THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 25, Issue of June 20, pp. 15898–15904, 1997

Printed in U.S.A.

A Plasma Membrane Sucrose-binding Protein That Mediates Sucrose Uptake Shares Structural and Sequence Similarity with Seed Storage Proteins but Remains Functionally Distinct*

(Received for publication, October 2, 1996, and in revised form, April 15, 1997)

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Photoaffinity labeling of a soybean cotyledon membrane fraction identified a sucrose-binding protein (SBP). Subsequent studies have shown that the SBP is a unique plasma membrane protein that mediates the linear uptake of sucrose in the presence of up to 30 mM external sucrose when ectopically expressed in yeast. Analysis of the SBP-deduced amino acid sequence indicates it lacks sequence similarity with other known transport proteins. Data presented here, however, indicate that the SBP shares significant sequence and structural homology with the vicilin-like seed storage proteins that organize into homotrimers. These similarities include a repeated sequence that forms the basis of the reiterated domain structure characteristic of the vicilin-like protein family. In addition, analytical ultracentrifugation and nonreducing SDS-polyacrylamide gel electrophoresis demonstrate that the SBP appears to be organized into oligomeric complexes with a M_r indicative of the existence of SBP homotrimers and homodimers. The structural similarity shared by the SBP and vicilin-like proteins provides a novel framework to explore the mechanistic basis of SBP-mediated sucrose uptake. Expression of the maize Glb protein (a vicilin-like protein closely related to the SBP) in yeast demonstrates that a closely related vicilin-like protein is unable to mediate sucrose uptake. Thus, despite sequence and structural similarities shared by the SBP and the vicilin-like protein family, the SBP is functionally divergent from other members of this group.

The development of plant seeds involves the accumulation of carbon and nitrogen reserves in proteinaceous forms that can both withstand desiccation and be utilized as an energy source by the developing embryo during germination. In legume species, these predominant seed storage proteins are found enclosed in membrane-bound organelles known as protein bodies (1, 2). The globulin seed storage proteins generally fall into two main classes: legumin-like and vicilin-like proteins (1, 3–6). Under nonreducing conditions, the legumin-like proteins are found as hexameric complexes with sedimentation coefficients of 11 S. The subunits of these complexes are derived from a precursor peptide containing two domains: an N-terminal acidic α chain and a C-terminal basic β chain. Following proteolytic processing, these domains remain associated through interchain disulfide links. In contrast, the vicilin-like proteins are found as 7 S trimers under nonreducing conditions. The vicilin-like monomers are 50–70-kDa polypeptides that undergo variable levels of post-translational proteolytic processing (1, 3).

X-ray crystallography of the vicilin-like proteins phaseolin (7, 8) and canavalin (9, 10) has permitted the formation of a canonical three-dimensional model for vicilin-like molecules (8). Each monomer consists of two very similar structural domains reflecting a tandem duplication observed at the nucleotide and amino acid sequences. These tandem domains are composed of two structural elements: a compact eight-stranded β barrel having the "Swiss roll" topology and an extended loop containing several short α helices. Trimers are formed by the apposition of a hydrophobic surface composed of side chains from one face of the N-terminal domain and a complementary hydrophobic surface of the C-terminal domain of the neighboring monomer. These intermonomeric interactions are further stabilized by several salt bridges. Once formed, the trimer is a disc-shaped molecule with a large internal channel of 18 Å (8, 10). The high degree of structural conservation shared by these two vicilin-like proteins is likely the result of several evolutionary constraints including subunit interactions that are required for holoprotein assembly, packaging of holoproteins into protein bodies, tolerance to desiccation, sorting in the secretory pathway, and general constraints for stability and solubility (11–14).

The accumulation of carbon in developing seeds is mediated by specific plasma membrane proteins (15–17). In most legume species, sucrose is the predominant form of assimilated carbon translocated from the photosynthetically active leaves to the developing cotyledons (18, 19). Photoaffinity labeling of membranes isolated from soybean cotyledon tissue with a photolyzable sucrose analog identified a distinct 62-kDa sucrose-binding protein (SBP)[1] (20). Analysis of the cDNA encoding the SBP and its deduced amino acid sequence indicates that the SBP contains a single hydrophobic domain at its N terminus but otherwise is a hydrophilic protein lacking the expected membrane-spanning hydrophobic segments typically present in transport proteins (21). Biochemical analysis of the topology of the SBP demonstrates that it is tightly associated with the external leaflet of the plasma membrane (22). The involvement of the SBP in sucrose uptake was implicated by immunolocal-

* This work was supported by National Science Foundation Grants IBN-9218811 and IBN-9514410. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) P10562 (canavalin) and P02853 (phaseolin).

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[1] The abbreviations used are: SBP, sucrose-binding protein; *sbp*, gene for SBP; PAGE, polyacrylamide gel electrophoresis; Mes, 4-morpholineethanesulfonic acid; DTSSP, 3,3′-dithiobis(sulfosuccinimidyl propionate).

ization experiments, which demonstrated that the SBP is exclusively associated with the plasma membrane of cells involved in active sucrose uptake (21). Kinetic analysis of SBP-mediated sucrose uptake in a yeast system indicates that the uptake is specific for sucrose but is proton-independent and relatively nonsaturable, thus defining a novel mechanism for sucrose uptake (15).

Despite strong evidence that the SBP mediates sucrose uptake, nucleotide and amino acid sequence analysis suggests that the SBP is distinct from other known transport proteins including binding protein-dependent transport systems of *Escherichia coli*. Here we show that a portion of the SBP amino acid sequence shares significant sequence homology with the vicilin-like seed storage proteins, including an internal sequence repeat. In addition, examination of plasma membrane fractions under nonreducing conditions show that the SBP exists in multimeric complexes that may represent dimeric and trimeric association of SBP monomers. Thus, the data presented here provide new insight into the three-dimensional organization of a unique plasma membrane protein capable of mediating sucrose uptake.

