Thesis Abstract

Protein Engineering of Sulfhydryl Groups and Methionine in Mungbean (*Vigna radiata* (L.) Wilczek) Vicilin and Effects on its Nutritional and Functional Properties

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The 8Sα globulin, the major storage protein of mungbean, was engineered to introduce sulfhydryl groups and disulfide bond to improve its structural stability and functional properties and methionine residues to improve nutritional quality. Using site-directed mutagenesis, mutants F59C, I99C and A213C were designed and prepared with free sulfhydryl group, I99C/A213C with disulfide bond and F59C/I99C/A213C with both a free sulfhydryl group and a disulfide bond, based on the established structure of mungbean 8Sα globulin. Mutants I99C/A213C and F59C/I99C/A213C formed a disulfide bond as expected which was confirmed by the Ellman method. Mutants with introduced disulfide bond exhibited greater stability to thermal denaturation and greater resistance to enzymatic digestion compared to the wild type (WT). All mutants showed greater hardness of heat-induced gels than WT, especially I99C/A213C and F59C/I99C/A213C mutants at 2% protein concentration. The results indicate that increasing sulfhydryl groups and disulfide bonds increases structural stability.

Eight methionine-mutants, IIa, IVa, IIa+b, IVa+b, II+4, IV+4, IVa+b+IIa and IVa+b+IIa+b, were designed and prepared using site-directed mutagenesis. Mutants IVa, IIa, II+4, IV+4, IIa+b, IVa+b, IVa+b+IIa and IVa+b+IIa+b contain 2, 3, 4, 5, 8 and 10 methionine residues, respectively. Mutants with introduced methionine exhibited greater stability in terms of thermal denaturation but similar pattern to enzymatic digestion compared to the wild type (WT). All mutants showed greater emulsifying ability than WT, specially IVa+b+IIa+b. Based on allergenicity prediction programs (BLASP and ADFS), WT and all mutants had no allergenic potential. The results indicate that increasing methionine content increases structural stability in terms of thermal denaturation and emulsifying ability.