Plant Physiol. (1995) 109: 505–511

Galactinol Synthase from Kidney Bean Cotyledon and Zucchini Leaf[1]

Purification and N-Terminal Sequences

Jingjie J. Liu, William Odegard, and Benito O. de Lumen*

Department of Nutritional Sciences, University of California, Berkeley, California 94720

Galactinol synthase (GS) was purified 1591-fold with a 3.9% recovery from the cotyledon of kidney bean (*Phaseolus vulgaris*) by a novel scheme consisting of ammonium sulfate fractionation followed by diethylaminoethyl, Affi-Gel Blue, and UDP-hexanolamine affinity chromatography. The purified enzyme had a specific activity of 8.75 μmol mg^{-1} min^{-1}, a pH optimum of 7.0, and requirements for manganese ion and DTT. The enzyme exhibited a K_m = 0.4 mM for UDP-galactose and a K_m = 4.5 mM for *myo*-inositol. It was identified as a 38-kD peptide that co-purified with a 41- and a 43-kD peptide as shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Purification to homogeneity was achieved by isolating the 38-kD peptide from the SDS-PAGE gel. To clarify conflicting reports in the literature about the relative molecular mass of purified GS from zucchini leaf (*Cucurbita pepo*), a similar scheme with modified eluting conditions was used to purify GS from this source. Zucchini leaf GS was purified to homogeneity and identified as a 36-kD peptide on SDS-PAGE. Partial N-terminal sequences of the 38-kD peptide from kidney bean cotyledon and the 36-kD peptide from zucchini leaf were obtained. To facilitate identification of GS during the purification, an assay utilizing thin-layer chromatography and an isotopic analytic imaging scanner was developed.

The raffinose family of GO are widely present in various higher plants (French, 1954; Lee et al., 1970). The biosynthesis of raffinose and stachyose occurs by sequential transfers of galactosyl units from galactinol to Suc and then to raffinose by specific transferases (Lehle and Tanner, 1972). Galactinol is synthesized by UDP-D-Gal:*myo*-inositol (1-α-D) galactosyltransferase (GS; EC 2.4.1.123), which catalyzes the following reaction: UDP-Gal + *myo*-inositol → galactinol + UDP. Since the only known function of galactinol is in the formation of GO, GS is believed to play a key role in the regulation of the synthesis of these sugars (Handley et al., 1983).

Partial purification of GS from mature cucumber and zucchini (*Cucurbita pepo*) leaves has been reported using similar techniques such as DEAE anion-exchange and size-exclusion chromatography (Pharr et al., 1981; Webb, 1982). A modified procedure using DEAE anion-exchange, hydrophobic interaction followed by size-exclusion chromatography as the final purification step gives a 752-fold purification of GS from zucchini leaf with minor contamination and identification of GS as a 42-kD protein (Smith et al., 1991). Two modifications of this scheme result in purification of GS to homogeneity. One, utilizing fast-protein liquid chromatography Mono-P chromatography and preparative SDS-PAGE, yields a 37-kD monomer (Kuo and Alexander, 1992), and the other, using fast-protein liquid chromatography Mono-Q chromatography, yields a 38-kD doublet. However, the procedure of purification has been described only briefly (Kuo and Alexander, 1992) or reported indirectly (Beebe and Turgeon, 1992).

Despite considerable interest in GS from zucchini leaf, the purification and biological roles of GS from kidney bean (*Phaseolus vulgaris*) seed have not been well studied. The accumulated evidence shows that GO may play an important role in providing desiccation tolerance to developing seeds. At the late stage of soybean seed development, raffinose and stachyose begin to accumulate as seeds lose water (Dey, 1985; Saravitz et al., 1987; Castillo et al., 1990). Suc and GO are consistently present in the desiccation-tolerant stage of seeds, and desiccation tolerance is lost when GO disappear, even when Suc is present (Koster and Leopold, 1988). Artificial slow drying of immature soybean seeds excised from pods that induces desiccation tolerance is strongly correlated (r^2 = 0.835) with a dramatic increase in stachyose content in the axes (Blackman et al., 1992). It has been suggested that the formation of a vitrified state (glassy, noncrystalline state) in the cytoplasm is an intrinsic mechanism of desiccation tolerance in developing seeds (Leopold and Vertucci, 1986). In this hypothesis, Suc is the principal protectant in desiccation, whereas GO serve to keep Suc from crystallizing.

We are interested in establishing GS as a key enzyme in controlling GO biosynthesis and elucidating the role of GO in seed desiccation tolerance. Toward this end, we purified GS from kidney bean cotyledon with the objective of cloning a cDNA encoding the enzyme.