EXPERIMENTAL PROCEDURES

Plant Material and Membrane Isolation—Soybean (*Glycine max* common version *Wye*) seeds were grown as described (21). Microsomal membranes from cotyledons were obtained at the indicated times after flowering or imbibition following the protocol of Overvoorde and Grimes (22). Highly enriched plasma membrane proteins were obtained by aqueous two-phase partitioning as described previously (22–25).

RNA Isolation and Gel Blot Analysis—Cotyledons were removed from the pod walls or parts of the germinating embryo, frozen in liquid nitrogen, and stored at −80 °C. RNA was isolated using the protocol described by Grimes *et al.* (21). For analysis of *sbp* mRNA levels, the probe was a 1.7-kilobase pair *Nco*I/*Spe*I fragment of the *sbp* cDNA (21) labeled with ^{32}P by random priming with a RadPrime (Life Technologies, Inc.) kit according to the manufacturer's directions. For analysis of β-conglycinin mRNA levels, a 350-base pair polymerase chain reaction fragment amplified from soybean genomic DNA was labeled with ^{32}P by random priming. Direct sequencing of this polymerase chain reaction fragment on an ABI 373 sequencer (Applied Biosystems, Inc.) confirmed the identity of this probe. Membranes containing the transferred RNA were prehybridized at 65 °C for 1 h in 1% SDS, 1 M sodium chloride, and 10% dextran sulfate. Hybridizations were carried out overnight at 65 °C in the same buffer containing 1–5 ng/ml denatured probe and 100 μg/ml denatured salmon sperm DNA. The blots were washed twice with 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) for 5 min each with constant agitation at room temperature, twice with 2 × SSC, 1% SDS for 30 min each at 65 °C, and twice with 0.1 × SSC for 15 min each at room temperature.

Analytical Ultracentrifugation—Purified plasma membrane proteins were solubilized in Buffer A (50 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA) supplemented with 1% Triton X-100 for 30 min on ice. This suspension was clarified by centrifugation at 100,000 × *g* (r_{avg}) for 24 min at 4 °C in a Beckman TL-100 ultracentrifuge. >85% of the membrane proteins were solubilized by this treatment. The supernatant was loaded onto a 5–25% sucrose gradient containing Buffer A supplemented with 0.1% Triton X-100. After centrifugation at 4 °C for 16 h at 130,000 × *g* (r_{avg}) in a Beckman SW40 rotor, fractions of 0.5 ml were collected from the bottom. One-fifth of each fraction was diluted with an equal volume of buffer containing 50 mM Tris, pH 6.8, 2% SDS, and 1 mM β-mercaptoethanol, collected on nitrocellulose using a slot-blot apparatus (Hoefer Scientific Instruments), and immunostained using affinity-purified antiserum described by Grimes *et al.* (21). Densitometry was performed as described earlier (22).

Sequence Analysis—Data base searches and sequence alignments were carried out using the GCG software package (26). Unrooted phylogenetic trees were generated using the neighbor-joining and fitch algorithms contained in the phylogenetic inference package, PHYLIP 3.57 (27).

Expression of SBP Forms and Glb1 in Yeast and Analysis of [^{14}C]Sucrose Uptake—To facilitate the expression of Glb1 in yeast, pYEGlb1 was constructed by excising the *glb1* cDNA from pGlb1 (28, 29) by digestion with *Sal*I and *Bam*HI and ligation of this fragment into the *Xho*I and *Bam*HI sites of pMK195 (15). The construct was confirmed by restriction digests. Two forms of the SBP were tested as well. A SBP with its C terminus truncated was excised from pSBP using *Xho*I and *Xba*I sites, and this fragment was ligated into the *Xba*I and *Xho*I sites of pMK195. This construct resulted in the deletion of 80 amino acids from the C terminus. A full-length SBP was excised from pSBP using *Sal*I and *Pst*I and ligated into the *Pst*I and *Xho*I sites of pMK195. The truncated form was used in a previous study (15).

susy7/ura3 yeast (15, 16) were transformed with 1 μg of pMK195, truncated pYESBP (15), full pYESBP, or pYEGlb1 using a small scale, lithium acetate-based protocol (30). To characterize the putative transformants, DNA was isolated (32) and analyzed either by Southern blot analysis (31) or by being electroporated into XL-I Blue *E. coli*. Plasmid DNA from transformed *E. coli* colonies growing on solidified Luria broth medium containing 100 μg/ml ampicillin was isolated (31) and used to confirm the structure of the re-isolated shuttle vectors by restriction digests. The yeast strain termed OG2 contains the C-terminal truncated SBP, whereas CG1 contains the full-length SBP. Both of these constructs were verified by DNA sequencing.

Colonies containing the appropriate vectors were grown to an A_{600} of 0.5–1.0 in liquid minimal medium supplemented with His, Trp (ura dropout). After harvesting by centrifugation, the cells were washed twice with 25 mM Mes-KOH buffer, pH 5.5, weighed, and resuspended in the same buffer to a density of 30–50 mg of cells/ml. [^{14}C]Sucrose uptake assays were performed as described by Overvoorde *et al.* (15).

