

Short Communication

Substantially Complete Removal of the 34 kDa Allergenic Soybean Protein, *Gly m Bd 30 K*, from Soy Milk of a Mutant Lacking the α - and α' -Subunits of Conglycinin

Masahiko SAMOTO,[†] Koji TAKAHASHI,* Yoichi FUKUDA, Shigeki NAKAMURA,* and Yukio KAWAMURA**,[†]

Central Research Institute, Fuji Oil Co., Ltd., 4-3 Kinunodai, Yawara, Tsukuba-gun, Ibaraki 300-24, Japan

*Tohoku National Agricultural Experiment Station, Nishisenboku-machi, Akita 019-21, Japan

**Protein Science Laboratory, National Food Research Institute, 2-1-2 Kannondai, Tsukuba, Ibaraki 305, Japan

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Substantially complete removal (99.78%) of the allergenic soybean protein, *Gly m Bd 30 K*, was attained by applying simple centrifugation at $10,000 \times g$ and at pH 4.5 in the presence of Na_2SO_4 to defatted soy milk from a genetically mutated soybean cultivar (Tohoku 124) which lacked the α - and α' -subunits of conglycinin. The removal rate was ten times higher than that (97.44%) from a normal soybean cultivar of I.O.M. grade.

Key words: allergen; soybean protein; soymilk

The incidence of allergy to soybean proteins is gradually increasing with the growing consumption of soybean products all over the world. The development of methods to reduce the allergenicity of soybean protein is urgently required, since no other food material is used as soybean is throughout the world. The 34 kDa oil-body associated protein, *Gly m Bd 30 K*, has recently been assigned as a major allergenic protein of soybean by Ogawa *et al.*^{1,2)} The most efficient way to avoid the allergy is to utilize soybean lacking *Gly m Bd 30 K*, but it has not become available yet. At present, some trials have been conducted to lower the allergenicity by enzymatic³⁾ and physical treatments.⁴⁾

Based on the insoluble nature of *Gly m Bd 30 K*, we have developed a simple but efficient procedure to remove it as a precipitate by conventional centrifugation.⁴⁾ This procedure successfully reduced it to less than 10% of the original level in the presence of Na_2SO_3 . The requirement of a reducing agent was proved to facilitate the precipitability of *Gly m Bd 30 K* by preventing a complex formation through the disulfide bond between *Gly m Bd 30 K* and the α - and α' -subunits of conglycinin, since the complex formed did not allow the effective removal of *Gly m Bd 30 K* in the precipitate.⁵⁾

This observation suggested that a soybean cultivar lacking the α - or α' -subunits of conglycinin or containing a lower amount of them would be a promising material for less-allergenic soybean products. Takahashi *et al.* have recently established a mutant line, Tohoku 124, lacking the α - and α' -subunits of conglycinin by subjecting the Kari-kei 434 cultivar to gamma-ray irradiation,⁶⁾ although it contained the normal level of *Gly m Bd 30 K*.

In the present communication, we prove that it was possible to remove *Gly m Bd 30 K* nearly completely from soy milk without any reducing agent by using the mutant soybean.

Defatted soybean meal by *n*-hexane was prepared from

a normal I.O.M. soybean (Indiana–Ohio–Michigan, 1995 crop), and the mutant soybean lacking the α -, α' -subunits. Soy milk was prepared by dispersing the meal in distilled water (1:15, w/w), adjusting the pH value to 7.5 with 1 N NaOH, and mixing at room temperature for 3 h. The mixture was centrifuged at $10,000 \times g$ for 10 min to remove the insoluble materials, the supernatant then being used as defatted soy milk.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was conducted by using a 12% gel (Tefco Co., Japan) and Laemmli's buffer system.⁷⁾ Proteins on the gel were stained with 0.1% Coomassie Brilliant Blue R-250 in a solution of methanol, acetic acid, and water (5:5:2, v/v/v). After western blotting of the gel on a PVDF membrane (Nippon Pole Co., Japan), the allergenic protein, *Gly m Bd 30 K*, was detected by using a monoclonal antibody specific to *Gly m Bd 30 K* (provided by Dr. Ogawa, University of Tokushima)⁸⁾ and ECL immunodetection reagents (Amersham Institute, U.K.). The percentage removal of *Gly m Bd 30 K* was calculated from a densitometric determination of the ECL immunofluorescent intensity on X-ray film.