[1] This work was supported in part by U.S. Department of Agriculture National Research Initiative Competitive Grants Program (grant No. 93-03045), the Hasselblad Foundation (Sweden), the California Dry Bean Board, and the Nebraska Dry Bean Commission.

* Corresponding author; e-mail nitto@nature.berkeley.edu; fax 1-510-642-0535.

Abbreviations: GO, Gal oligosaccharide(s); GS, galactinol synthase; RB, running buffer (50 mM Hepes-Na, 1 mM DTT, pH 7.0).

We present here a novel purification scheme that combines ammonium sulfate fractionation, DEAE anion-exchange chromatography, Affi-Gel Blue, and UDP-hexanolamine affinity chromatography. This procedure led to the identification of kidney bean seed GS as a 38-kD peptide and further purification to homogeneity by SDS-PAGE for sequencing. To resolve the discrepancy in the reported molecular mass of GS from zucchini leaf, we also purified GS from this source and confirmed its molecular mass as 36 kD. The N-terminal sequences of these two GS's show homology neither with each other nor with any other protein sequences reported in the data base.

MATERIALS AND METHODS

Chemicals

UDP-[U-^{14}C]Gal was purchased from Amersham. Affi-Gel Blue gel, Econo-Pac 10DG desalting columns, and the protein assay kit were obtained from Bio-Rad. TOYOPEARL DEAE-650 M was from Supelco (Montgomeryville, PA). UDP-Gal, *myo*-inositol, Hepes, DTT, and UDP-hexanolamine agarose gel were purchased from Sigma. All other chemicals were obtained from Fisher.

Plant Materials

Seeds of red kidney bean (*Phaseolus vulgaris*, var Big Red) were acquired from Dr. Paul Gepts (Agronomy Department at University of California, Davis). Plants were grown in a greenhouse under a 16-h light/8-h dark cycle. Seeds were harvested 25 DAF.

Zucchini plants (*Cucurbita pepo*, var Black Beauty, from Advance Seed Co., Fulton, KY) were grown under greenhouse conditions as above and mature leaves were collected.

Enzyme Assay

At the end of each purification step, GS was assayed by a modified procedure of Handley and Pharr (1982). A 25-μL reaction mixture contained buffer (50 mM Hepes-Na, 2 mM DTT, pH 7.0), 4 mM $MnCl_2$, 4 mM UDP-[U-^{14}C]Gal (0.25 μCi/μmol), 20 mM *myo*-inositol, 4 μg of BSA, and 10 μL of enzyme dialyzed against the reaction buffer before each assay. The enzyme was preincubated with the buffer and $MnCl_2$ at 30°C for 15 min and then incubated with the substrates at 30°C for 15 min. The reaction was stopped by adding 50 μL of ice-cold 100% ethanol and centrifuging in a microcentrifuge. An aliquot of 15 μL was spotted on a TLC plate (silica gel 60) and air dried. The plate was developed in a sealed tank containing 1-propanol:ethyl acetate:water in the ratio of 7:1:2 (v/v/v) for 4 h to overnight, air dried, and redeveloped in the same tank for another 4 h. An analytic imaging scanner (System 200, Bioscan, Inc., Washington, DC) was used to identify and quantify labeled galactinol. The identity of galactinol was established by running authentic galactinol (courtesy of Dr. D. Pharr) on TLC and staining for sugar with the silver nitrate-alkaline method (Zweig and Sherma, 1972).

Purification of GS from Kidney Bean Cotyledon

All purification steps were carried out on ice or at 4°C with an RB. EDTA (disodium salt, 0.5 M stock solution, pH 8.0) was added to RB to a final concentration of 25 mM without adjusting the pH of RB during affinity chromatography.

Preliminary experiments showed that kidney bean cotyledon has the highest GS activity among all of the components of seed (de Lumen et al., 1991). For this reason, seed coats and axes were removed from seeds that had been collected at 25 DAF. One hundred grams of cotyledon were frozen in liquid nitrogen, ground to a fine powder in a mortar and pestle, and homogenized in 400 mL of cold extraction buffer (1 mM PMSF in RB) with a Polytron (Brinkmann) at 6000 rpm for 30 s. The homogenate was filtered through four layers of cheesecloth and centrifuged at 28,000*g* for 15 min. The supernatant was fractionated by the addition of solid ammonium sulfate, and the fraction that precipitated between 25 and 40% saturation was collected by centrifugation at 10,000*g* for 10 min. The precipitate was resuspended in 50 mL of RB and dialyzed against the same buffer overnight.