Analytical Methods—Protein concentration was assayed with the bicinchoninic acid reagent according to the manufacturer's directions (Pierce). Polyacrylamide gels were run according to Laemmli (33), except that a 7.5–15% acrylamide gradient was used with an accompanying 7.5–15% glycerol gradient. To determine the mobility of the SBP under nonreducing conditions, the samples were solubilized in sample buffer lacking β-mercaptoethanol but containing SDS and were not heated before loading. Gels were stained with 0.2% Coomassie Brilliant Blue R-250. For immunostaining, proteins resolved by SDS-PAGE were electroblotted to nitrocellulose (34) and immunodecorated using affinity-purified anti-SBP antisera as described by Grimes *et al.* (21). Color development was performed using ECL according to the manufacturer's directions (Amersham Corp.).

RESULTS

SBP Homology with Vicilin-like Proteins—Analysis of the deduced amino acid sequence of the SBP shows that it lacks similarity with any other known transport protein identified from other species (21). Searches of the GenBank™ data base for SBP homologues, however, reveal that the SBP shares 44–61% similarity and 20–37% identity with the vicilin-like seed storage proteins (Fig. 1). The vicilin-like family of proteins shares several conserved features, and a detailed comparison of the SBP sequence with other members of this family highlights the close relationship of the SBP with this group of polypeptides.

Seed storage proteins typically contain a hydrophobic signal sequence that targets the newly synthesized polypeptides to the endoplasmic reticulum where the protein undergoes a variety of modifications, including the proteolytic removal of the signal sequence, glycosylation, and sorting for transit through the remainder of the secretory pathway. Similarly, the SBP contains a hydrophobic stretch of amino acids at its N terminus that may function as a signal sequence. Microsequencing of the N terminus of the mature SBP (21), however, shows that if this hydrophobic domain is removed, the site of processing (indicated by an *asterisk* in Fig. 1) is different from the highly conserved site of processing shared by both vicilin- and leguminlike proteins (indicated by a *down arrow* in Fig. 1; Ref. 4).

The vicilin-like proteins can be roughly grouped into two size classes depending on the length of a variable highly charged segment that follows the hydrophobic N terminus (35). Storage proteins containing this hydrophilic segment (which ranges in size from 90 to 183 residues) are approximately 70 kDa, whereas those lacking this region are approximately 50 kDa. Although considerably smaller (73 residues) than the 70-kDa proteins, the SBP contains a similar hydrophilic segment that is rich in charged amino acids, especially Glu and Arg (Fig. 1;

