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Sesame oleosin and prepro-2S albumin expressed as a fusion polypeptide in transgenic rice were split, processed and separately assembled into oil bodies and protein bodies

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Abstract

A recombinant polypeptide containing the precursor protein of a sesame storage protein, 2S albumin, fused to the C-terminus of a sesame oleosin was expressed in transgenic rice seeds under the control of a rice glutelin promoter. The recombinant polypeptide of 32 kDa, equivalent to the resultant molecular mass of sesame oleosin (15 kDa) and prepro-2S albumin (17 kDa), was detected in the endoplasmic reticulum fraction of maturing transgenic rice seeds, but not in the purified oil bodies or the soluble extract of transgenic seeds. However, sesame oleosin presumably fused with a 2 kDa C-terminal appendix originating from the signal sequence of prepro-2S albumin, was found in the purified oil bodies, and mature sesame 2S albumin apparently processed into two subunits (9 and 4 kDa) linked by disulfide bonds was detected in extracts of transgenic seeds. Immunogold labeling revealed that the sesame oleosin and 2S albumin were separately located in oil bodies and protein bodies of embryo cells of transgenic rice seeds. While sesame 2S albumin was also detected in protein bodies of endosperm cells of transgenic seeds, the co-expressed sesame oleosin, probably degraded due to the lack of oil bodies in this tissue, and was not detected. The results provide a new technique for introducing two recombinant polypeptides separately into rice oil bodies and protein bodies from one expression construct.

Keywords: 2S albumin; Oleosin; Sesame; Signal sequence; Transgenic rice

1. Introduction

Sesame 2S albumin (S2SA), the major soluble storage protein in seed, has been identified as a sulfur-rich protein, which apparently accounts for the high nutritional quality of sesame (Tai et al., 1999). As with other known seed 2S albumins, S2SA is first synthesized as a precursor protein (17 kDa) with a cleavable *N*-terminal signal sequence essential for ER targeting via the signal-recognition particle (SRP) dependent pathway (Shewry and Halford, 2002). After cleavage of the signal sequence and further post-translational processing, the resulting mature S2SA is composed of two subunits of 9 and 4 kDa linked by disulfide bonds, and is accumulated in the seed protein bodies (Brown et al., 2003). Similar results were found when the sesame S2SA precursor protein was expressed in transgenic rice seeds (Lee et al., 2003, 2005). Obviously, the machinery responsible for recognition and cleavage of the *N*-terminal signal sequence as well as that responsible for the translocation, packaging, transportation, proteolytic cleavage, and folding of storage proteins is compatible between sesame and rice seeds.

Oleosin is a structural protein protecting oil bodies of plant seeds by steric hindrance and electronegative repulsion (Tzen et al., 1992). The structure of oleosin has been proposed to consist of three domains: an *N*-terminal amphipathic domain of 20–60 residues, a central hydrophobic domain of approximately 70 residues, and a C-terminal amphipathic α-helix domain of 30–40 residues. During seed maturation, oleosin is proposed to

Abbreviations: ER, endoplasmic reticulum; S2SA, sesame 2S albumin; SRP, signal-recognition particle; preproS2SA, precursor protein of sesame 2S albumin; Ab-SOL15, antibodies against sesame oleosin; Ab-S2SL, antibodies against the large subunit of S2SA.

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co-translationally target to the ER membrane from which oil bodies are derived under the assistance of SRP (Abell et al., 2002; Beaudoin and Napier, 2000). Since oleosin does not possess an *N*-terminal signal sequence, it is assumed that the SRP interacts with the central hydrophobic domain of the protein.

It is not known how a recombinant polypeptide is directed, processed and translocated when it contains both targeting signals to seed oil body and protein body. Knowing that oleosin co-translationally targets to the ER membrane during seed maturation, we speculated that the leader signal sequence of preproS2SA would be recognized and cleaved properly in the ER membrane and initiate the follow-up processing of mature S2SA when an oleosin polypeptide is linked to its *N*-terminus. In this study, a recombinant polypeptide containing preproS2SA fused to the C-terminus of a sesame oleosin was specifically expressed in transgenic rice seeds under the control of a rice glutelin promoter. The expression and accumulation of the recombinant polypeptide and its processed products in maturing and mature seeds of transgenic rice plants were analyzed.

