Improvement of Soybean Protein Functions by Protein Engineering

Chan-Shick Kim and Se-Kwang Ko
Department of Agricultural Chemistry
Cheju National University, Cheju 690-756, Korea

ABSTRACT

Glycinin is one of the dominant storage proteins of soybean seeds and plays an important role in their utilization in human and animal food systems. We attempted to create modified proglycinins designed to improve their nutritional and/or functional properties by protein engineering using an *Escherichia coli* expression system for models of modified glycinins. The modifications were based on structure-function relationships and genetically variable regions found by comparison of amino acid sequences of glycinin-type globulins from various legumes and nonlegumes. All modified proglycinins were accumulated as soluble proteins in the cells at a high level and self-assembled. Modified proglycinins purified to near homogeneity by salt precipitation, ion-exchange chromatography, and cryoprecipitation. The functional properties of the modified proteins were compared with those of native glycinin.

[Supported by a grant from Genetic Engineering Research Program 1995, Ministry of Education, Republic of Korea]

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 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.

We used *Escherichia coli* strain JM105 as the host cells(Yanisch-Perron et al., 1985) and LB medium for culture. Plasmids employed here were pKK233-2 (Pharmacia), and $pKGA_{1a}B_{1b}-3$ (Kim et al., 1990). Modified expression plasmid $pKGA_{1a}B_{1b}V+4$ Met, $pKGA_{1a}B_{1b}V+P$ were constructed using $pKGA_{1a}B_{1b}-3$.

Expression and detection of modified proteins from *E. coli*

Five hundred milliliters of LB medium containing 25 μg/ml ampicilin was inoculated with 5 ml of a full-grown culture of JM105 harboring individual expression plasmids and cultured at 37℃ as described previously (Kim *et al.*, 1990). At A600=0.3 isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1mM. After incubation for 20 h at 37℃, the induced cells were harvested by centrifugation. The cells were disrupted by sonication as described previously (Kim et al., 1990). The cell debris and the supernatant were fractionated by centrifugation. Aliquots of the total cells, the cell debris and the supernatant were dissolved in SDS-sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 20% glycerol and 0.2% 2-mercaptoethanol) and analyzed by SDS-PAGE to determine whether the expressed proteins were soluble.

SDS-PAGE was carried out according to the method of Laemmli(1970) using 11% polyacrylamide gels. Proteins were visualized with Coomssie Brilliant Blue R-250.

Purification of expressed proteins from *E.coli*

Escherichaia coli cells from 9×500 ml culture were disrupted by sonication and the cell debris and unbroken cells were removed by centrifugation as described previously. From the resultant supernatant the expressed proteins were purified by ammonium sulfate fractionation, Q-Sepharose column chromatography and cryoprecipitation as described previously (Kim *et al.*, 1990)

Protein determination

Protein was determined by the method of Bradford(1976) with bovine serum albumin as a standard. Figure 1. (A) The variable and conserved domains of glycinin $A_{1a}B_{1b}$ subunit aligned by Wright(1988). The numbers of the residues from the N-terminus are described for the variable domains above the alignment. Black and open areas are variable and conserved regions, respectively. Acidic and basic refer to the acidic and basic polypepties, respectively. (B) Construction of normal expressed proglycinin $A_{1a}B_{1b}-3$ and modified proglycinin $A_{1a}B_{1b}V+P$. $A_{1a}B_{1b}-3$ lacks the N-teminal three amino acids and retains the initiation methionine.

RESULTS AND DICUSSION

Construction of expression plasmids for modified proteins

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

Abilities of modified proteins to form the correct conformation

To improve the glycinin qualities by means of protein engineering, the modified proteins should be able to assume the correct conformation. Production of modified proteins in JM105 cells harboring individual expression plasmids was analyzed by SDS-PAGE. $A_{1a}B_{1b}-3$ was accumulated in the cells at a high level, and $A_{1a}B_{1b}V+4Met$, and $A_{1a}B_{1b}V+P$ at a moderately high level. After the disruption of the cells harboring individual expression plasmids by sonication, the debris and the soluble fractions were applied to SDS-PAGE and the expressed proteins were detected by Coomassie Brilliant Blue staining or immunoblotting. $A_{1a}B_{1b}V+4Met$ and $A_{1a}B_{1b}V+P$ were predominantly detected in the soluble fraction.

Purification and functional properties of modified proteins

$A_{1a}B_{1b}V+P$ 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 $A_{1a}B_{1b}-3$. $A_{1a}B_{1b}V+P$ gave predominantly the band with a mol.wt of~55kd. The functional properties of the modified proteins were compared with those of native glycinin.

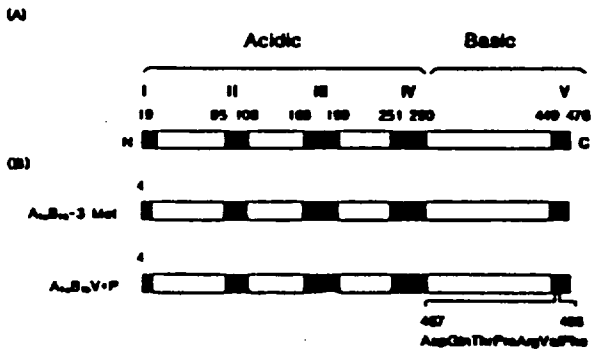

Figure 1. (A) The variable and conserved domains of glycinin $A_{1a}B_{1b}$ subunit aligned by wright(1988). The numbers of the residues from the N-terminus are described for the variable domains above the alignment. Black and open areas are variable and conserved regions, respectively. Acidic and basic refer to the acidic and basic polypeptides, respectively. (B) Construction of normal expressed proglycinin $A_{1a}B_{1b}-3$ and modified proglycinin $A_{1a}B_{1b}V+P$. $A_{1a}B_{1b}-3$ lacks the N-terinal three amino acids and retains the initiation methionine.

REFERENCES

1. Utsumi, S. et al.(1988) Gene, 71, 349-358.
2. Kim, C.-S. et al.(1990) Protein Engineering 3, 725-731.
3. Wright, D. J. The Seed Globulins. In Developments in Food Proteins ; Hudson, B.J.F., Ed. ; Elsevier : London, 1988 ; Vol. 6, 119-178.