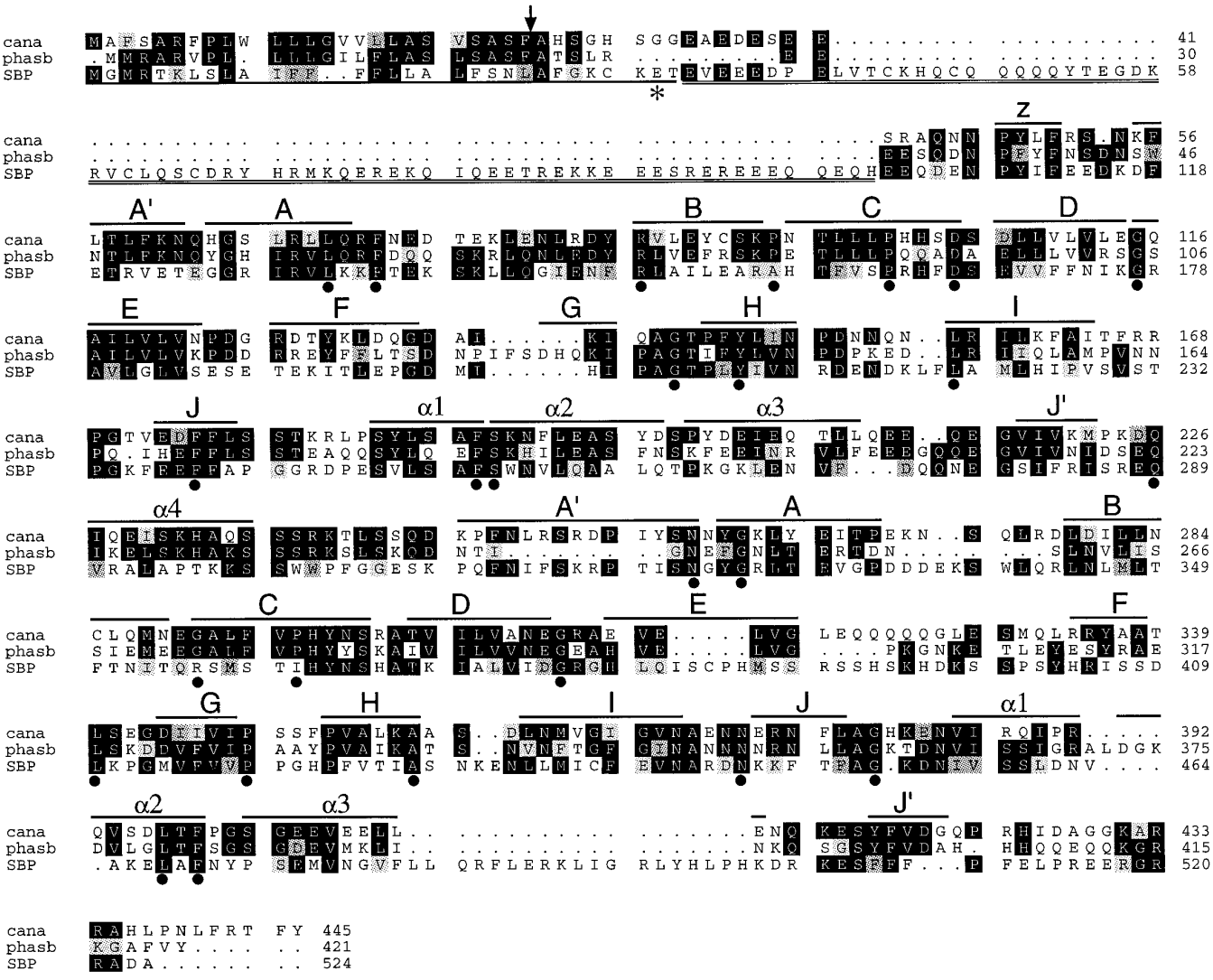

FIG. 1. **Multiple sequence alignment of the SBP with canavalin (accession number P10562) and phaseolin (accession number P02853), two vicilin-like proteins whose three-dimensional structure is known.** The known secondary structure elements of phaseolin are indicated *above* the pileup (β sheets *A–J* and α helices *1–4*). The hydrophobic N-terminal domain is indicated (——), and the hydrophilic segment of the SBP is shown (═). Residues important for maintaining the three-dimensional organization of the vicilin-like protein that are hyperconserved among vicilin-like and legumin-like polypeptides according to Lawrence *et al.* (8) are indicated (●). The conserved site for proteolytic removal of the hydrophobic N terminus is indicated (↓), and the N-terminal residue of SBP, as determined by microsequencing, is also shown (*).

Ref. 21). In addition, the C*XXX*C motif that is repeated six times in this portion of the cotton vicilin and twice in the vicilin-like storage proteins of cocoa, barley, soybean, wheat, and maize is also present twice in the SBP sequence (residues 43–47 and 61–65 using SBP numbering in Fig. 1; Refs. 28 and 35–39). Thus, the SBP shares both the charged hydrophilic domain and the conserved C*XXX*C motif within this domain with other members of the vicilin-like proteins.

A third shared feature of the SBP with the vicilin-like proteins is the presence of a repeated sequence that is thought to have arisen through the duplication of a primordial gene (5, 6, 8, 40). The three-dimensional structure of both phaseolin and canavalin, vicilin-like proteins from *Phaseolus vulgaris* and *Canavalia ensiformis*, respectively, demonstrates that this duplicated sequence forms the basis for the symmetrically reiterated Swiss roll domain structure (7–10). Alignment of the SBP sequence with phaseolin and canavalin shows that the SBP contains a similar internal repeat (residues 117–299 and 310–509; Fig. 1).

Lawrence *et al.* (8) identified 26 strictly conserved residues present in all members of the vicilin-like protein family (indicated by a *black circle* under the sequences in Fig. 1). Of these 26 residues, 23 (88%) are also present in the SBP sequence (Fig. 1). Analysis of these conserved residues in the context of the known three-dimensional structures demonstrates their importance in maintaining the overall structure and intramonomer contacts (8). As such, the SBP appears likely to contain similar tertiary motifs and to be organized in similar three-dimensional structures. Two potentially significant departures from this conservation are the substitution of Ile for Pro at position 361 of the SBP (36) and the substitution of Ala for Pro at position 157 of the SBP. Both of these Pro residues result in a β bulge forming in the respective β sheets, and these β bulges are postulated to be important in mediating oligomeric formation (10).

Within the vicilin-like sequence, there are segments that highlight the relatedness of the reiterated motif (10). For instance, residues 173–181 (canavalin numbering) in the N-terminal domain of canavalin share 67% similarity (44% identity) with its C-terminal counterpart, residues 375–383 (10). Such similarity and that of adjoining regions support the hypothesis that these domains have arisen by tandem duplication of a smaller ancestral protein (5–10). Interestingly, the SBP contains residues that are conserved between its two domains that

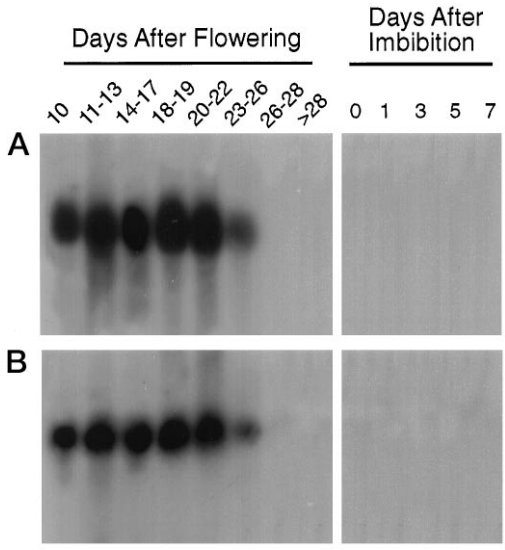

FIG. 2. **The temporal pattern of SBP and β-conglycinin expression during cotyledon development and seedling germination.** RNA isolated from cotyledons at the indicated times after flowering or imbibition was subjected to RNA gel blot analysis using either radiolabeled *sbp* cDNA (*panel A*) or a radiolabeled β-conglycinin polymerase chain reaction fragment (*panel B*) as a probe. The SBP blot was exposed to x-ray film for 18 h at −70 °C, and the β-conglycinin blot was exposed for 6 h under the same conditions.

