

Rapid Report**A Pumpkin 72-kDa Membrane Protein of Precursor-Accumulating Vesicles Has Characteristics of a Vacuolar Sorting Receptor**Tomoo Shimada¹, Miwa Kuroyanagi¹, Mikio Nishimura^{1,2} and Ikuko Hara-Nishimura^{1,2,3}¹ Department of Cell Biology, National Institute for Basic Biology, Okazaki, 444 Japan² Department of Molecular Biomechanics, School of Life Science, The Graduate University for Advanced Studies, Okazaki, 444 Japan

Precursor-accumulating (PAC) vesicles were previously shown to mediate the transport of the precursor of a major storage protein (pro2S albumin) to protein-storage vacuoles in developing pumpkin cotyledons. In this study, we characterized two homologous proteins from PAC vesicles, a 72 kDa protein (PV72) and an 82 kDa protein (PV82). PV72 and PV82 showed an ability to bind to peptides derived from both an internal propeptide and a C-terminal peptide of pro2S albumin. PV72 was predicted to be a type I integral membrane protein with epidermal growth factor (EGF)-like motifs. These results suggest that PV72 and PV82 are potential sorting receptors for 2S albumin to protein-storage vacuoles.

Key words: EGF-like motif — Precursor-accumulating vesicle (PAC vesicle) — Pumpkin (*Cucurbita* sp.) — Sorting receptor — Targeting signal — Vacuolar protein.

Vacuolar proteins are synthesized on the rough endoplasmic reticulum and are then delivered to the vacuoles via vesicle-mediated transport systems (Okita and Rogers 1996). Sorting and targeting them to vacuoles require the presence of a specific signal and a receptor. The best characterized sorting system is mannose 6-phosphate (M6P)-dependent targeting of lysosomal proteins to the lysosomes in animal cells (Pfeffer and Rothman 1987). Lysosomal proteins with M6P-linked oligosaccharide chains bind to two transmembrane M6P receptors (MPR46 and MPR300), to be delivered to the lysosomes. Plant and yeast vacuoles have been regarded as being analogous to lysosomes. In

plant and yeast cells, however, vacuolar targeting signals reside in amino acid sequences, rather than in an oligosaccharide such as M6P. A short stretch sequence of amino acids, QRPL, found in the propeptide of carboxypeptidase Y has been shown to function as a targeting signal to the vacuoles of yeast (Valls et al. 1987). Marcusson et al. (1994) reported that the VPS10 gene encoded a sorting receptor for the targeting signal of carboxypeptidase Y.

In plants, two types of the targeting signals of vacuolar proteins have been described. One is an N-terminal propeptide (NTPP) and the other is a C-terminal propeptide (CTPP). The NTPPs of barley (*Hordeum vulgare*) thiol protease, aleurain, (Holwerda et al. 1992) and sweet potato (*Ipomoea batatas*) sporamin (Matsuoka and Nakamura 1991) have been shown to function as a targeting signal of the proteins. An NPIR sequence is conserved between these two NTPPs. On the other hand, the CTPPs of barley lectin (Bednarek and Raikhel 1991) and tobacco (*Nicotiana tabacum*) chitinase (Neuhaus et al. 1991) are also known to be necessary and sufficient for the targeting of the proteins to the vacuole. No significant homology, however, was observed between these two CTPPs.

Although much has been learned about the targeting signals of vacuolar proteins, the sorting receptors for the signals are poorly understood in plants. Previous studies have identified a potential receptor protein, BP-80, from the membranes of clathrin-coated vesicles (CCVs) of developing cotyledons of pea (*Pisum sativum*, Kirsch et al. 1994). BP-80 specifically bound to the NTPPs of aleurain and sporamin at neutral pH but not at acidic pH (Kirsch et al. 1994, 1996). Treatment of intact CCVs with a protease demonstrated that BP-80 was a transmembrane protein with a 5-kDa cytoplasmic domain (Kirsch et al. 1994). Although BP-80 is suggested to be a vacuolar sorting receptor, the molecular details of the protein remain to be established. Recently, Ahmed et al. (1997) described an 80-kDa *Arabidopsis thaliana* epidermal growth factor receptor-like protein (AtELP), which was predicted to be a type I integral membrane protein with three repeats of an epidermal growth factor (EGF)-like motif. They suggested that AtELP might be a homologue of pea BP-80 and is likely involved in intracellular protein trafficking in the plant cell. The exact function of AtELP, however, is not known.

