Biotechnology Advances 30 (2012) 1309–1317

Contents lists available at SciVerse ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Multiple biological functions of sporamin related to stress tolerance in sweet potato (*Ipomoea batatas* Lam)

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ARTICLE INFO

Available online 28 January 2012

Keywords:
Insect resistance
Sporamin
Sweet potato
Trypsin inhibitor
Storage protein

ABSTRACT

The initial investigation of the nature of the proteins in the tuber of sweet potato (*Ipomoea batatas* Lam.) revealed a globulin-designated "ipomoein," which was reported by Jones and Gersdorff, (1931). Later, "ipomoein" was renamed "sporamin" and was found to be a major storage protein that accounted for over 80% of the total protein in the tuberous root. To date, sporamin has been studied by a series of biochemical and molecular approaches. The first purification of sporamin into two major fractions, A and B, was successfully completed in 1985. Several characteristics of the protein, such as the diversification of the nucleotide sequences in the gene family, the protein structure, the biological functions of storage, defense, inhibitory activity and ROS scavenging, were identified. In the past decade, sporamin was classified as a Kunitz-type trypsin inhibitor, and its insect-resistance capability has been examined in transgenic tobacco and cauliflower plants, indicating the multiple functions of this protein has evolved to facilitate the growth and development of sweet potato. Sporamin is constitutively expressed in the tuberous root and is not normally expressed in the stem or leaves. However, this protein is expressed systemically in response to wounding and other abiotic stresses. These dual expression patterns at the transcriptional level revealed that the complex regulatory mechanism of sporamin was modulated by environmental stresses. The versatile functions of sporamin make this storage protein a good research model to study molecular evolution, regulatory mechanisms and physiological functions in plants. This review summarizes and discusses recent approaches and future perspectives in agricultural biotechnology.

Contents

1. Sporamin as a storage protein

Storage proteins are the most abundant proteins in storage tissue. In general, storage proteins display a tissue-specific expression pattern, such as in seeds, tubers and bark. Storage proteins serve as a nutritional resource for plants by providing an essential source of nitrogen and

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doi:10.1016/j.biotechadv.2012.01.022

amino acids during growth and development, especially during seed germination and tuber regrowth. Moreover, many storage proteins have been shown to possess the enzymatic activities that are related to plant defenses against threats such as insects, plant diseases and abiotic stress (Meuriot et al., 2004a, 2004b; Shewry, 2003; Van Damme et al., 2002). The sporamin of sweet potato is an example. Initially known as ipomoein (Jones and Gersdorff, 1931), sporamin was first described and purified from the total soluble protein of a sweet potato tuber by assaying the dominant protein band in a 25-kDa SDS-PAGE (Maeshima et al., 1985). Considered to be the major storage protein in sweet potato tubers, sporamin accounts for 60–80% of the total soluble protein. Its expression has been shown to be primarily associated with the tubers, with a very low amount in the stems and none in the leaves (Chen et al., 2006).

1.1. The molecular characterization of sporamin

Sporamin genes were reported to be intronless genes that comprised a large multi-gene family. Based on nucleotide sequence homology, Hattori and Nakamura assessed a group of 47 sporamin cDNAs in 1989 (Hattori et al., 1989) and divided the sporamin genes into two subfamilies, sporamin A and sporamin B. Within the same subfamily, the nucleotide identity reached as high as 94–98%, while the identity between the subfamilies was up to 82–84% (Shewry, 2003). The differences in the nucleotide sequence resulted from nucleotide substitutions in the translated region and 3–6 bp nucleotide insertions or deletions in the 5′- and 3′-untranslated regions (Hattori et al., 1989). Moreover, in a phylogeny of published sporamin cDNAs, the sporamin A subfamily is further differentiated into two subgroups, sporamin A1 and sporamin A2 (Hattori et al., 1989). Sporamin size varies depending on the conditions. Under denaturing conditions, the protein size of sporamin is identified as 25 kDa, whereas in native gel analysis, sporamin separated into two protein bands, 31 kDa and 22 kDa, which correspond to sporamin A and sporamin B, respectively (Maeshima et al., 1985). Sporamin can only be separated into its subfamilies while in its native state. In addition, the differences in behavior of sporamin A and sporamin B under non-reducing conditions has not yet been fully elucidated. The structures of sporamin A and sporamin B are similar but not identical to each other. In contrast, no obvious homology of the amino acid sequences was found between sporamin and patatin, a potato storage protein (Hattori et al., 1985). The deduced amino acid sequence identity of sporamin A and sporamin B with other trypsin inhibitors, excluding the signal peptide, was 30% with soybean trypsin inhibitor (STI), *Erythrinia variegata* trypsin inhibitor (ETIa), wing bean trypsin inhibitor (WTI-1A) and *Acacia confusa* trypsin inhibitor (ACTI) (Fig. 1), which indicates that sporamin are quite different from other plant trypsin inhibitors (Yao et al., 2001). All of the sequences contain four conserved cysteine residues and a negatively charged A4-B1 loop that confers trypsin inhibitory activity (Fig. 1) (Yao et al., 2001). It has been postulated that the divergence of sporamin A and sporamin B from their common ancestral gene occurred on different chromosomes carried by different progenitors of sweet potato during the course of evolution (Murakami et al., 1986). However, the analysis of sporamin from wild-type species, including *Ipomoea leucantha* (2×), *Ipomoea littoralis* (4×) and *Ipomoea trifida* (3× and 6×) will be desirable for understanding the promiscuous evolutionary activities that, served as a starting point for the divergence leading to the multi-gene family (Murakami et al., 1986).