The dialysate was centrifuged at 10,000*g* for 10 min and the supernatant was applied to a TOYOPEARL DEAE-650 M column (2.5 × 18 cm) previously equilibrated with RB. Proteins bound to the column were washed with 3 bed volumes of RB at a flow rate of 1.5 mL/min. GS activity was eluted with 0.1 M NaCl in RB. The active fractions were precipitated again with ammonium sulfate at 70% saturation. The precipitate was resuspended immediately in a small volume of RB and dialyzed on an Econo-Pac 10DG desalting column (Bio-Rad) previously equilibrated with 25 mM $MnCl_2$ in RB.

The desalted sample was applied to an Affi-Gel Blue gel column (1.5 × 8.5 cm) pre-equilibrated with 25 mM $MnCl_2$ in RB at a flow rate of 0.3 mL/min. The sample was allowed to stay in the column for at least 1 h by stopping the flow. The column was washed first with 25 mM $MnCl_2$ in RB, followed by RB only, and then with 25 mM EDTA in RB. GS activity was detected during the wash with 25 mM EDTA in RB. Active fractions were pooled and concentrated to 3 mL by Ultrafree-CL filter (Millipore) and dialyzed on an Econo-Pac 10DG desalting column pre-equilibrated with 25 mM $MnCl_2$ in RB.

The desalted sample (4 mL) was applied to a UDP-hexanolamine agarose gel column (1.5 × 6 cm) pre-equilibrated with 25 mM $MnCl_2$ in RB at a flow rate of 0.3 mL/min. The sample was allowed to stay in the column for at least 1 h by stopping the flow. Washing with 25 mM $MnCl_2$ in RB eluted the unbound proteins, and then elution with 25 mM EDTA in RB released the GS activity from the column in a sharp peak. Highly purified GS from this step was dialyzed in RB and used for enzyme kinetics studies or prepared for isolating homogeneous peptide by SDS-PAGE.

Purification of GS from Zucchini Leaf

Extraction was the same as above except 100 g of mature leaves were chopped and homogenized in a blender (War-

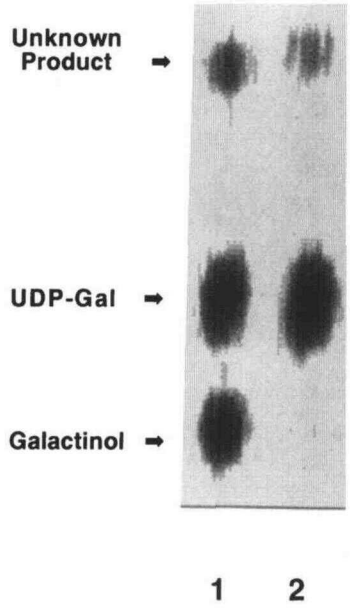

Figure 1. GS assay. Spots representing radioactive UDP-Gal, galactinol, and an unknown product from the reaction mixture (see "Materials and Methods" for details) were located and quantitated using the Bioscan analytic imaging scanner. Lane 1, Active fraction from DEAE preparation; lane 2, reaction mixture with boiled enzyme as negative control.

ing). The purification of zucchini leaf GS differed from that of kidney bean GS in the following modifications: (1) Ammonium sulfate fractionation was carried out between 35 and 55% saturation. (2) Proteins bound to the DEAE column were washed with 0.1 M NaCl in RB first. GS was eluted with 0.175 M NaCl in RB. (3) No $MnCl_2$ was added to either desalting buffer or equilibration buffer for Affi-Gel Blue affinity chromatography. GS was present in unbound fractions. (4) Pooled active fractions from the Affi-Gel Blue column were supplemented with $MnCl_2$ to a final concentration of 25 mM and applied to a UDP-hexanolamine column pre-equilibrated with 25 mM $MnCl_2$ in RB.

SDS-PAGE

SDS-PAGE was performed using a 12.5% separating gel with a Hoefer minigel apparatus (Hoefer Scientific, San Francisco, CA), according to the method of Laemmli (1970). Proteins were stained with 0.1% Coomassie brilliant blue R or silver reagent. The relative molecular mass of highly purified enzyme was determined by SDS-PAGE based on the mobility of the peptide. Molecular mass markers used were rabbit muscle phosphorylase *b* (97.4 kD), BSA (66.2 kD), hen egg white ovalbumin (45.0 kD), bovine carbonic anhydrase (31.0 kD), soybean trypsin inhibitor (21.5 kD), and hen egg white lysozyme (14.4 kD) (Bio-Rad).

Protein Blotting and Sequencing

Purified GS sample was concentrated with an Ultrafree filter and subjected to SDS-PAGE as described above. Proteins were blotted onto a Transblot membrane (Bio-Rad) using the Milliblot semidry electroblotting system (Millipore) with 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid buffer containing 10% methanol, pH 8.5, and stained with 0.1% Coomassie brilliant blue R. The band containing GS was cut from the membrane and sequenced on an Applied Biosystems 477 sequencer by the Protein Structure Laboratory at the University of California, Davis.