Abbreviations: AtELP, *Arabidopsis thaliana* epidermal growth factor receptor-like protein; CCV, clathrin-coated vesicle; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CTPP, C-terminal propeptide; EGF, epidermal growth factor; EST, expressed sequence tag; LDL, low-density lipoprotein; MPR, M6P receptor; M6P, mannose 6-phosphate; NTPP, N-terminal propeptide; PAC vesicle, precursor-accumulating vesicle; PV72/82, PV72 and PV82; VPS, vacuolar protein sorting.

The nucleotide sequence reported in this paper has been submitted to the DDBJ, EMBL and GenBank with the accession number AB006809.

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PAC vesicles have been shown to mediate the transport of a precursor of 2S albumin, one of the major storage proteins, to protein-storage vacuoles in developing pumpkin cotyledons (Hara-Nishimura et al. 1993a). The vesicles could contain a receptor protein for the storage protein. Here we describe two PAC membrane components with receptor-like features, designated PV72 and PV82. In the following, "PV72 and PV82" is abbreviated as "PV72/82".

Materials and Methods—Seeds of pumpkin (*Cucurbita* sp. cv. Kurokawa Amakuri Nankin) were grown in the field during the summer season, and the developing cotyledons of 25–35 d after fertilization were used. PAC vesicles were isolated from developing pumpkin cotyledons by Percoll self-gradient centrifugation as described previously (Shimada et al. 1994b). The vesicle fraction was subjected to SDS-PAGE with an 8 or 15% gel. The separated proteins were then stained with Coomassie blue or blotted to a GVHP membrane (Millipore, Bedford, MA, U.S.A.). To determine N-terminal amino acid sequences, the bands corresponding to PV72 and PV82 were cut out from the blot and subjected to automatic Edman degradation on a peptide sequencer (model 492; Applied Biosystems Inc., Foster City, CA, U.S.A.). An immunoblot analysis was performed with specific antibodies against 2S albumin or PV72/82 and an ECL system (Amersham Japan, Tokyo, Japan).

To isolate a cDNA for PV72, we searched sequence databases with the N-terminal sequences of PV72 and PV82 that we determined (Fig. 1C). These sequences showed a high homology to only one deduced amino acid sequence of an Arabidopsis expressed sequence tag (EST) clone (Fig. 1C, EMBL accession number Z38123). We determined the complete nucleotide sequence of the clone Z38123 and found that it encoded a 68,913-Da protein of 623 amino acids. The deduced sequence showed a homology to other EST clones including Arabidopsis, rice (*Oryza sativa*) and maize (*Zea mays*). We synthesized two degenerate primers, 5'-GGATCCAGATT(TC)GT(ATGC)GT(ATGC)GA(AG)AA(AG)AA-3' and 5'-CTGCAGGA(AG)-(TAG)AT(ATGC)GT(ATGC)AC(GA)TC(ATGC)CC-3', based on the amino acid sequences, RFVVEKN and GDVTIL, respectively. RFVVEKN is a consensus sequence among the N-terminal sequences of PV72, PV82 and the Arabidopsis EST clone Z38123. GDVTIL is a consensus sequence between the Arabidopsis EST clone Z38123 and rice EST clone D41226. PCR was conducted using a cDNA library from developing pumpkin cotyledons (Hara-Nishimura et al. 1993b) and these primers, and yielded DNA fragments of a size of about 1 kb. Sequence analysis revealed that two DNA fragments encoding PV72 and PV82, respectively, were amplified. The amplified DNA fragments were used for screening of the cDNA library as described previously (Shimada et al. 1994a). Sequencing of both strands of the insert of the isolated cDNA was performed with a DNA sequencer (model 377; Applied Biosystems

Inc.) and -21M13 forward and M13 reverse fluorescent primers in accordance with the manufacturer's directions.