2. Experimental

2.1. Plasmid construction

A chimeric plasmid, pGlu-OlePP2S, carrying a rice seed-specific glutelin promoter (1200 bp, Wu et al., 1996), a cDNA fragment encoding sesame oleosin 15 (420 bp, Tai et al., 2002), and a cDNA fragment encoding the precursor polypeptide of S2SA (450 bp, Tai et al., 1999), was ligated into pCAMBIA 1300 (accession no. AF234296) between the restriction enzyme sites of *Hin*dIII and *Eco*RI (Fig. 1). For the construction, sesame oleosin 15 gene was first amplified using a pair of primers, 5′-CGATCCCATGGCTGAGCATTATGGTCAACAA-3′ and 5′-GCGTACCATGGAACAGGCTGCTGCGAGAAC-3′ to create *Nco*I restriction enzyme sites (underlined) on both ends of the clone. The amplified fragment was then introduced into the *Nco*I site of the plasmid pGlu-PP2S containing the glutelin promoter and the preproS2SA clone constructed previously (Lee et al., 2003), and the resulting plasmid, pGlu-OlePP2S harboring a selectable marker hygromycin phosphotransferase gene was transferred into *Agrobacterium tumefaciens* strain EHA105 by electroporation. Accuracy of the plasmid construction, particularly the direction of sesame oleosin gene, was checked by PCR amplification of the OlePP2S fragment and confirmed by DNA sequencing. The expected recombinant polypeptide (32 kDa) comprised the sesame oleosin (15 kDa) lacking its last six amino acid residues (AGSQTS) and the preproS2SA (17 kDa).

2.2. Plant transformation and selection

Oryza sativa L., *japonica* cv. TNG67, a major rice variety cultured in Taiwan, was selected as the target plant for transformation. Rice calli derived from immature embryos were used as the target material for *Agrobacterium*-mediated transformation according to Hiei et al. (1997) with slight modifications. Rice calli were incubated in a suspension of *Agrobacteria tumefaciens* containing pCAMBIA 1300 for 15–30 min. Transformants were removed from the suspension and transferred to solid 2N6-AS medium (two-fold N6 salts, 2,4-dichlorophenoxyacetic acid 2 ppm, casamino acid 1 g/l, sucrose 30 g/l, pH 5.2), and cocultivated in a plant incubator at 28 °C for 72 h in the dark. After cocultivation, transformants were washed in a liquid medium with cefotaxime (250 mg/l) and plated on solid 2N6-CH medium (two-fold N6 salts, 2,4-dichlorophenoxyacetic acid 2 ppm, sucrose 30 g/l, pH 5.7) for 3 weeks. Subcultured transformants were then transferred to RS-selection medium (MS salts, casamino acid 1 g/l, naphthaleneacetic acid 0.02 ppm, kinetin 2 ppm, sorbitol 30 g/l, sucrose 30 g/l, pH 5.7) containing hygromycin 50 ppm every ten days until shoot regenerated. Regenerated plantlets (T0 plants) were transplanted to soil in pots in a restricted greenhouse in the Taiwan Agricultural Research Institute, Taichung. Transgenic lines were detected by screening with hygromycin resistance as reported previously (Lee et al., 2005).

2.3. Southern and Northern analyses

Rice genomic DNA was isolated from 3-week-old seedling according to Dellaporta et al. (1984). Isolated DNA of 15 μg was digested with *Hin*dIII/*Eco*RI or *Xho*I, resolved on a 1% agarose gel, transferred onto a Zeta-probe GT membrane (BioRed, USA), and hybridized with a ^{32}P-labeled cDNA probe containing the coding sequence of sesame oleosin fused with preproS2SA. Hybridization,

Fig. 1. Diagram of the construction of pGlu-OlePP2S. The glutelin promoter (1200 bp, accession number D26365), sesame oleosin clone (420 bp, accession number AF091840), 2S albumin precursor gene (450 bp, accession number AF091841), and NOS termini (300 bp) were ligated into pCAMBIA 1300 (8985 kb) between the restriction enzyme sites of *Hin*dIII and *Eco*RI. LB, RB, and *hpt* represent left border, right border, and hygromycin phosphotransferase gene, respectively.