Protein Determination and Enzyme Characterization

Protein concentration was determined with a Bio-Rad kit with BSA as the standard, using the method of Bradford (1976). Estimation of K_m values was based on the Michaelis-Menten equation. The optimal pH for GS assay was determined using the following buffers: pH 4.0 to 5.0, Na-acetate buffer; pH 5.5 to 6.5, Mes-Na buffer; pH 7.0 to 8.0, Hepes-Na buffer.

RESULTS AND DISCUSSION

GS Assay

Figure 1 shows a typical two-dimensional image of separated reaction mixture on TLC plate. Labeled galactinol was clearly resolved from labeled UDP-Gal and an unidentified product and quantitated by the Bioscan analytic imaging scanner. The enzyme samples taken at different stages of purification were regularly diluted before assay to ensure that the reaction rates were in a linear relationship with time. BSA was essential to preserve GS activity during the assay, especially when the protein concentration was low at the final step. The enzyme samples were usually dialyzed with a multiple-well microdialysis system (GIBCO-BRL) before assays. Fractions containing GS activity were subjected to SDS-PAGE to analyze protein patterns.

Table I. *Purification of GS from kidney bean cotyledon*

One hundred grams of kidney bean cotyledon were processed.

Purification Step	Total Protein	Total Activity	Specific Activity	Purification	Yield
	mg	*μmol min^{-1}*	*μmol min^{-1} mg^{-1}*	*fold*	*%*
Crude extract	2,565	14.22	0.0055		
Ammonium sulfate precipitation	455	9.69	0.021	3.8	68.1
DEAE	99	11.09	0.111	20.2	78.0
Affi-Gel Blue	1.62	2.43	1.50	273	17.1
UDP-hexanolamine	0.064	0.56	8.75	1591	3.9