To prepare specific antibodies against each of PV72 and PV82, the respective DNA fragments described above were inserted into an expression vector pQE-30 (QIAGEN, Chatsworth, CA, U.S.A.). The expressed histidine-tagged proteins were subjected to SDS-PAGE followed by staining with Coomassie blue and the bands corresponding to the PV72 or PV82 fragments were cut out from the gel and injected into rabbits, as described previously (Inoue et al. 1995). Prepared antibodies against PV72 and PV82 were mixed and used in an immunoblot analysis (each dilution 1 : 1000).

A binding assay of PV72/82 was performed by affinity column chromatography. To prepare affinity columns, peptides were synthesized by a peptide synthesizer (model 431A; Applied Biosystems Inc.): SSSFADSNPIRPVTDRAASTYC (proaleurain), YRTTITTEVEENRQGR-E (2S-N), SRDVLQMRGIENPWRRREG (2S-I), KARNLPSMCGIRPQRCDF (2S-C), MRGIENPWRRREG (2S-I/ Δ N), SRDVLQMRGIEN (2S-I/ Δ C), SRDVLQMRGGENPWRRREG (I72G), SRDVLQMRGIGNPWRRREG (E73G), SRDVLQMRGIEGPWRREG (N74G), SRDVLQMRGIENGWRREG (P75G), SRDVLQMRGIENPGRREG (W76G), SRDVLQMRGIENPWGREG (R77G), SRDVLQMRGIENPWREG (R78G), SRDVLQMRGIENPWRGG (E79G), SRDVLQMRGIEGGGGGRER (2S-I/4G), SRDVLQMRGIENPWGGGG (2S-I/3G), KARGGGGMCIRPQRCDF (2S-C/4G). Each peptide (5 mg) was immobilized to coupling gels according to the manufacturer's protocols (2 ml of a bed volume; Pierce, Rockford, IL, U.S.A.), Sulfolink gel for proaleurain peptide and Aminolink gels for the other peptides. Affinity chromatography was carried out essentially as described by Kirsch et al. (1994). We prepared the microsome fraction from developing pumpkin cotyledons as described previously (Hatano et al. 1997). The fraction in 20 mM HEPES-NaOH, pH 7.1, 150 mM NaCl, 1 mM MgCl₂ and 1 mM CaCl₂ was sonicated and centrifuged at 100,000 $\times$ g for 1 h. For extraction of membrane proteins, the pellet was resuspended and sonicated again in the above buffer with 1% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and 10% (v/v) glycerol, and subsequently centrifuged at 100,000 $\times$ g for 1 h. The resultant supernatant (CHAPS extract) was used as a crude source of PV72/82. Five ml of the CHAPS extract (containing 5 or 20 mg of proteins) was applied to the affinity columns, followed by washing the column with 5 ml of CHAPS buffer, and then with 5 ml of CHAPS buffer containing 500 mM NaCl and 5 ml of CHAPS buffer again. Finally, bound proteins were eluted by the addition of 0.2 ml of each peptide in the CHAPS buffer (25 mg ml⁻¹).

The PAC vesicles contain the two homologous proteins PV72 and PV82 together with 2S albumin precursor

