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Full Paper

Hypocholesterolemic Activity of Mungbean 8Sα Globulin Engineered with Lactostatin

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Protein engineering has been the major tool in enhancing proteins and their functional properties throughout the years. Using this technology, the improvement of proteins of important food crops such as the major storage protein of mungbean (8sα globulin) was made possible. In this study, the 8sα globulin of mungbean was engineered with the hypocholesterolemic peptide called lactostatin (originally derived from bovine β-lactoglobulin) with the sequence I-I-A-E-K (Ile-Ile-Ala-Glu-Lys), through substitution mutation, specifically using site-directed mutagenesis approach. Initially, in silico approach was done in order to design models for the wild type (WT) and the mutant mungbean 8sα globulin protein for comparison purposes and this preliminary approach checked that the mutation plotted to the mungbean 8sα globulin gene is stable considering the relationship between the protein's structure and function. After the mutation, the mutated gene in pET21d vector was transformed and expressed in E. coli BL21 (DE3) cells. The average total protein concentration attained in WT and mutant 8sα globulins were 746.36 ± 5.71 µg mL^{-1} and 1066.02 ± 3.76 µg mL^{-1}, respectively. Based on the densitometric analysis, the expression of the mutant 8sα globulin is slightly higher than the wild type 8sα globulin. Hydrophobic Interaction Chromatography (HIC) was used to purify the WT and mutant 8Sα globulin, which were later on digested using trypsin and chymotrypsin enzymes at different hours interval. Peptide mapping and detection using Liquid Chromatography–Mass Spectrometry (LC-MS) revealed the successful recombinant production, expression and release of the IIAEK peptide from the mutated mungbean 8sα globulin. The percent (%) reduction of bound sodium taurocholate of HIC purified WT and HIC purified mutant 8sα globulin were 31.62 ± 0.56 - 33.49 ± 1.62; and 27.54 ± 1.82 - 40.29 ± 6.29, respectively. Results showed significant difference on the activity of the HIC-purified mutant protein between hours digests, and the maximum % bile-acid reduction was observed in the 24th hr digest of the HIC purified mutant 8sα globulin (40.29 ± 6.29), indicating the presence of the hypocholesterolemic activity of the released target peptide.

Keywords: hypocholesterolemic, lactostatin, protein engineering, site-directed mutagenesis, 8Sα globulin