanding of the interactions between genetically distinct cell layers in chimeric plants is known primarily from morphological observations (Hake and Freeling, 1986; Stewart et al., 1972; Szymkowiak and Sussex, 1989) and molecular studies (Lucas et al., 1995). Additional studies of metabolism in periclinal chimeras may provide a means to examine how genetically distinct metabolic control systems interact between cell layers.

DISCUSSION

The first intermediate in the biosynthesis of 2,3,4-tri-*O*-acylglucose in *L. pennellii*, 1-*O*-acyl-β-Glc, is synthesized from UDP-Glc and fatty acid. The activated acyl group of the 1-*O*-β-acylglucose serves as an acyl donor in subsequent acyl transfer reactions. In this paper we show that there are at least two operationally soluble UDP-Glc:fatty acid glucosyltransferases in *L. pennellii*, GTI and GTII. GTI expresses optimal activity toward short-chain fatty acids (4:0), whereas GTII prefers medium-chain fatty acids (8:0 and 12:0). These activities are consistent with the mainly short- (4:0 and 5:0) and medium-chain-length (10:0 and 12:0) fatty acid composition of *L. pennellii* 2,3,4-tri-*O*-acylglucoses (Burke et al., 1987; Walters and Steffens, 1990). The strong activity of GTII toward 8:0 indicates that the mechanism of 6:0 to 9:0 fatty acid exclusion from *L. pennellii* triacylglucoses is unlikely to act at the level of fatty acid activation. In addition, the transacylation step also proceeds with little specificity for fatty acyl chain length of the 1-*O*-β-acylglucose donor (Ghangas and Steffens, 1995; G.S. Ghangas and J.C. Steffens, unpublished data). Therefore, the acyl chain-length composition of 2,3,4-tri-*O*-acylglucoses is likely to be controlled at the level of elongation or termination of fatty acid synthesis.

Unlike *L. pennellii*, the leaves of *L. esculentum* do not produce significant amounts of polyacylated sugars. However, they possess higher UDP-Glc:fatty acid glucosyltransferase activity than *L. pennellii* leaves, especially when medium-chain fatty acids are used as the substrates. The localization of the triacylglucose biosynthetic pathway remains unclear. Because 2,3,4-tri-*O*-acylglucose accumulation can be shown to behave in a *L. pennellii* layer-1 autonomous fashion (Table IV; Goffreda et al., 1990b), it has been concluded that the epidermis of *L. pennellii* is sufficient to specify the synthesis of these compounds. The observation that UDP-Glc:4:0 glucosyltransferase activity is not layer autonomous and that strong UDP-Glc:fatty acid transglucosylation activities are present in layers 2 and 3 in addition to layer 1 may argue against an exclusively epidermal localization of 2,3,4-tri-*O*-acylglucose biosynthesis. Alternatively, these UDP-Glc-dependent activities may be utilizing fatty acids as adventitious substrates and may have little relevance to the specific enzymes responsible for activation of fatty acids destined to be incorporated into triacylglucoses. However, *L. pennellii* trichome extracts do not exhibit significantly higher GTI- and GTII-specific activities than leaf extracts, again suggesting that this portion of the pathway may not be epidermis specific. The organization and localization of the pathway by which *L. pennellii*

trichomes secrete about 20% of leaf dry weight as 2,3,4-tri-*O*-acylglucoses remains an open question.

The high levels of nonepidermal UDP-Glc:fatty acid transglucosylation activities may also be related to other biosynthetic activities. Leaf extracts of many nonacylsugar-producing monocot and dicot species also possess UDP-Glc:fatty acid transglucosylase activities toward a range of fatty acids (G.S. Ghangas and J.C. Steffens, unpublished data). However, to date there is only one other report of 1-*O*-β-fatty acylglucoses as plant natural products, that being the identification of 1-*O*-β-D-Glc esters of 16:0, 18:1, 18:2, and 18:3 acids from *Brassica napus* pollen (Mandava and Mitchell, 1972). These results imply that UDP-Glc:fatty acid transglucosylases and their 1-*O*-β-fatty acylglucose products may have functions in plant metabolism in addition to their role in synthesis of 2,3,4-tri-*O*-acylglucoses or related polyacylated sugars.

UDP-Glc:fatty acid glucosyltransferases resemble the UDP-Glc-dependent glucosyltransferases responsible for synthesis of Glc or quinic acid esters of phenolic acids (Strack, 1980; Gross, 1982, 1983, 1992; Shimizu and Kojima, 1984). It is not known whether purified UDP-Glc:phenolic acid glucosyltransferases are capable of UDP-Glc-dependent fatty acid glucosylation (or vice versa), or whether plants possess a discrete repertoire of glucosyl transferases specific for either fatty acids or phenolic acids. Answers to these questions may provide insight into the evolution of the acylsugar biosynthetic pathway and secondary metabolism.