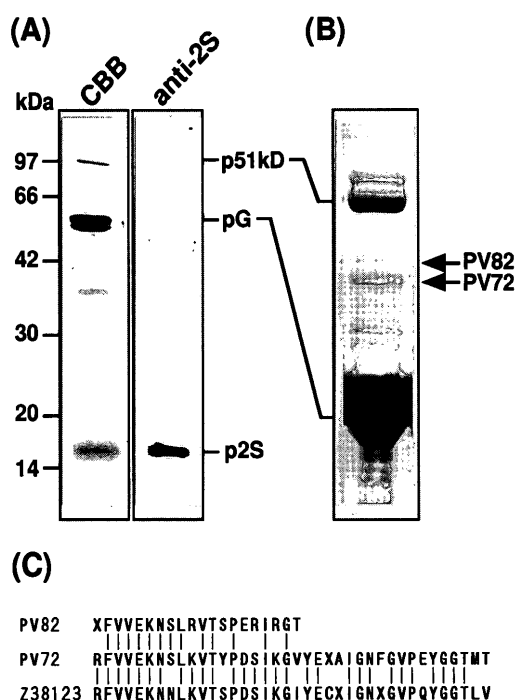


Fig. 1 Protein components of the PAC vesicles. (A) The PAC vesicles (5 µg) were subjected to SDS-PAGE (15% acrylamide) and then to staining with Coomassie blue (CBB) or to immunoblot analysis with anti-2S albumin antibodies (anti-2S). p2S, pG and p51 kD indicate pro2S albumin, proglobulin and pro51-kDa protein, respectively. (B) The PAC vesicles (100 µg) were subjected to SDS-PAGE (8% acrylamide) and then to staining with CBB to detect PV72 and PV82. (C) Comparison of N-terminal amino acid sequences of PV82 (20 residues) and PV72 (38 residues) with the sequence of an Arabidopsis EST clone (EMBL accession number Z38123).

—We previously reported that the PAC vesicles mediate the transport of the proprotein precursor of 2S albumin to the protein-storage vacuoles in developing pumpkin cotyledons (Hara-Nishimura et al. 1993a). To study protein components of the vesicles, we subjected the isolated vesicles to SDS-PAGE on a 15% gel and then either to staining with Coomassie blue or to immunoblotting with anti-2S albumin antibodies, as shown in Fig. 1A. One of the major proteins was a 15-kDa pro2S albumin (p2S in Fig. 1A). The other two major proteins were pro51-kDa protein and proglobulin (Hara-Nishimura et al. 1991).

To characterize the minor protein components with high molecular masses in greater detail, we subjected the isolated PAC vesicles to SDS-PAGE on an 8% gel, as shown in Fig. 1B. Two minor proteins with apparent molecular masses of 72 kDa and 82 kDa were designated PV72 and PV82, respectively. Their N-terminal amino acid sequences were very similar to each other and also to the deduced sequence of Arabidopsis EST clone Z38123 (Fig. 1C).

Molecular structure of PV72—Based on the conserved

amino acid sequences (see Materials and Methods), we isolated a cDNA from the library of developing pumpkin cotyledons. This clone encoded a 68,950-Da protein of 624 amino acids (Fig. 2A). The N-terminal sequence of PV72 was identical to a sequence following a putative signal peptide of the deduced sequence (double underlined in Fig. 2A), indicating that this clone was a cDNA for PV72. A structural analysis suggested that PV72 was a type I integral membrane protein composed of a luminal domain of 549 amino acids followed by a transmembrane domain of 17 amino acids and a cytoplasmic tail of 37 amino acids. The membrane topology of PV72 was supported by the results that PV72 bound to concanavalin A (data not shown) and that two possible glycosylation sites were found in the luminal domain, NES and NIS (dots in Fig. 2A).

Recently, Ahmed et al. (1997) reported an Arabidopsis receptor-like protein, AtELP, from the nucleotide sequence of two contiguous EST clones. The amino acid sequence of AtELP was identical to the sequence of the EST clone Z38123 that we determined. PV72 and AtELP showed 74% identity at the protein level and some common features of a receptor-like protein. Both PV72 and AtELP were predicted to be type I integral membrane proteins. At the C-terminal region of the luminal domain, they contained three repeats of an EGF-like motif that were presumed to mediate protein-protein interactions (boxed sequences in Fig. 2A). Each motif was composed of about 50 amino acids and contained six conserved Cys residues that could form three intrachain disulfide bonds in the region (Campbell and Bork 1993). The third motif showed the characteristics of a calcium-binding EGF-like motif. The cytoplasmic tail contained a YMPL sequence (asterisks in Fig. 2A). This is a potential Tyr-based signal, YXXΦ, in which X represents any amino acid and Φ represents a hydrophobic residue with a bulky side chain. Tyr-based signals are known to be recognized by the adapter complex (AP-1 and/or AP-2) of CCV components. Structural analyses suggested that PV72 and AtELP might function as sorting receptors in plant cells.