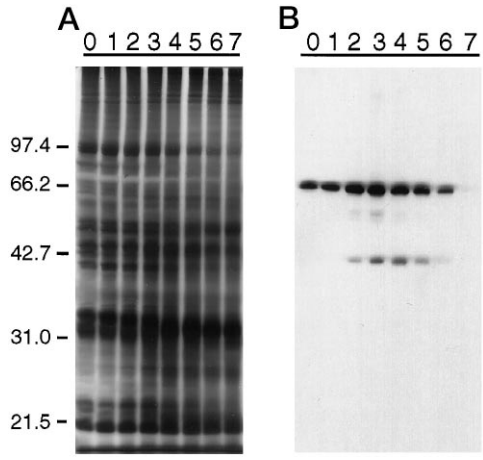

FIG. 3. **SDS-PAGE and immunoblot analysis of microsomal membrane proteins from soybean cotyledons at 0–7 days after imbibition.** Proteins present in the microsomal fraction at 0–7 days after imbibition were resolved by SDS-PAGE and stained with Coomassie Brilliant Blue (*panel A*). Proteins present in the microsomal fraction at 0–7 days after imbibition were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with affinity-purified anti-SBP antiserum (*panel B*). Units for the indicated molecular mass markers are in kilodaltons.

are not conserved in other vicilin-like sequences (*e.g.* Phe-239 and Gly-243 (SBP numbering) in the N-terminal domain are found in the same register at positions 449 and 453, respectively, as in the C-terminal domain). The significance of these observations for inferring the evolutionary relationship of the SBP and other proteins that contain vestiges of this repeated domain remains unclear.

An additional conserved feature of the vicilin-like proteins is the LQRF motif at positions 70–73 of the canavalin sequence and positions 60–63 of the phaseolin sequence. This LQ(R/K)F motif is similar to the yeast carboxypeptidase Y vacuolar targeting signal and has been proposed to function as a vacuolar targeting motif in the vicilin-like proteins as well (9). This region in the SBP is LKKF, which may partially explain the targeting of the SBP to the plasma membrane rather than vacuoles or protein storage bodies.

SBP and β-Conglycinin Expression in Cotyledons—β-Conglycinins are vicilin-like storage proteins found in soybean cotyledons (14, 41). The expression, processing, localization, and hydrolysis during germination of these storage proteins are well defined (14, 42). A direct comparison of β-conglycinin and SBP mRNA levels shows that their expression patterns are regulated in a similar manner during seed development. Both transcripts are present in cotyledons 10 days after flowering and reach a peak at 18–22 days after flowering (Fig. 2). After this time, both *sbp* and β-conglycinin mRNA levels decrease to undetectable levels at 26–28 days after flowering. During germination, neither of these genes is expressed, as the mRNA levels remain undetectable during embryo emergence (Fig. 2).

Seed storage proteins like β-conglycinin are present in dehydrated seeds and are degraded during the first 7–10 days after imbibition. The hydrolysis of these proteins contributes to the energetic and structural requirements of the rapidly growing embryo (1–3, 42). Since the SBP mRNA is absent in newly emerging cotyledons, it was of interest to examine whether the SBP protein was present in dehydrated seeds and, if so, whether it was degraded after imbibition in a time frame similar to other storage proteins. To examine these questions, microsomal proteins from seeds at 2–7 days after imbibition were isolated and separated by SDS-PAGE, and the levels of the SBP were probed by immunoblotting. Fig. 3 shows that the SBP is present in imbibed seeds. Analysis of total membrane protein extracts from dehydrated seeds confirms the presence of the SBP in this dormant tissue (data not shown). After imbibition, the level of SBP decreases with kinetics similar to other storage proteins and reaches undetectable levels 7 days after imbibition. The slight increase in the amount of SBP at 3–4 days is probably indicative of more protein being loaded on the gel in these lanes and does not represent *de novo* synthesis, as no SBP mRNA is present in these tissues. The smaller band at ~42 kDa represents a proteolytic product that sometimes forms during protein extraction and SDS-PAGE. These data show that despite the fact that the SBP is localized to the plasma membrane, it is regulated in a manner similar to the seed storage proteins. Probing of the purified plasma membrane proteins with antibodies specific for β-conglycinin showed that no plasma membrane proteins shared antigenic sites with this storage protein (data not shown).

Oligomeric Organization of the SBP—Examination of vicilin-like proteins using analytical ultracentrifugation or size-exclusion chromatography shows that these proteins are organized as stable homotrimers (43–47). In addition, the three-dimensional structure of phaseolin and canavalin shows that the trimer is composed of monomers arranged around a 3-fold axis of symmetry (7, 8, 10). Since detailed structural information is available for the storage proteins and the SBP shares strong sequence similarity with the vicilin-like storage proteins, we examined the organization of SBP in membrane preparations from soybean cotyledons using analytical ultracentrifugation in the absence or presence of reducing reagents. The results of these analyses show that under nonreducing conditions, the SBP is part of two multimeric complexes (Fig. 4*A*). Using proteins of known size and organization as standards, the molecular masses of SBP-containing complexes were determined; these findings suggest that the two complexes represent dimers (125 kDa) and trimers (190 kDa) of the SBP. When the sample is denatured by incubation with a strong reducing reagent (β-mercaptoethanol), the multimeric complexes dissociate, and only monomers of SBP are observed (Fig. 4*A*), suggesting that interactions between subunits of this complex are mediated by disulfide linkages. These experiments, however, fail to exclude