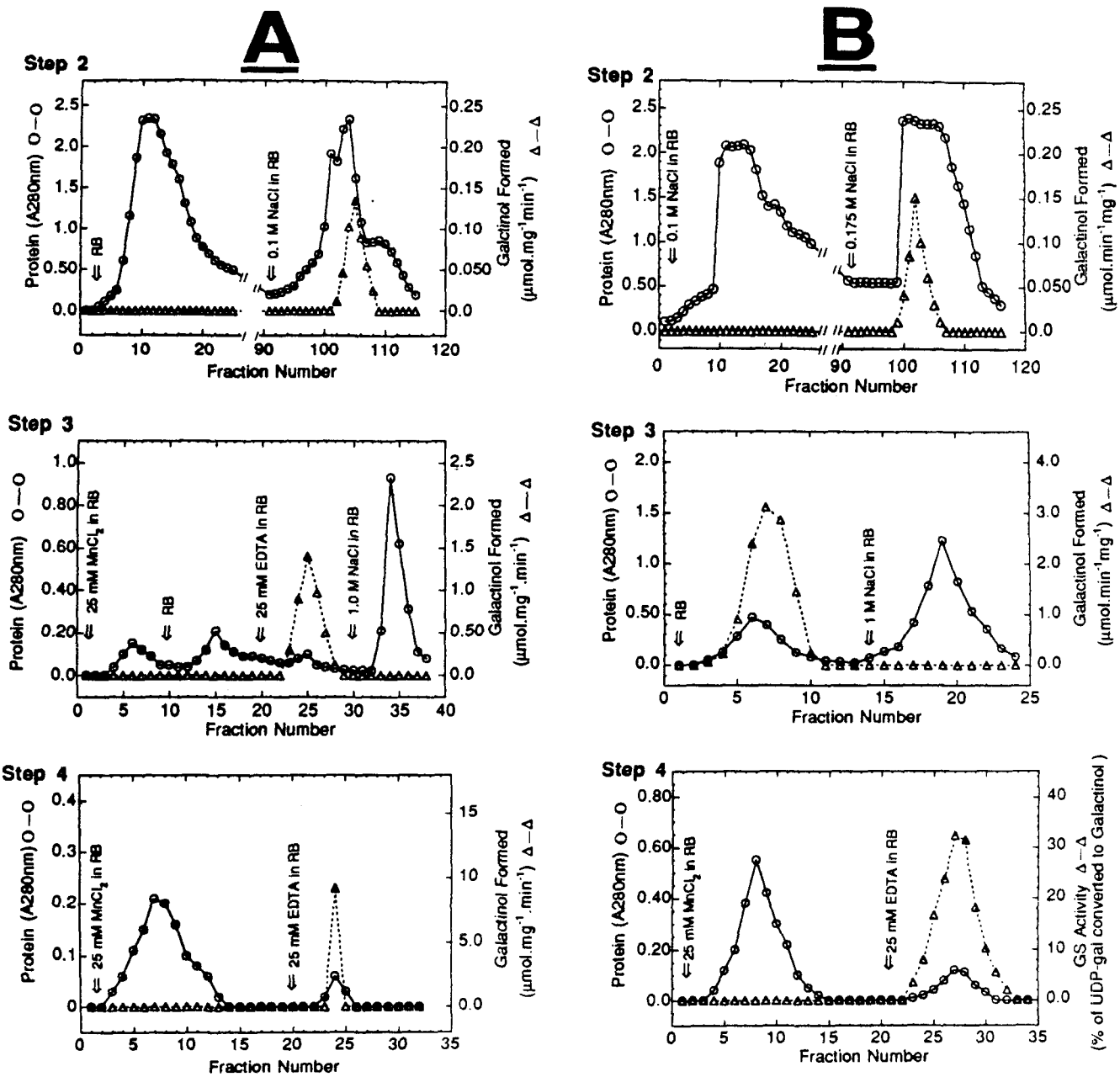

Figure 2. Purification of GS from kidney bean cotyledon (A) and zucchini leaf (B). Step 2, DEAE chromatography; step 3, Affi-Gel Blue affinity chromatography; step 4, UDP-hexanolamine affinity chromatography.

Purification of GS from Kidney Bean Cotyledon

A typical purification of GS from kidney bean cotyledon is summarized in Table I. This procedure resulted in a 1591-fold purification and 3.9% recovery of GS. The purified GS had a specific activity of 8.75 μmol mg^{-1} min^{-1}. Figure 2A shows chromatograms of this purification scheme. Several aspects of the procedure are noteworthy.

Step 1. Ammonium Sulfate Precipitation

Although ammonium sulfate fractionation caused the loss of GS activity by 32%, it removed 82% of the total protein and lipid contaminants from the crude extract and reduced the volume of sample applied to the DEAE column. GS activity was stable in the ammonium sulfate precipitate form at −20°C for 6 to 8 weeks. This was a convenient stopping point during the purification and resulted in no significant loss of GS activity during storage. Ammonium sulfate fractionation has been reported to inhibit GS activity during GS purification from zucchini leaf (Pharr et al., 1981). It was used in this scheme to clear up the crude extract before DEAE preparation and to concentrate the sample for fast dialysis by a desalting column before Affi-Gel Blue affinity chromatography. Recovery of enzyme activity was sacrificed

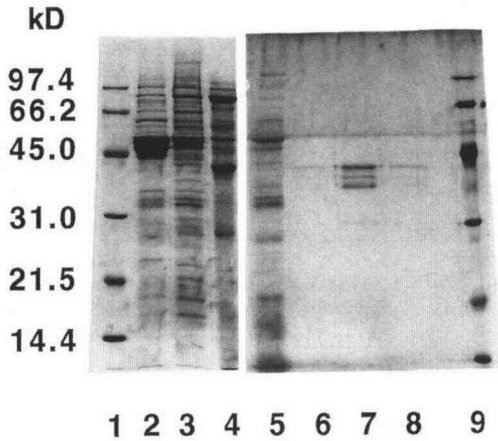

Figure 3. SDS-PAGE of samples containing GS activity taken at different stages of purification from kidney bean cotyledon. Lanes 1 and 9, Molecular mass standards (Bio-Rad); lane 2, crude extract; lane 3, ammonium sulfate precipitate; lane 4, DEAE eluate; lane 5, Affi-Gel Blue eluate; lanes 6 to 8, UDP-hexanolamine eluates. Fractions in lanes 6 and 8 were inactive. Samples were run on the same SDS-PAGE gel. The gel was cut into two pieces and stained separately with Coomassie blue (lanes 1–4) or with silver reagent (lanes 5–9).

because preservation of GS activity by a rapid purification was crucial for the identification of GS. It has been reported that GS activity is lost during the extensive process involving hydrophobic and size-exclusion chromatography (Smith et al., 1991). Precipitate of DEAE active fractions had to be resuspended in the buffer immediately. GS in the pellet could not be dissolved very well in the buffer when the pellet had been frozen and thawed at this stage.

Step 2. DEAE Anion-Exchange Chromatography

In contrast to zucchini leaf GS, which was eluted at 0.175 M NaCl from the DEAE column (Smith et al., 1991), kidney bean GS was eluted at 0.1 M NaCl in RB using the bulk elution mode. A brownish color was found in the most active fractions, which could be used as an indication of GS-containing fractions.