PV72/82 bind to the peptides derived from both an internal propeptide and a C-terminal peptide of pro2S albumin—To clarify the function of PV72/82, we investigated the binding abilities of PV72/82 to a vacuolar targeting sequence by affinity column chromatography. Pea BP-80 has been shown to have an ability to bind vacuolar targeting sequences (Kirsch et al. 1994, 1996), suggesting that it is a potential receptor for vacuolar proteins. The microsomal membrane proteins that contained PV72/82 were solubilized and were applied to a proaleurain column with the targeting sequence of barley aleurain, as shown in Fig. 3. In contrast to the predominance of PV72 in the PAC vesicles (Fig. 1B), PV82 was predominant in the microsome membrane proteins (data not shown). PV72/82

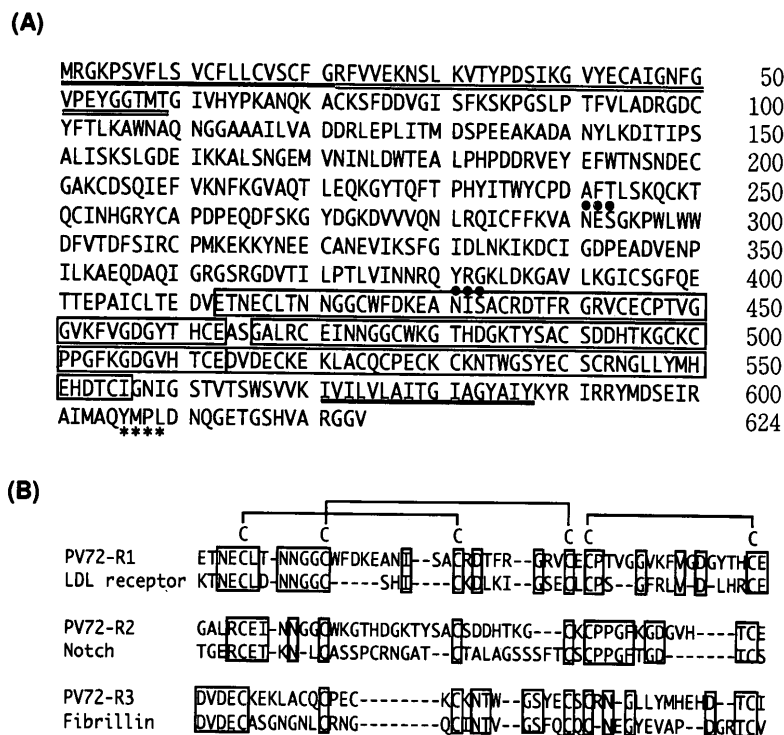


Fig. 2 Structural characterization of pumpkin PV72. (A) Deduced amino acid sequence from the isolated cDNA. The putative signal peptide (starting at position 1) and the transmembrane domain (starting at position 571) are indicated by single underlines. The N-terminal sequence of PV72 shown in Fig. 1C is indicated by a double underline. Three EGF-like motifs are boxed (starting at positions 413, 466 and 514). A potential tyrosine motif, YMPL, is indicated by asterisks. Two possible glycosylation sites are indicated by dots. (B) Comparison of each EGF-like motif with the known EGF-like motif from three proteins (LDL receptor, PIR accession number I48623; notch, Swiss-Prot accession number P07207 and fibrillin, Swiss-Prot accession number P35555). Identical amino acids are boxed. Three possible disulfide bonds of six conserved Cys residues are linked by lines.

bound to the column and then specifically eluted with the addition of the same peptide at excess concentration. This