1.2. The biosynthesis of sporamin

Sporamin is stored in the vacuole in the form of monomers (Hattori et al., 1985; Schroeder et al., 1993). Mechanism for the biosynthesis of sporamin and its transportation to the vacuole were proposed by Nakamura et al. (1993). Sporamin is initially produced as preprosporamin, which is synthesized by the membrane-bound polysome in the endoplasmic reticulum (ER). This precursor, preprosporamin, gives rise to prosporamin by removing the prepropeptide. The prepropeptide is cleaved from the amino terminus of the preprosporamin as a cotranslational process, thus generating prosporamin, which is transported to the lumen of the ER (Nakumura and Matsuoka, 1993). Prosporamin is shorter than its precursor by a signal peptide (or propeptide) of 25 amino acid residues. Next, the highly conserved sequence [Asn-Pro-Ile-Arg-Leu] (NPIRL) within the 16-aa C-terminal propeptide is recognized, which labels prosporamin for transport to the vacuole for its final post-translational processing (Shewry, 2003). The removal of a 16-amino-acid propeptide from the N-terminus of prosporamin completes the synthesis of sporamin, allowing the mature protein to be stored and accumulated in the vacuole.

The pre-propeptide at the N-terminus of preprosporamin is composed of two parts. One part is a hydrophobic signal peptide at the N-terminus, with 21 amino acids in the A subfamily and 19 amino acids in the B subfamily (Murakami et al., 1986); the other is a propeptide at the C-terminus with 16 charged amino acids that acts as a vacuole-sorting domain (Matsuoka and Nakamura, 1991; Matsuoka et al., 1990; Nakamura and Matsuoka, 1993). The mature sporamin is then active in its function. However, Matsuoka et al. (1995) also suggested that sporamin has another active isoform with its Ser^{39} modified by the addition of a galactosyl residue, also known as O-glycosylation. Using the yeast expression system, two sporamin proteins with the same sequence but different sizes were produced as a result of glycosylation.

1.3. The biochemical activities of sporamin

In addition to its role as a storage protein, sporamin has a defense role as a trypsin inhibitor. Sporamin has 30% identity and 50% similarity to the soybean Kunitz-type trypsin inhibitor (Hattori et al., 1991). Several reports have provided indirect evidence that the trypsin inhibitory activity is correlated with the concentration of soluble tuberous protein in sweet potato tubers (Lin and Tsu, 1987). In 1997, Yeh and colleagues demonstrated the in vivo trypsin inhibitory activity of sporamin by using an in-gel activity assay with recombinant protein from *Escherichia coli*. Several biochemical properties of sporamin were recognized: In the kinetics analysis, sporamin exhibits mix-type inhibition; the PI of sporamin is 5.0; the thermostability of sporamin reaches 65 °C for 10 min; its optimal reaction temperature is between 35 and 45 °C; and its optimal reaction pH ranges from pH 7–11 (Hilder et al., 1993). Sporamin-trypsin inhibitor was also shown to have various antioxidant functions related to stress tolerance, such as DHA and MDA reductase activities (Hou and Lin, 1997), existing in leaves and other plant tissues (Foyer and Mullineaux, 1998). In addition, sporamin possesses scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals as well as glutathione peroxidase-like activity (Hou et al., 2001, 2004). Further studies showed that the degree of tolerance or defense was dependent on trypsin inhibitors isolated from the cultivars. Hydrolysis with pepsin and chymotrypsin increased the scavenging activity of the trypsin inhibitors against DPPH, indicating that digestion did not eliminate the antioxidant properties (Hou et al., 2005).

In a structural analysis based on homology modeling principles (Yao et al., 2001), sporamin was predicted to have a secondary and tertiary structure similar to the *Erythrina caffra* trypsin inhibitor DE-3 (ETI), a member of the soybean Kunitz-type trypsin inhibitors. The inhibitory mechanism of the Kunitz-type trypsin inhibitors is through a substrate-like interaction with the protease catalytic sites of specific cognate enzymes. Although members of the Kunitz-type trypsin inhibitors exhibit a high degree of variation in their amino acid sequences, all of them contain a reactive site loop that bears the inhibitory residues. Additionally, the sites of four cysteine residues (two disulfide bond formations), Cys^{83}, Cys^{133}, Cys^{186} and