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

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Abstract

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-beta-D-thiogalactoside, at 37 degrees 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.

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