The protein composition of soy milk samples from the normal I.O.M. cultivar and the mutant lacking the α - and α' -subunits of conglycinin was analyzed by SDS–PAGE as shown in Fig. 1. Both soy milk samples (lanes a and b) had

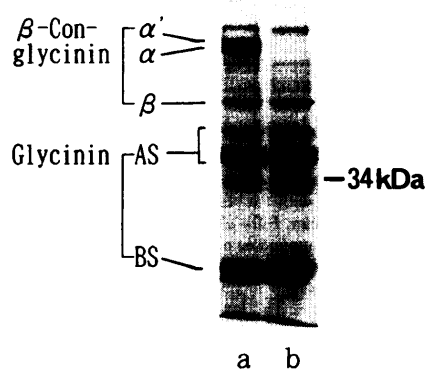


Fig. 1. SDS–PAGE Patterns of Proteins in Defatted Soy Milk Samples Prepared from I.O.M. Soybean and the Mutant Lacking the α and α' -Subunits.

Fifty μl of defatted soy milk prepared from I.O.M. soybean or the mutant lacking the α - and α' -subunits was mixed with 450 μl of an SDS–PAGE sample buffer. Ten μl of the mixture was then applied to a 12% gel. Lane a, I.O.M.; lane b, the mutant (Tohoku 124). AS, acidic subunits; BS, basic subunits.

[†] To whom correspondence should be addressed.

Abbreviations: SDS, sodium dodecyl sulfate; PVDF, polyvinyliden fluoride; ECL, enzyme chemiluminescence.

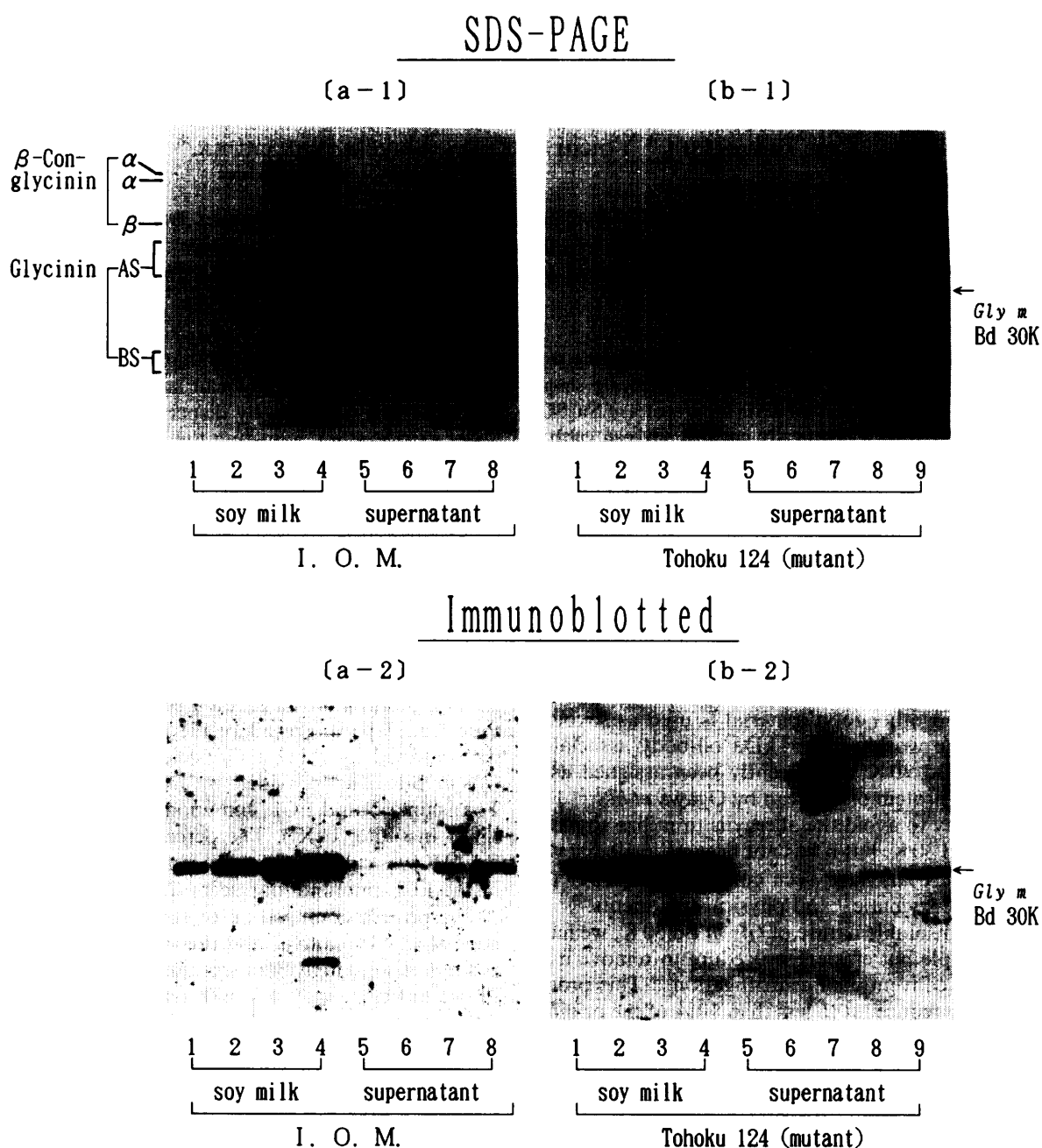


Fig. 2. SDS-PAGE and Immunoblotted Patterns of the Defatted Soy Milk Samples and the Supernatants Centrifuged in 1 M Na₂SO₄ at pH 4.5 Prepared from I.O.M. Soybean and the Mutant Lacking the α - and α' -Subunits.