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LITERATURE CITED

Burke BA, Goldsby G, Mudd JB (1987) Polar epicuticular lipids of *Lycopersicon pennellii*. Phytochemistry **26:** 2567–2571

Clayberg CC (1975) Insect resistance in a graft-induced periclinal chimera of tomato. Hortic Sci **10:** 13–15

Fobes JF, Mudd JB, Marsden MPF (1985) Epicuticular lipids on the leaves of *Lycopersicon pennellii* and *Lycopersicon esculentum*. Plant Physiol **77:** 567–570

Ghangas GS, Steffens JC (1993) UDPglucose:fatty acid transglucosylation and transacylation in triacylglucose biosynthesis. Proc Natl Acad Sci USA **90:** 9911–9915

Ghangas GS, Steffens JC (1995) 1-*O*-acyl-β-D-glucoses as fatty acid donors in transacylation reactions. Arch Biochem Biophys **318:** 370–377

Goffreda JC, Ave DA, Mutschler MA, Tingey WM, Steffens JC (1989) Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, *Lycopersicon pennellii*. J Chem Ecol **15:** 2135–2147

Goffreda JC, Steffens JC, Mutshler MA (1990a) Association of epicuticular sugars with aphid resistance in hybrids with wild tomato. J Am Soc Hortic Sci **115:** 161–165

Goffreda JC, Szymkowiak EJ, Sussex IM, Mutschler MA (1990b) Chimaeric tomato plants show that aphid resistance and triacylglucose production are epidermal autonomous characters. Plant Cell **2:** 643–649

Gross GG (1982) Synthesis of β-glucogallin from UDP-glucose and gallic acid by an enzyme preparation from oak leaves. FEBS Lett **148:** 67–70

Gross GG (1983) Partial purification and properties of UDP-glucose:vanillate 1-*O*- glucosyl transferase from oak leaves. Phytochemistry **22:** 2179–2182

Gross GG (1992) Enzymatic synthesis of gallotannins and related compounds. *In* HA Stafford, RK Ibrahim, eds, Phenolic Metabolism in Plants. Plenum Press, New York, pp 297–323

Hake S, Freeling M (1986) Analysis of genetic mosaics shows that the extra epidermal cell divisions in *Knotted* mutant maize plants are induced by adjacent cells. Nature **320:** 621–623

Hawthorne DJ, Shapiro JA, Tingey WM, Mutschler MA (1992) Trichome-borne and artificially applied acylsugars of wild tomato deter feeding and oviposition of the leafminer *Liriomyza trifolii*. Entomol Exp Appl **65:** 65–73

Latchinian L, Khouri HE, Ibrahim RK (1987) Fast protein affinity chromatography of two flavonoid glucosyltransferases. J Chromatogr **388:** 235–242

Leznicki J, Bandurski RS (1988) Enzymatic synthesis of indole-3-acetyl-1-*O*-β-D-glucose. I. Partial purification and characterization. Plant Physiol **88:** 1474–1480

Liedl BE, Lawson DM, White KK, Shapiro JA, Cohen DE, Carson WG, Trumble JT, Mutschler MA (1995) Acylsugars of wild tomato *Lycopersicon pennellii* alter settling and reduce oviposition of *Bemisia argentifolii* (Homoptera:Aleyrodidae). J Econ Entomol **88:** 742–748

Lucas WJ, Bouche-Pillon S, Jackson DP, Nguyen L, Baker L, Ding B, Hake S (1995) Selective trafficking of KNOTTED-1 homeodomain protein and its βmRNA through plasmodesmata. Science **270:** 1980–1983

Mandava N, Mitchell JW (1972) Structural elucidation of brassins. Chem Ind 930–931

McIntosh CA, Latchinian L, Mansell RL (1990) Flavanone-specific 7-glucosyltransferase activity in *Citrus paradisi* seedlings: purification and characterization. Arch Biochem Biophys **282:** 50–57

Mutschler MA, Steffens JC, Tingey WM (1993) Broad-based insect resistance from *L. pennellii*: its nature and potential for pest control in tomato. *In* JI Yoder, ed, Molecular Biology of the Tomato. Technomic Publishing, Lancaster, PA, pp 285–291

Shimizu T, Kojima M (1984) Partial purification and characterization of UDPG:*t*-cinnamate glucosyltransferase in the root of sweet potato, *Ipomoea batatas* Lam. J Biochem **95:** 205–212

Stewart RN, Meyer FG, Dermen H (1972) Camellia + "Daisy Eagleson", a graft chimera of *Camellia sasanqua* and *C. japonica*. Am J Bot **59:** 515–524

Strack D (1980) Enzymatic synthesis of 1-sinapoylglucose from free sinapic acid and UDP-glucose by a cell-free system from *Raphanus sativus* seedlings. Z Naturforsch **35c:** 204–208

Szymkowiak EJ, Sussex IM (1989) Chimeric analysis of cell layer interactions during development of the flower pedicel abscission zone. NATO ASI Adv Sci Inst Ser Ser H Cell Biol **35:** 362–368

Walters DS, Steffens JC (1990) Branched chain amino acid metabolism in the biosynthesis of *Lycopersicon pennellii* glucose esters. Plant Physiol **93:** 1544–1551