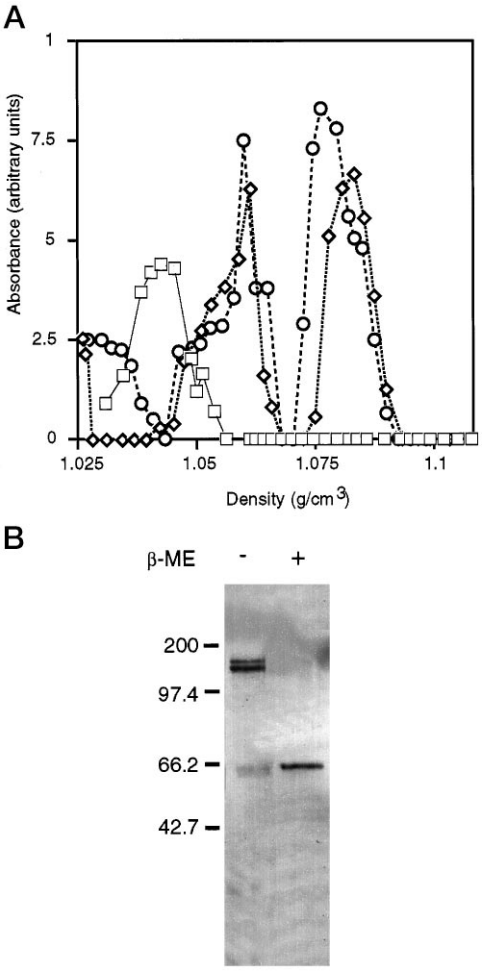

FIG. 4. **Analytical ultracentrifugation and SDS-PAGE analysis of microsomal and purified plasma membrane proteins.** Microsomal membranes (*panel A*) obtained from cotyledons (18 days after flowering) that were treated with buffer alone (○) or with the cross-linking reagent DTSSP (◇) were solubilized in 1% Triton in the presence (□) or absence (○, ◇) of β-mercaptoethanol. After clarifying the solution by centrifugation at 100,000 × *g* (r_{avg}) for 30 min in a TLA100.1 rotor, the solubilized proteins were applied to a 5–25% sucrose gradient. The distribution of the SBP in the gradient was determined by assaying one-fifth of each 500-μl fraction by immunostaining of a slot-blot. Plasma membrane proteins (*panel B*) were solubilized in sample buffer prepared with (+) or without (−) β-mercaptoethanol (*β-ME*), separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-SBP antiserum. Units for the indicated molecular mass markers are in kilodaltons.

the possibility that other proteins may be present in these higher order complexes.

One explanation for the presence of two SBP-containing complexes is that the larger complex (190 kDa) is unstable and dissociates to form the smaller complex (125 kDa) during sample preparation. Since the SBP is present on the extracellular surface of the plasma membrane (22), treatment of soybean cotyledons with the membrane-impermeant cross-linking reagent DTSSP followed by analysis of the microsomal proteins using ultracentrifugation should address whether the larger complex is labile. The results of this experiment show that both SBP-containing complexes are observed, even when the proteins are covalently cross-linked before membrane purification (Fig. 4*A*). It may still be possible that the DTSSP is unable to cross-link all available SBP complexes because the intact cotyledons are relatively large and contain cell walls. Alternatively, the smaller complex may represent intermediates in the process of forming the larger complexes. The legumin-like proteins exhibit a similar accumulation of partially assembled oligomers at different times during seed development (2).

To further examine the multimeric complexes observed using ultracentrifugation, plasma membrane proteins isolated from soybean cotyledons were separated by SDS-PAGE either in the presence or absence of reducing reagent, transferred to nitrocellulose, and immunostained. Fig. 4*B* shows that in the presence of reducing reagent, the SBP is present as a single 62-kDa band. However, if the plasma membrane proteins are resolved in the absence of reducing reagent, the SBP is present as two slower migrating complexes with molecular masses of 125 ± 7.5 kDa and 190 ± 10 kDa (Fig. 4*B*). These observations are consistent with the results obtained via analytical ultracentrifugation and suggest that the SBP is associated as dimers and trimers *in vivo*.

Functional Divergence of a Vicilin-like Protein and [^{14}C]Sucrose Uptake in Full-length and Truncated Forms of the SBP—Vicilin-like storage proteins have been identified in distantly related plant species including angiosperms and gymnosperms (36, 48). Examination of relatedness among vicilin-like storage proteins using an unrooted phylogenetic tree shows that the SBP is more closely related to the vicilin-like proteins than to the legumin-like proteins (11 S) (data not shown). The overall sequence identity between individual members of the family is highly variable (28–82%) with the SBP, sharing the highest levels of identity in the less variable region (*i.e.* residues 108–482) with the wheat (33%), cacao (33%), and maize (34%) sequences.

The observations that the SBP is present in dehydrated seeds and shares significant homology with the vicilin-like seed storage proteins are unexpected because the SBP is known to bind sucrose (as evidenced by the photoaffinity labeling originally used to identify this protein), is tightly associated with the plasma membrane of cells involved in active sucrose uptake, and is able to mediate sucrose uptake when ectopically expressed in yeast (15, 20–22). However, because the SBP shares a high degree of sequence and, presumably, structural conservation with vicilin-like proteins, it remains possible that other vicilin-like proteins may also mediate sucrose uptake or that overexpression of a vicilin-like protein in yeast may induce a cryptic sucrose transport activity. To test these possibilities, the cDNA encoding a closely related vicilin-like protein, *glb1* from maize, was subcloned in front of the constitutive yeast alcohol dehydrogenase 1 (ADH1) promoter of the pMK195 yeast expression vector. This vector was transformed into the *susy7/ura3* yeast strain (15, 16), and the ability of yeast expressing the Glb1 protein to mediate [^{14}C]sucrose uptake was determined. The *susy7/ura3* strain has the plant enzyme sucrose synthase, a cytosolic enzyme that hydrolyzes sucrose into UDP-glucose and fructose, stably integrated into its genome and contains mutations in both invertase and the *MALO* transporter (the latter has a low affinity for sucrose (15, 16)). Fig. 5 shows that yeast expressing the full-length SBP (CG1) are able to mediate [^{14}C]sucrose uptake. In contrast, yeast containing either the pMK195 vector alone (OG1) or expressing the Glb1 protein are unable to mediate the uptake of [^{14}C]sucrose. These results show that sucrose uptake mediated by the SBP does not result from the overexpression of a vicilin-like protein in yeast and furthermore suggest that the SBP is functionally divergent from other members of the vicilin-like protein family.