Defatted soy milk with 1 M Na₂SO₄ and 10 mM Na₂SO₃ prepared from I.O.M. soybean was centrifuged at 10,000 $\times$ g for 10 min at pH 4.5 to remove *Gly m Bd 30 K* as the residue. Defatted soy milk with 1 M Na₂SO₄ without reducing agents prepared from the mutant lacking the α - and α' -subunits was centrifuged under the same conditions as those used for I.O.M. soybean. Fifty μ l of each defatted soy milk and the supernatant were mixed with 450 μ l of an SDS-PAGE sample buffer. Each amount of defatted soy milk and the supernatant applied to a 12% gel is indicated as follows: defatted soy milk prepared from I.O.M. soybean (Fig. 2, a-1 and a-2, lane 1, 0.1 μ l; lane 2, 0.3 μ l; lane 3, 1 μ l; lane 4, 3 μ l); the supernatant prepared from I.O.M. soybean (Fig. 2, a-1 and a-2, lane 5, 0.1 μ l; lane 6, 0.3 μ l; lane 7, 1 μ l; lane 8, 3 μ l); defatted soy milk prepared from the mutant (Fig. 2, b-1 and b-2, lane 1, 0.1 μ l; lane 2, 0.3 μ l; lane 3, 1 μ l; lane 4, 3 μ l); the supernatant prepared from the mutant (Fig. 2, b-1 and b-2, lane 5, 0.1 μ l; lane 6, 0.3 μ l; lane 7, 1 μ l; lane 8, 3 μ l; lane 9, 30 μ l).

a 34 kDa band containing *Gly m Bd 30 K* at a similar level, but the α - and α' -subunits of conglycinin existed only in the soy milk from I.O.M. (lane, a), being completely lacking in that from the mutant (lane, b).

The efficiency of removal of *Gly m Bd 30 K* from both the soy milk samples was evaluated by comparing the SDS-PAGE patterns of the original soy milk, the supernatant from I.O.M. (a-1 and -2) and the mutant (b-1 and -2) as shown in Fig. 2. The supernatants were prepared from the original soy milk samples by adjusting the pH to 4.5 with

1 N HCl, and centrifuging them at 10,000 $\times$ g for 10 min. Various amounts of the original soy milk samples (lanes 1-4) and the resulting supernatants (lanes 5-8 or 9) were applied to the gel to achieve precise densitometric quantification.

The removal rate of *Gly m Bd 30 K* from the I.O.M. milk (a-1) was approximately 75%, judging from a comparison of the protein-staining intensity between lanes 4 and 8, while that from the mutant milk (b-1) was over 84% by comparing between lanes 4 and 9. This evaluation, however,

has the possible ambiguity that CBB staining is a non-specific protein detection method and may not distinguish *Gly m Bd 30 K* from other proteins that comigrated to the same position.

Consequently, a precise evaluation of *Gly m Bd 30 K* was conducted by immunostaining with a monoclonal antibody specific to *Gly m Bd 30 K* (a-2 and b-2). In the case of I.O.M., the fluorescence intensity of 3 μ l of the supernatant (a-2, lane 8) and of 0.1 μ l of the soy milk (a-2, lane 1), enabled the calculation that $97.44 \pm 0.73\%^{*1}$ of *Gly m Bd 30 K* in the soy milk had been removed from the supernatant in the presence of a reducing agent with an overall yield of 65–70% of the total soy proteins. In the case of the mutant, a comparison of the fluorescence intensity of 30 μ l of the supernatant (b-2, lane 9) and of 0.1 μ l of soy milk (b-2, lane 1), enabled the evaluation that $99.78 \pm 0.05\%^{*1}$ of *Gly m Bd 30 K* in the soy milk had been removed from the supernatant even in the absence of a reducing agent with an overall yield of about 60% of the total soy proteins.

Accordingly, a soybean mutant genetically lacking the α - and α' -subunits of conglycinin was proved to be a very promising material for a non- or low-allergenic soybean protein or product.

In this paper, we propose a much more efficient procedure to remove the allergenic soybean protein, *Gly m Bd*

30 K, than that reported previously,⁴⁾ by combining both the genetically selected soybean cultivar and the physico-chemical separation method. This is a very simple, efficient and industrially applicable procedure to produce non- or very low-allergenic soybean proteins.

We are now trying to extend this procedure to large-scale production for use with allergic patients.

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*1 Values are each the mean $\pm$ standard deviation from triplicate determinations.