Step 3. Affi-Gel Blue Affinity Chromatography

Affi-Gel Blue gel has been particularly useful in the purification of kinase, dehydrogenase, and other nucleotide-dependent enzymes. It has been suggested that enzymes containing a "dinucleotide fold" bind specifically to the blue dye (Thompson et al., 1975). GS bound to the column in the presence of Mn^{2+} ions when a stepwise wash was used and was released in a sharp peak with 25 mM EDTA in RB. GS activity was not found in the further elution of tightly bound proteins with 1 M NaCl in RB. Attempts to omit Mn^{2+} ions from the pre-equilibration buffer resulted in the release of GS activity over a broad range of fractions. Dialysis of a sample, supplementation with Mn^{2+} ions in the buffer, and slowing down the flow rate were essential for the separation during this step (Fig. 2A, step 3).

Step 4. UDP-Hexanolamine Affinity Chromatography

UDP-hexanolamine affinity chromatography has been used to purify a number of UDP-galactosyl transferases. These enzymes are adsorbed specifically to the UDP-hexanolamine affinity column in the presence of Mn^{2+} ions and released by elution with EDTA in the presence of a competing ligand, such as GlcNAc (Barker et al., 1972), or with a competing ligand alone, such as UDP (Elices et al., 1986). In this experiment, GS and possibly other UDP-binding proteins were adsorbed to the affinity column only in the presence of Mn^{2+} ions. In preliminary experiments, a two-step elution scheme using RB followed by 25 mM EDTA in RB resulted in a broad peak of GS activity. In contrast, elution with 25 mM EDTA in RB immediately after the initial wash (25 mM $MnCl_2$ in RB) resulted in a sharp peak of GS activity (Fig. 2A, step 4).

The silver-stained SDS-PAGE showed that a 38-kD peptide co-purified with a 41- and a 43-kD peptide in the active fraction from the last step (Fig. 3, lane 7). At different stages of the purification, the 38-kD peptide was always present in the active fractions, whereas the 41- and the 43-kD peptide were also present in the inactive fractions, such as tightly bound fractions from Affi-Gel Blue affinity chromatography (data not shown) and neighboring inactive fractions from the last step (Fig. 3, lanes 6 and 8). Furthermore, the increase in activity as measured by the rate of galactinol formation coincided with the enrichment of the 38-kD peptide but not with either the 41- or the 43-kD peptide. For these reasons, we concluded that the 38-kD peptide was GS.

Preliminary purification without Affi-Gel Blue chromatography in the scheme resulted in an inefficient separation of GS with the 41- and 43-kD peptides as major co-purified components in the active fractions. This suggested that 41- and 43-kD peptides might be other UDP-binding proteins

Table II. *Purification of GS from mature zucchini leaf*

One hundred grams of mature zucchini leaf were processed.

Purification Step	Total Protein	Total Activity	Specific Activity	Purification	Yield
	mg	*μmol min^{-1}*	*μmol min^{-1} mg^{-1}*	*fold*	*%*
Crude extract	990	12.9	0.013		
Ammonium sulfate precipitation	635	12.0	0.019	1.5	93
DEAE	173	14.4	0.083	6.4	112
Affi-Gel Blue	1.98	7.0	3.5	270	54
UDP-hexanolamine	0.056	1.8	32.1	2472	14

that could compete with GS for UDP-binding sites in the UDP-hexanolamine agarose column.

Purification of GS from Zucchini Leaf

Affi-Gel Blue and UDP-hexanolamine affinity chromatography separated GS on the basis of its biological properties. By using a similar scheme as that for kidney bean GS, we were able to purify GS from mature zucchini leaf to homogeneity (Fig. 2B; Table II). These two steps improved the purification significantly from previously published methods (Pharr et al., 1981; Smith et al., 1991). Silver-stained SDS-PAGE revealed that there was only a 36-kD peptide in the active fractions from UDP-hexanolamine chromatography (Fig. 4, lanes 4–9). The intensities of protein stain in lanes 4 through 9 paralleled the activities as measured by the rate of galactinol formation, i.e. lane 6, showing the most intense band by silver staining, also showed the highest activity. This relative molecular mass is consistent with that reported by Kuo and Alexander (1992) (37 kD) and Kerr et al. (1992) (38 kD), determined by SDS-PAGE. Zucchini leaf is a much richer source of GS activity. Less starting plant material was needed to obtain a sufficient amount of homogeneous protein for sequencing from the zucchini leaf sample than from the kidney bean cotyledon.