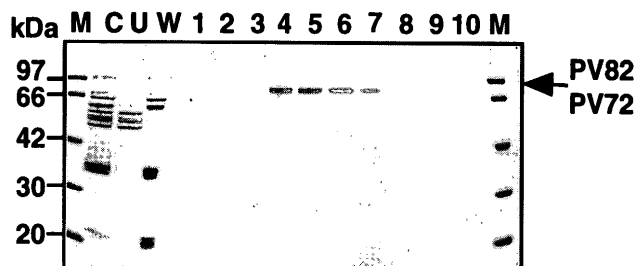
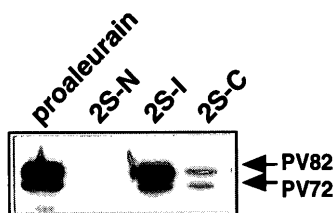


Fig. 3 Affinity chromatographic analysis of PV72/82 showing binding to a vacuolar targeting sequence of barley aleurain (pro-aleurain peptide). CHAPS extract (5 ml) containing 20 mg of protein from the microsome membrane fraction was applied to the proaleurain column. After washing with 5 ml of CHAPS buffer containing 500 mM NaCl, bound proteins were eluted by the addition of 0.2 ml of the same peptide in CHAPS buffer (25 mg ml⁻¹). The eluate was collected in 0.5-ml fractions. Each 5 μ l of CHAPS extract (C), unbound fraction (U) and wash fraction (W), and each 50 μ l of eluted fractions (1 to 10) were subjected to SDS-PAGE followed by Coomassie blue staining. M, molecular mass markers. Both PV72 and PV82 are indicated by an arrow.

result suggested that PV72/82 might function as sorting receptors for vacuolar proteins.

PV72/82 and pro2S albumin co-exist in the PAC vesicles (Fig. 1A, B). This raised the question of whether PV72/82 could function as sorting receptors for pro2S albumin to target them to protein-storage vacuoles. To examine the affinity of PV72/82 for pro2S albumin, we prepared affinity columns with three peptides derived from pro2S albumin: 2S-N, which contains the N-terminal propeptide, 2S-I, which contains the internal propeptide, and 2S-C, which contains the C-terminal region. PV72/82 specifically bound to the 2S-I column and the 2S-C column, but not to the 2S-N column (Fig. 4A). These results suggested that the 2S-I peptide and/or the 2S-C peptide might contain the signal for pro2S albumin that binds with the putative receptors PV72/82. The 2S-C column retained a smaller amount of PV72/82 compared with the proaleurain and the 2S-I columns. The poor binding of PV72/82 to the 2S-C peptide may have been due to an inaccessibility of the binding site. The binding site was expected to be located in the N-terminal region (see Fig. 6), but this region might have been inaccessible because the 2S-C peptide was linked to the column at its N-terminus.

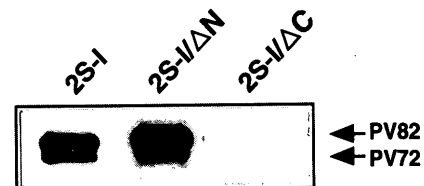
(A) Binding assay**(B) Competition assay (2S-I column)**

2S-N YRTTITTVEVEENRQGRE
2S-I SRDVLQMRGIENPWRREG
2S-C KARNLPSMCGIRPQRDCF
proaleurain
 SSSFADSNPIRPVTDRAASTYC

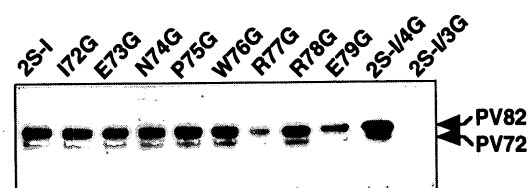
Fig. 4 Binding of PV72/82 to the peptides derived from pro2S albumin. (A) Binding assay using affinity columns with the peptides. The procedure was almost the same as in Figure 3. CHAPS extract containing 5 mg of proteins was applied to each column with the indicated peptide and the respective peptide was used as an eluent. Eluted fraction number 5 (50 μ l) was subjected to SDS-PAGE followed by immunoblot analysis with antibodies against PV72 and PV82. (B) Competition assay using the indicated peptide as an eluent on the 2S-I column. CHAPS extract containing 5 mg of proteins was applied to the 2S-I column. The elution was performed with each of the indicated peptides. The subsequent procedures were the same as in A. Both PV72 and PV82 are indicated by arrows.

