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Research Article

Expression and characterization of the acidic subunit from 11S Amaranth seed protein

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Amarantin acidic subunit has the potential to be employed as a functional and a nutraceutical protein. To evaluate both possibilities this protein was produced in recombinant *Escherichia coli* Origami (DE3) harboring the expression plasmid pET-AC6His. Three different expression factors were assayed: inductor concentration, temperature and time of the amarantin acidic subunit accumulation. The results indicated that a 0.3 mmol/L concentration of isopropyl-β-D-thiogalactoside, at 37°C and 6 h after induction were favorable for high expression of amarantin acidic subunit, mostly in the form of inclusion bodies. The protein was purified from soluble fraction by immobilized metal affinity chromatography, up to 30 mg amarantin acidic subunit/L Terrific broth culture were obtained. Sucrose density gradient ultracentrifugation analysis of the expressed soluble amarantin acidic subunit revealed that it was assembled in monomers. The expression of the amarantin acidic subunit, together with the one-step purification will facilitate further investigation of this storage protein through site-directed mutagenesis.

Keywords: Amaranth globulin · *E. coli*-expressed protein · 11S acidic subunit · Seed storage protein

1 Introduction

Amarantin is one of the most predominant storage proteins in *Amaranthus hypochondriacus* seeds, according to the Osborne classification [1], and by sedimentation coefficient it belongs to the 11S globulin class, with the advantage of showing high content of essential amino acids, making this protein important from a nutritional point of view [2]. Amarantin extracted from seeds has a hexameric structure with a molecular mass of 398 kDa and an 11.9S sedimentation coefficient. SDS-PAGE analysis under reducing conditions resolved three different bands: one of 50–52 kDa, corresponding to proamarantin, and two more bands of 32–34 and 22–24 kDa corresponding to acidic and basic chains, respectively [2, 3].

The biogenesis of 11S globulins has been detailed by different authors [4, 5]. During seed maturation, proteins that accumulate in the protein storage vacuoles, such as the 11S globulins, are synthesized in the endoplasmic reticulum; they are initialized by the synthesis of a single polypeptide, the precursor, which consists of covalently linked acidic and basic polypeptides with a signal sequence. The signal sequence is cotranslationally removed, and the resultant proglobulin subunits self-assemble into trimers of about 7S in the endoplasmic reticulum and are transported by vesicles to the protein storage vacuoles [4]. Vacuolar sorting determinants, which are usually amino acid sequences of short or moderate length, direct the proteins to this pathway [5–7]. The proteins migrate to

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Abbreviations: IMAC, immobilized metal affinity chromatography; IPTG, isopropyl-β-D-thiogalactoside