Protein Sequencing

The N-terminal sequences of kidney bean GS and zucchini leaf GS are shown in Figure 5. No homology was found between the N-terminal sequences of GS from kidney bean cotyledon and zucchini leaf. The 10-residue N-terminal sequence of zucchini leaf GS was identical with that reported by Kerr et al. (1992). We concluded therefore that GS from zucchini leaf is a 36-kD peptide when the relative molecular mass is determined by SDS-PAGE. There is no homology among the N-terminal sequences of

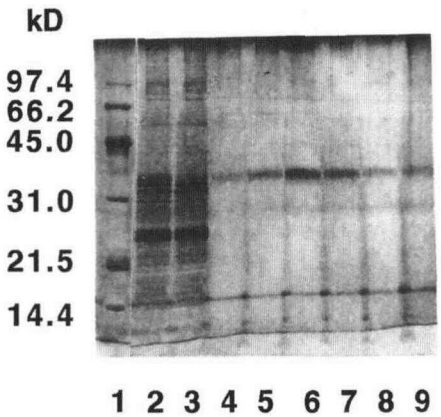

Figure 4. SDS-PAGE of samples containing GS activity taken at different stages of purification from zucchini leaf. Lane 1, Molecular mass standards (Bio-Rad); lane 2, Affi-Gel Blue eluate; lane 3, inactive unbound UDP-hexanolamine eluate; lanes 4 to 9, active bound fractions from UDP-hexanolamine chromatography, corresponding to fractions Nos. 25–30 in chromatogram in Figure 2B. The gel was stained with silver reagent.

Amino Acid Position	1									10										20
Kidney Bean GS:	–	N	K	V	I	N	V	P	A	G	F	?	Y	E	L	Y	N	R	N	R
Zucchini Leaf GS:	P	A	A	T	E	T	A	I	E											

Figure 5. N-terminal sequences of kidney bean cotyledon GS and zucchini leaf GS. ?, Uncertain amino acid residue.

GS from kidney bean cotyledon, reported in this article, and GS from zucchini leaf and soybean seed reported by Kerr et al. (1992). High homology regions were found in the middle of the GS sequences from zucchini leaf and soybean seed.

Since the 43-kD peptide from kidney bean cotyledon was blocked at the N terminus, an internal peptide sequence (VINVPAGFDYELYNR) was obtained after trypsin digestion of the 43-kD peptide and compared with the protein sequences of GS reported by Kerr et al. (1992). No significant homology was found in this comparison. It is interesting that the internal sequence of the 43-kD peptide was found to be identical with the N-terminal sequence of the 38-kD kidney bean GS beginning at position 4 except that position 12 was uncertain in the 38-kD GS. Considering the fact that the 43-kD protein was co-eluted with the 38-kD GS throughout purification, we propose that these two proteins may be closely related, possibly as UDP-galactosyl transferases with different acceptors. The other possibility is that the 43-kD protein is the precursor of the 38-kD GS. This remains to be established until cDNA encoding kidney bean GS is cloned and the cDNA sequence is analyzed. When this 15-residue internal sequence was compared with other protein sequences in the data base, it showed 57.1% homology over 14 amino acid residues with one region of glycinin, a major seed storage protein of soybean. However, there is no significant homology between the 15-residue internal sequence and phaseolin, the major storage protein of kidney bean seed. The N-terminal sequence of GS from kidney bean seed will be used to clone its cDNA.

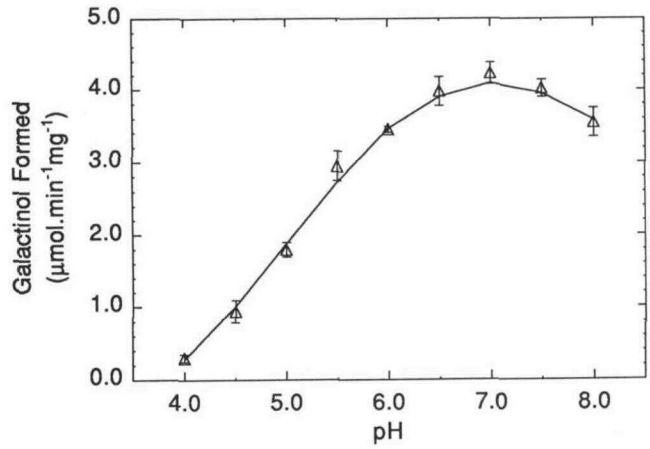

Figure 6. Activity-pH profile of GS from kidney bean cotyledon.

Kinetic Properties of GS from Kidney Bean Cotyledon

Kinetics properties of highly purified GS from kidney bean cotyledon were determined by measuring the reaction rate under standard assay conditions as described in "Materials and Methods" except for variations in buffers or concentrations of UDP-Gal or *myo*-inositol. GS from kidney bean cotyledon had a pH optimum of 7.0 (Fig. 6). Mn^{2+} ion and DTT were absolutely required for GS activity. The enzyme exhibited a high specific affinity for UDP-Gal and *myo*-inositol with K_m values of 0.4 and 4.5 mM, respectively.