To examine which peptides could function as a competitor with 2S-I peptide, we next performed competition experiments with the 2S-I column and an excess amount of each peptide of proaleurain, 2S-N, 2S-I or 2S-C (Fig. 4B). PV72/82 were eluted specifically by the addition of each proaleurain peptide, 2S-I and 2S-C, but not by the addition of 2S-N. In this experiment, the 2S-C peptide was approximately equal to the proaleurain peptide or 2S-I peptide in its ability to bind PV72/82. These results suggested that each of these three peptides bound to the same binding site of PV72/82.

To more clearly define the parts of the 2S-I and 2S-C peptides that are responsible for binding PV72/82, further binding experiments were done using variants of these peptides. Deletion of the six C-terminal amino acids completely abolished its ability to bind to PV72/82, whereas dele-

(A) Competition assay (2S-I column)

2S-I SRDVLQMRGIENPWRREG
2S-I/ΔN MRGIENPWRREG
2S-I/ΔC SRDVLQMRGIEN

(B) Binding assay

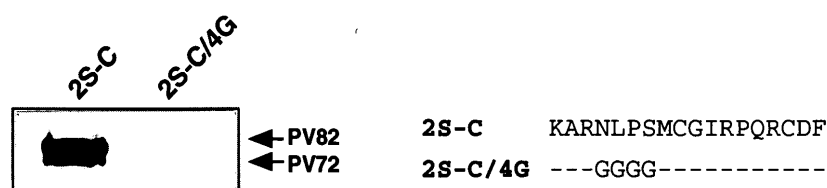
2S-I SRDVLQMRGIENPWRREG
I72G -----G-----
E73G -----G-----
N74G -----G-----
P75G -----G-----
W76G -----G-----
R77G -----G-----
R78G -----G-----
E79G -----G-----
2S-I/4G -----GGGG-----
2S-I/3G -----GGG-----

Fig. 5 Essential elements in the 2S-I peptide for PV72/82 binding. (A) Competition assay on the 2S-I column. The mutated peptide 2S-I/ΔN and 2S-I/ΔC were separately used as an eluent. Procedure was the same as in Fig. 4B. (B) Binding assay using affinity columns with the mutated peptides, as indicated. Procedure was the same as in Fig. 4A. Both PV72 and PV82 are indicated by arrows.

tion of the six N-terminal amino acids showed no effect (Fig. 5A). This result indicated that the binding site for PV72/82 resided in the C-terminal region of the 2S-I peptide. To investigate the essential residue(s) for binding to PV72/82, each amino acid in the region was substituted with a Gly residue. Substitutions of Gly for either Arg-77 (R77G) or Glu-79 (E79G) reduced the peptide's ability to binding to PV72/82, whereas the other substitutions showed no obvious effects, as shown in Fig. 5B.

The 2S-I peptide contains the NPWR sequence that resembles the NPIR motif of a vacuolar targeting signal reported by Holwerda et al. (1992) and Matsuoka and Nakamura (1991). We substituted the NPWR of the 2S-I peptide with GGGG to produce the 2S-I/4G peptide and prepared a 2S-I/4G affinity column. Unexpectedly, PV72/82 specifically bound to the mutated peptide (Fig. 5B). This

(A) Competition assay (2S-I column)



(B) Alignment of C-terminal regions of 2S albumins

			*	*	
Pumpkin-1	QARGQ--EGRQMLQ	<i>KARNLPSMCGIRP-QRCDF</i>			141
Pumpkin-2	QGRMQGIDVGQMLERAT	<i>NLPSVCRLSQ-RRCELRSSRW</i>			139
<i>R. com.</i>	QGQLQGQDVFEAFRTAA	<i>NLPSMCGVSP-TECRF</i>			258
<i>A. tha.</i>	QGQHQPMPQVRKIYQTAKHLPNVCDIPQVDVCPF	<i>NIPSPFSFY</i>			164
<i>B. exc.</i>	EMOPRGEOMRRMMRLAEN	<i>IPSRCNLSP-MRCPMGGSIAGF</i>			146