A truncated form of the SBP has previously been shown to mediate sucrose uptake in a proton-independent manner and not adhere to Michaelis-Menten kinetic parameters (15). This modified SBP was a chimeric protein consisting of residues 1–444 of the SBP and a portion of the yeast alcohol dehydrogenase 3′-noncoding region. Fig. 5 also shows that the truncated form of the SBP (OG2) is more efficient at mediating

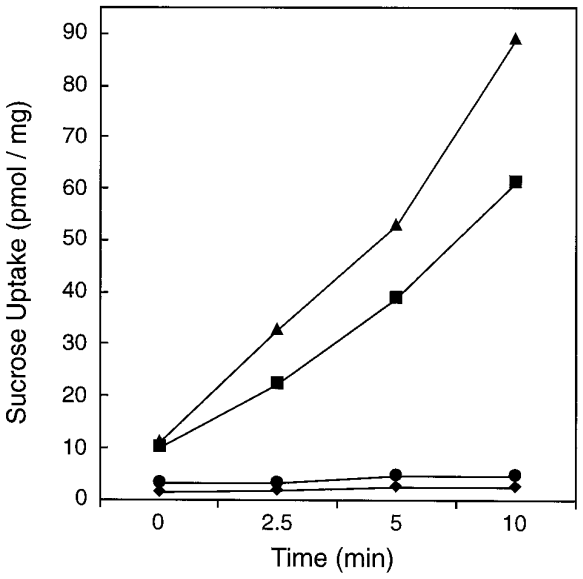

FIG. 5. **Analysis of [^{14}C]sucrose uptake by *susy7/ura3* yeast containing pMK195, pYEGlb1, pYESBP, and truncated pYESBP.** *A*, yeast strains harboring the pMK195 vector (●), pYEGlb1 (♦), truncated pYESBP (▲), or full-length pYESBP (■) were grown overnight in selective media, harvested, and incubated in a solution containing [^{14}C]sucrose. The amount of radioactivity accumulated at the indicated times was determined and used to calculate the amount of [^{14}C]sucrose uptake. The data shown are the mean of three replicates. The standard error in all cases was smaller than the size of the symbols used.

sucrose uptake than the full-length protein (CG1). There are at least two interpretations of these results. First, the truncated form may be processed more efficiently, resulting in more functional protein arriving at the plasma membrane to mediate uptake. Alternatively, deletion of the C-terminal 80 amino acids from the SBP may diminish its ability to form oligomers.

DISCUSSION

The current study shows that the SBP shares significant structural and sequence similarity with the vicilin-like seed storage proteins. In addition, the SBP and the soybean vicilin-like storage protein, β-conglycinin, are expressed coordinately during seed development. Although the SBP protein accumulates in developing cotyledons, the presence of the SBP in dried, mature seeds and its rapid degradation during germination provide additional insight into its relationship with the vicilin-like protein family.

Sequence analysis reveals that the SBP shares several primary elements that are conserved among members of the vicilin-like protein family. These include a hydrophobic domain at the N terminus, a putative vacuolar signal sequence, a hydrophilic segment enriched in charged amino acids, and an internally repeated domain consisting of approximately 170 residues. The three-dimensional structures of phaseolin and canavalin demonstrate that these repeated segments form α + β structural motifs consisting of a β barrel with a Swiss roll topology followed by an extended loop containing several short α helices. Within these structural motifs, many identical or conservatively replaced amino acids occupying equivalent positions can be identified by aligning the primary sequences of the vicilin-like protein family (4–10). In fact, the N-terminal domain of the SBP contains 12 of 13 residues that are conserved across the vicilin-like family, the C-terminal domain contains 10 of 12 strictly conserved residues, and the intervening segments contain a strictly conserved residue (Gln-289). Each of these residues is involved in stabilizing inter- or intramolecular contacts that coordinate the three-dimensional structure of the vicilin-like molecules (8, 10).

Interestingly, there are only two residues, a Pro and a Gly, in each α + β motif that are conserved in both the N- and C-terminal domains of all proteins belonging to the vicilin-like and legumin-like families (8). The N-terminal α + β domain of the SBP contains these highly conserved residues in proper register (Pro-163 and Gly-177, using SBP numbering). However, as has been noted before, the C-terminal α + β domain of the SBP contains a Ile for Pro substitution (Ile-361), but Gly-376 remains conserved (Fig. 1; Ref. 36). Additionally, the N′-terminal α + β domain of the SBP contains an Ala for Pro substitution (Ala-157). Both of these Pro residues contribute to the formation of β bulges in both domains of the canavalin and phaseolin proteins (7, 8, 10). In the case of phaseolin, the β bulge in the C-terminal domain (49) mediates the interaction of the J and J′ β sheets that flank the extended α-helical segment of each domain. Alignment of the SBP with canavalin and phaseolin suggests that there is a 19-residue insertion present in the primary sequence of the SBP in this region (Fig. 1). Since the three-dimensional structure has not been defined, the effects of this insertion remain unknown; however, it is tempting to speculate that the Ile for Pro substitution at position 361 in the C-terminal domain of the SBP might coordinate the interaction of this extended portion of the protein with the β barrel. In addition, this 19-amino acid insertion in the C-terminal domain may be important for the functional divergence of the SBP from other members of the vicilin-like protein family. Another departure from the vicilin-like proteins present in the SBP is a 5-residue insertion following residue Gln-381 that corresponds to the EF loop of the C-terminal domain of phaseolin and canavalin (Fig. 1). This structural comparison, then, provides insight into which residues and domains may be altered to examine the impact on SBP-mediated sucrose uptake.