ACKNOWLEDGMENTS

We wish to thank Dr. David Pharr of North Carolina State University for kindly providing purified galactinol and Dr. Young Moo Lee and Dr. Jack Presley of the Protein Structure Laboratory at the University of California, Davis, for assistance with protein sequencing. We also appreciate Dr. Tsung Min Kuo of the U.S. Department of Agriculture, Agriculture Research Service (Illinois) for his valuable suggestions and discussions, and Dr. Alfred Galvez and Jay Revilleza for their technical assistance and reviewing of the manuscript.

Received April 7, 1995; accepted June 30, 1995.

Copyright Clearance Center: 0032–0889/95/109/0505/07.

LITERATURE CITED

Barker R, Olsen KW, Shaper JH, Hill R (1972) Agarose derivatives of uridine diphosphate and N-acetylglucosamine for the purification of a galactosyltransferase. J Biol Chem **247:** 7135–7147

Beebe DU, Turgeon R (1992) Localization of galactinol, raffinose and stachyose synthesis in *Cucurbita pepo* leaves. Planta **188:** 354–361

Blackman SA, Obendorf RL, Leopold AC (1992) Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. Plant Physiol **100:** 225–230

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Castillo EM, de Lumen BO, Reyes PS, de Lumen HZ (1990) Raffinose synthase and galactinol synthase in developing seeds and leaves of legumes. J Agric Food Chem **38:** 351–355

de Lumen BO, Liu JJ, Jone GT, Nanagas F (1991) A genetic engineering strategy to reduce flatulence in beans. *In* University of California Dry Bean Research 1991 Progress Report. California Dry Bean Advisory Board, Dinuba, CA, pp 37–42

Dey PM (1985) D-Galactose containing oligosaccharides. *In* PM Dey, RA Dixon, eds, Biochemistry of Storage Carbohydrates in Green Plants. Academic Press, New York, pp 53–130

Elices MJ, Blake DA, Goldstein IJ (1986) Purification and characterization of a UDP-Gal:β-D-Gal(1,4)-D-GlcNAc α(1,3)-galactosyltransferase from Ehrlich ascites tumor cells. J Biol Chem **261:** 6064–6072

French D (1954) The raffinose family of oligosaccharides. Adv Carbohydr Chem **9:** 149–184

Handley LW, Pharr DM (1982) Ion stimulation, UDP inhibition and effects of sulfhydryl reagents on the activity of galactinol synthase from leaves of cucumber, *Cucumis sativus L.* Z Pflanzenphysiol **108:** 447–455

Handley LW, Pharr DM, McFeeters RF (1983) Relationship between galactinol synthase activity and sugar composition of leaves and seeds of several crop species. J Am Soc Hortic Sci **108:** 600–605

Kerr PS, Pearlstein RW, Schweiger BJ, Becker-Manley MF, Pierce JW (1992) Nucleotide sequences of galactinol synthase from zucchini and soybean. International Patent Application Number PCT/US92/06057

Koster KL, Leopold AC (1988) Sugars and desiccation tolerance in seeds. Plant Physiol **88:** 829–832

Kuo TM, Alexander NJ (1992) Partial protein sequence of galactinol synthase from zucchini leaves (abstract No. 430). Plant Physiol **99:** S-72

Laemmli UK (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature **227:** 680–685

Lee CY, Shallenberger RS, Vittum MT (1970) Free sugars in fruits and vegetables. NY Food Life Sci Bull **1:** 1–12

Lehle L, Tanner W (1972) Synthesis of raffinose-type sugars. Methods Enzymol **28:** 522–530

Leopold AC, Vertucci CW (1986) Physical attributes of desiccation seeds. *In* AC Leopold, ed, Membranes, Metabolism and Dry Organisms. Comstock Press, Ithaca, NY, pp 22–34

Pharr DM, Sox HN, Locy RD, Huber SC (1981) Partial purification of the galactinol forming enzyme from leaves of *Cucumis sativus L.* Plant Sci Lett **23:** 25–33

Saravitz DM, Pharr DM, Carter TE (1987) Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes. Plant Physiol **83:** 185–189

Smith PT, Kuo TM, Crawford CG (1991) Purification and characterization of galactinol synthase from mature zucchini squash leaves. Plant Physiol **96:** 693–698

Thompson ST, Cass KH, Stellwagen E (1975) Blue dextran-Sepharose: an affinity column for the dinucleotide fold in proteins. Proc Natl Acad Sci USA **72:** 669–672

Webb JA (1982) Partial purification of galactinol synthase from leaves of *Cucurbita pepo.* Can J Bot **60:** 1054–1059

Zweig G, Sherma J (1972) Thin-layer chromatography. *In* G Zweig, J Sherma, eds, Handbook of Chromatography, Vol II. CRC Press, Boca Raton, FL, pp 160–161