Fig. 6 Essential elements in the 2S-C peptide for PV72/82 binding. (A) Competition assay using the mutated peptide, 2S-C/4G, as an eluent on the 2S-I column. Procedure was the same as in Fig. 4B. Both PV72 and PV82 are indicated by arrows. (B) Comparison of the C-terminal region among pro2S albumins from various plants. Sequences are derived from 2S albumin of pumpkin isoform-1 (Pumpkin-1; Hara-Nishimura et al. 1993a), pumpkin isoform-2 (pumpkin-2; Shimada et al., unpublished data); castor bean (*R. com.*; Irwin et al. 1990), Arabidopsis (*A. tha.*; Krebbers et al. 1988), Brazil nut (*B. exc.*; Altenbach et al. 1992). The 2S-C sequence is underlined. NL/IPS sequences are indicated by bold-faced and italic characters. Two conserved Cys residues are indicated by asterisks.

result indicated that the NPWR sequence was not involved in the binding of PV72/82, in spite of the similarity of the sequence to the reported targeting signal. In contrast, when the RRE sequence of the 2S-I peptide was substituted with GGG to produce the 2S-I/3G peptide, PV72/82 could not bind to the mutated peptide (Fig. 5B). This result was very consistent with the results obtained with the mutated peptides, R77G and E79G (Fig. 5A), indicating that the RRE sequence was essential for the binding of PV72/82 to the 2S-I peptide.

In the next step, we examined the effect of mutation in the 2S-C peptide on PV72/82 binding. An NL/IPS sequence was conserved in the C-terminal regions of 2S albumins from several plants (Fig. 6B). We substituted the NLPS with GGGG to produce a 2S-C/4G peptide (Fig. 6A) and used it as a competitor with 2S-I in the PV72/82 binding. PV72/82 were eluted with 2S-C, but not with 2S-C/4G (Fig. 6A), indicating that the NLPS sequence was essential for PV72/82 binding to the 2S-C peptide.

The overall results suggested that each of the 2S-I, 2S-C and proaleurain peptides might bind to the same binding site of PV72/82. However, no obvious sequence homology was observed among these three peptides. Moreover, the essential elements for the binding to PV72/82 were quite different: RRE in the 2S-I peptide and NLPS in the 2S-C peptide. Taken together, these results suggested that PV72/82 could recognize the physicochemical properties of the peptides rather than the primary structure of them. Further characterization of peptides that are able to bind PV72/82 is necessary to clarify the mechanism of molecu-

lar recognition between PV72/82 and the peptides.

Recently, the vacuolar targeting signal of pro2S albumin from Brazil nut (*Bertholletia excelsa*) was determined to reside at its C-terminus (Saalbach et al. 1996). Pea BP-80 has been shown to bind to a synthetic peptide derived from the C-terminal sequence of pro2S albumin of Brazil nut (Kirsch et al. 1996). These findings suggest that a protein homologous to BP-80 might act as a sorting receptor for pro2S albumin in the developing seeds of Brazil nut. PV72/82 also bound to 2S-I and 2S-C peptides derived from pumpkin pro2S albumin, suggesting that the 2S-I peptide and/or the 2S-C peptide might contain the vacuolar targeting signal. Thus, it is possible that PV72/82 might mediate the sorting of pro2S albumin to the vacuole in developing pumpkin seeds. This possibility is strongly supported by the fact that PV72/82 and pro2S albumin co-exist in the PAC vesicles which mediate the transport of the precursor of 2S albumin to protein-storage vacuoles. The overall results suggest that pumpkin PV72/82, pea BP-80 and AtELP are sorting receptors for vacuolar proteins in plant cells.

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(Received August 25, 1997; Accepted October 17, 1997)