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Modification of the amaranth 11S globulin storage protein to produce an inhibitory peptide of the angiotensin I converting enzyme, and its expression in *Escherichia coli*

Silvia Luna-Suárez[a], Sergio Medina-Godoy[b], Andrés Cruz-Hernández[c], Octavio Paredes-López[c,*]

[a] *Centro de Investigación en Biotecnología Aplicada, CIBA-IPN, Tepetitla, Tlaxcala, Mexico*
[b] *Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, CIDIIR-IPN, Guasave, Sinaloa, Mexico*
[c] *Departamento de Biotecnología y Bioquímica, Centro de Investigación y de Estudios Avanzados del IPN, Apdo. Postal 629, 36821 Irapuato, Guanajuato, Mexico*

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ABSTRACT

Amarantin is the predominant seed storage protein from amaranth. It shows a high content of essential amino acids, making this protein important from a nutritional viewpoint. The protein has two disulfide linked subunits: acidic and basic. Acidic subunit has the potential as a functional and nutraceutical protein, and it is structurally a good candidate for modification. In order to improve its functionality, the primary structure was modified in the third variable region of globulins 11S, by inserting four Val-Tyr antihypertensive peptides in tandem. The designed plasmid was expressed in *Escherichia coli* Origami (DE3) and then the expressed protein was purified. Mass spectrometry analysis was used to corroborate the identity of the protein by peptide mass fingerprinting; also, the modified peptide was fragmented and sequenced by mass spectrometry, corroborating thus the inserted residues. The hydrolyzed protein showed a high inhibitory activity of the angiotensin converting enzyme (IC_{50} 0.064 mg ml^{-1}); it was nearly eightfold more active than the nonmodified protein. In spite that the nonmodified subunit is less active, its activity is comparable with other hydrolyzed proteins reported as high active inhibitors. The expressed and purified subunit after its engineered modification, may be useful for preventing hypertension and for other medical purposes.

1. Introduction

The globulin seed storage proteins have been proposed as excellent candidates to be incorporated into major crops in order to impact both functional and nutraceutical properties (Shewry, 1998). Amarantin, an 11S seed globulin, is the most predominant storage protein in *Amaranthus hypochondriacus* seeds; it contains a good balance of essential amino acids, remarkable heat stability and emulsifying properties (Romero-Zepeda and Paredes-López, 1996); these features make this protein a suitable model to generate transgenic crops (Rascón-Cruz et al., 2004). Moreover, this type of proteins are susceptible to protein engineering to improve further some characteristics, such as those described by Matoba et al. (2001) and Prak et al. (2006).

Mature amarantin extracted from seeds has a hexameric structure with a molecular mass of 398 kDa. 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 (Barba de la Rosa et al., 1996; Chen and Paredes-López, 1997). A His-tagged version was expressed and accumulated in *Escherichia coli* as a trimer, and proamarantin was purified by immobilized metal affinity chromatography (Medina-Godoy et al., 2004). Expression in plants was also performed including tobacco and maize, resulting in a proper accumulation pattern and in important increases of seed protein content with no-allergenic reaction in mice fed with transgenic plants (Rascón-Cruz et al., 2004; Sinagawa-García et al., 2004; Valdez-Ortiz et al., 2005). Moreover, the acidic subunit of amarantin is the candidate for protein modification. This fraction harbors four hypervariable regions of the five detected in the 11S seed globulins (Wright, 1988; Dickinson et al., 1990; Adachi et al., 2003). A His-tagged version of the acidic subunit was expressed in *E. coli* and was purified by immobilized metal affinity chromatography (Luna-Suárez et al., 2008). Using protein engineering, further characteristics could be incorporated to this high-nutritional protein, such as biopeptides or modified amino acid sequence, to enhance functional and nutraceutical properties.

On the other hand, hypertension is a major risk factor for arteriosclerosis, stroke, myocardial infarction, and end-stage renal disease. Angiotensin I converting enzyme (EC 3.4.15.1; ACE) is the

* Corresponding author at: Km. 9.6 Libramiento Norte Carr. Irapuato-León, Apdo. Postal 629, C.P. 36821, Irapuato, Guanajuato, Mexico. Tel.: +52 462 6239641; fax: +52 462 6245996.
E-mail address: oparedes@ira.cinvestav.mx (O. Paredes-López).

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