CONFORMATION AND FUNCTIONAL PROPERTIES OF MODIFIED SOYBEAN GLYCININS

Chan-Shick Kim, Se-Kwang Ko, Kyung-Cheal Ko, Kwon-Min Yang, Soon-Sean Kang and Sam-Pin Lee

Department of Agricultural Chemistry Cheju National University, Cheju 690-756.

Department of Food Science and Technology Keimyung University. Taegu 704-701.

To improve the nutritional and functional properties of soybean proglycinin, cDNA coding soybean proglycinin was manipulated using the synthetic oligonucleotides. Modified expression plasmids pKGA$_{1a}$B$_{1b}$ Ⅳ+4Met was used as template for mutation. Each amino acid sequence of Gln-Leu-Ile-Phe-Lys-Leu and Gln-Leu-Phe-Asp-Gln-Thr-Pro-Arg-Val -Phe was inserted between Pro467 and Gln468 in the variable domain Ⅴ. The insertion of new amino acids was confirmed by the creation of *Pvu* II restriction site. Expression plasmids carrying a modified cDNA were constructed and expressed in *E. Coli.* Modified soybean glycinins were purified by salt precipitation, ion-exchange chromatography and cryoprecipitation. The physico-chemical and functional properties of modified soybean glycinins were determined.

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INTRODUCTION

Improvement of functional properties and nutritional value of soybean proteins is a major objective in the food industry. Glycinin is a suitable target for the improvement of the functional properties, since it is the dominant storage protein in soybean seeds. Protein engineering is a powerful approach to attain this goal. However, the effects of protein engineering of the stability and self-assembly of glycinin should be considered from the standpoint of its high a cumulative level in the protein bodies of the beans. It is necessary to evaluate whether protein-engineered glycinins are able to form proper confirmation and to exhibit expected functional properties before the modified genes are transferred to the soybean plant. In this study, we describe the creation of novel glycinins designed to improve their nutritional and functional properties.

MATERIALS AND METHODS

Bacterial Strain, Medium and Plasmids.

E. coli JM 105 and *E. coli* DH5α were used as the host cells for gene expression and plasmid manipulation, respectively. Both strains were grown in LB medium at 37℃. Plamid pkG4MS and pkG4MP constructed were derived from an expression plasmid pKGA$_{1a}$B$_{1b}$ Ⅳ+Met, which contained cDNA encoding a modified proglycinin with Met-Met-Met-Met in variable region Ⅳ.

Construction of recombinant plasmids

A clone coding proglycinin contained a unique *Eco*81 I restriction site in variable region V. The synthetic oligonucleotides were inserted in this region. Oligonucleotides used for mutation were constructed for the purpose of increasing the hydrophobicity of glycinin as well as fortifying the biologically active oligopeptide sequence.

Four oligonucleotides were synthesized from Korea Biotech. Inc. To make the double strand oligonucleotide, a pair of oligonucleotides prepared for the synthesis of each oligopeptide sequence was mixed with annealing buffer (0.1M NaCl, 10mM Tris-HCl, pH 7.8, 1.0 mM EDTA) at a final concentration of 1 ng/$\mu\ell$. The reaction mixture was heated at 65℃ for 5 min and then cooled it down for 30 min to room temperature. To construct recombinant plasmids, pKG5S and pKG5P containing new synthetic oligonucleotide, an expression plasmid pKGA$_{1a}$B$_{1b}$-3 was digested with *Eco*81 I and then ligated with each paired oligonucleotide at 16℃ for 16 h. A 30 $\mu\ell$ of ligation mixture was treated with ice cold ethanol and 3 M sodium acetate at -20℃ for 6 h. The ligated DNA was harvested by centrifugation at 12,000 rpm for 10 min. In order to reduce the self-ligated expression plasmid DNA, the resuspended DNA was again digested with *Eco*81 I and then transformed into *E. coli* DH5α.

Transformants were selected on LB agar containing 50 ug/ml of ampicillin. Plasmid was prepared with 2.0 ml of LB culture broth by SDS alkaline method. A new *Pvu* II restriction site was confirmed between an expression plasmid pKG$A_{1a}B_{1b}$-3 and recombinant plasmids pKG5S and pKG5P.

Purification of modified proglycinins from E. coli

The expressed proglycinin protein in supernatant was concentrated by ammonium sulfate fractionation in the condition of 40-65 % saturation. Q - Sepharose column was equilibrated with buffer A (35 mM potassium phosphate buffer, pH 7.0 containing 0.15 M NaCl, 1.5 mM PMSF, 10 mM 2-ME and 0.02 % (w/v) sodium azide). A protein sample loaded was eluted with a 500 ml of salt gradient buffer (0.15 M-0.5M NaCl in buffer A). Finally, the collected protein solution was purified by cryoprecipitation. Amounts of purified proglycinins were measured by densitomertic analysis after staining a gel with coomassie brilliant blue solution.

RESULT AND DISCUSSION

Construction of expression plasmids for modified proteins

Figure 1(A) shows variable and conserved domains of the $A_{1a}B_{1b}$-3 proglycinin aligned by Wright(1988). To improve the functional properties of glycinin, we inserted synthetic DNAs to the variable domains V indicated by Wright(1988), and constructed pKG $A_{1a}B_{1b}$MV+P and pKG $A_{1a}B_{1b}$MV+S as shown in Figure 1(B).

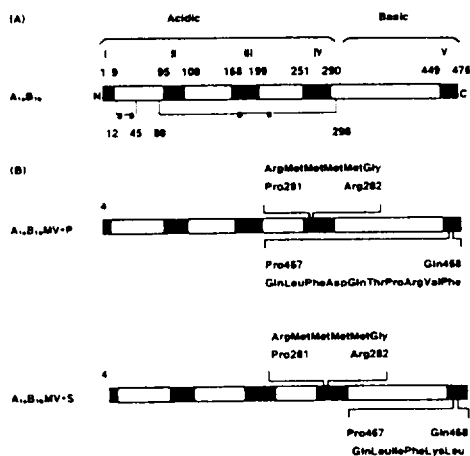

Fig.1. (A) Schematic representation of the proglycinin $A_{1a}B_{1b}$ subunit. (B) Construction of modified proglycinins.

Purification and functional properties of modified proteins

$A_{1a}B_{1b}$MV+P expressed in *E. coli* strain JM105 was purified according to the procedure employed for the purification of $A_{1a}B_{1b}$-3.

Every protein exhibited the same behavior during the course of purification as that of $A_{1a}B_{1b}$-3. $A_{1a}B_{1b}$MV+P gave predominantly the band with a mol. wt of ~55 kb. The functional properties of the modified proteins were compared with those of native glycinin.

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