Canavalin, phaseolin, and other vicilin-like storage proteins exist as homotrimeric complexes with molecular masses of 150–200 kDa (7, 13, 45, 46). The three monomers assemble around an exact 3-fold axis to form a toroid complex with an outside diameter of 86–88 Å and an internal hole of 18 Å (8, 10). Although the effects of the Ile (Ile-361 of the SBP) for Pro substitution and the Ala for Pro substitution at position 157 on the oligomeric organization of the SBP remains unclear, it appears that these substitutions do not prevent the SBP from forming higher order complexes. Analytical ultracentrifugation and nonreducing SDS-PAGE indicate that the SBP is present in higher order complexes of a size consistent with trimers and support the notion that the SBP is structurally related to the vicilin-like proteins. These results also indicate that the SBP may be present as dimers *in vivo*. In this regard, it is interesting that the C-terminal truncated form of the SBP is more efficient than the full-length in mediating sucrose uptake into yeast, as it might be expected that this deletion would interfere with the formation of oligomers. Alternatively, this difference in activity may be due to different amounts of the SBP or the truncated SBP arriving at the plasma membrane. Analyses of SBP concentration at the plasma membrane and the oligomeric form of SBP in yeast are under way to address these questions. Although the dimers observed *in vivo* are consistently present and cross-linking the proteins before sample preparation does not increase the trimer:dimer ratio, the significance of these dimeric complexes remains obscure. It remains possible, however, that the native oligomer is present as a trimer and that disruption of the cells results in some degradation of this higher order structure that cannot be prevented by the cross-linking reagent used, perhaps because the DTSSP is unable to effectively penetrate the entire cotyledonary tissue.

Despite the sequence and structural similarity the SBP shares with the vicilin-like proteins, it remains distinct from

these seed storage proteins in several ways. First, unlike the storage proteins whose distribution is limited to the seeds (1, 2), expression of the SBP is not limited to this tissue. In fact, the SBP is expressed in leaves and is associated with the plasma membrane of cells comprising the mature phloem (21). Second, while the vicilin-like storage proteins are contained in membrane-bound protein bodies in seeds, the SBP is associated with the plasma membrane in this tissue (21, 22). This may be related to the LKKF motif found in the SBP corresponding to the LQ(R/K)F motif postulated in the processing of vicilin-like proteins through the secretory system and into vacuoles and protein bodies. Third, while the vicilin-like proteins comprise up to 30% of the protein found in seeds, the SBP is a low abundance protein comprising <0.1% of the total protein present in cotyledons.

Finally, the ability of the SBP to mediate proton-independent, relatively nonsaturable uptake of sucrose when ectopically expressed in yeast clearly distinguishes it from the vicilin-like storage proteins. It is possible that SBP-mediated sucrose uptake might result from the aberrant overexpression of a vicilin-like plant protein in yeast cells. To examine this possibility, Glb1, a vicilin-like protein from maize (28, 29), was expressed in yeast, and the capacity of the yeast to mediate [^{14}C]sucrose uptake was assessed. The inability of yeast cells expressing Glb1 to accumulate sucrose demonstrates that a closely related vicilin-like protein is unable to mediate sucrose uptake and that SBP-mediated sucrose uptake does not result from the ectopic expression of a vicilin-like protein in yeast. It is clear, therefore, that the SBP protein is functionally distinct from the Glb1 protein, and its targeting to the plasma membrane and association with cells of the mature phloem demonstrate that the SBP has diverged significantly from other members of this family.

Since it is unlikely that the similarities between the SBP and the vicilin-like proteins at the primary level are the result of convergent evolution, it appears that the SBP belongs to a superfamily of proteins containing the globulin storage proteins and the spherulin-like proteins of Myxomycetes that share homology with the germins of cereals (36, 39, 50, 51). The evolutionary relationship between the SBP and the vicilin-like storage proteins may have important implications for elucidating the mechanism of SBP-mediated sucrose uptake. Kinetic analysis of SBP-mediated sucrose uptake in yeast indicates that this process is both proton-independent and relatively nonsaturable (15). These unique attributes closely parallel prior physiological observations of a nonsaturable sucrose uptake system in plants (19, 52, 53). Thus, the mechanism of SBP-mediated uptake is biochemically distinct from other established membrane transport mechanisms. Ko *et al.* (10) have suggested that the 18-Å channel formed in the canavalin trimer may play a role in the transport of solvents, metabolites, or water. They further envision the disc-shaped trimers to be stacked like plates so that the molecules form a network of solvent-filled microtubules (10). Although we do not yet know if trimers of the SBP are required for SBP-mediated sucrose uptake or whether such channels are formed by interacting SBP monomers, such a model remains an attractive hypothesis that may explain the unique mechanism of SBP-mediated sucrose uptake into plant cells. As such, the hypothesized structural relatedness of the SBP with the vicilin-like proteins provides a novel framework for probing the mechanism of sucrose uptake mediated by the SBP.

Acknowledgments—We thank Steve Thompson (Visualization, Analysis, and Design in the Molecular Sciences Center, Washington State University) for assistance in performing sequence analysis. The gift of *glb1* cDNA and anti-Glb1 antiserum from Dr. Alan Kriz (Dekalb Plant Genetics) is gratefully acknowledged. We also thank Joerg Riesmeier, Marion Kwart, and Dr. Wolf Frommer for supplying the original yeast strain and vector.

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A Plasma Membrane Sucrose-binding Protein That Mediates Sucrose Uptake Shares Structural and Sequence Similarity with Seed Storage Proteins but Remains Functionally Distinct

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J. Biol. Chem. 1997, 272:15898-15904.
doi: 10.1074/jbc.272.25.15898