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Action on the 7S Globulin of *Bacillus* sp. IJ-3 Enzyme

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Abstract

7S globulin has been isolated from the defatted soybean meal (*glycine max. merill*) by Sepharose-6B column chromatography and CM-Sephadex column chromatography. Coagulum of 7S globulin formed at a temperature of 65°C in microbial enzyme treatment. In order to characterize the structure of the coagulum, three kinds of coagulum (enzyme-, calcium- and acid-induced coagulum) were compared through the Scanning Electron Microscope (SEM). The network structure was found to be of two levels. First, there was an appearence on the molecular level in the form of strands. Second, there was a denser network with a fine structure.

Key words: soy protein, 7S globulin, IJ-3 strain enzyme, coagulum

INTRODUCTION

Soy proteins have a complex quaternary structure which easily degradates association-dissociation reactions depending on several conditions such as high temperature, ionic strength and metal ions, etc. The gel-forming ability of soy protein is of significance with respect to their usage in food systems. Pioneers (1-3) have studied the basic factors affecting gelation of soy proteins.

Among the soy proteins contents, 7S globulin is a major storage protein (about 35%) of soybean seed together with 11S globulin. 7S globulin makes up 90% of the conglycinin fraction and can be divided into six isomers comprising three subunits α, α′ and β.

The molecular weights of the subunits are 57,000 for α,α′ and 42,000 for β subunit. 7S globulin has a very low sulfur content with no intermolecular S-S bond between subunits.

Several studies (4-8) investigated the gelation of soy proteins, especially the gelation of 11S globulin. Most of the studies on the 7S globulin compared the gelation characteristics to those found in 11S globulin. A lot of works deal with the gelation of soy protein by means of physical conditions, especially heating and metal ions in many food manufactures.

Authors (9-11) reported in the preceding papers that the microbial enzyme from *Bacillus* sp. IJ-3 strain was able to coagulate soy protein, and that the digestion properties of the soy protein were important to understand the enzymatic digestion of soy protein in food usage. Therefore, author's attention was paid to how the 7S globulin gelation occurred in microbial enzyme.

The aim of this work was to characterize the gel structure of the major soy protein 7S globulin, when the gel was formed by microbial enzyme, secreted IJ-3 strain, at pH 6.0 and 65°C.

MATERIALS AND METHODS

Preparation of enzyme

Two liters of enzyme production medium {yeast extract (0.2%)-peptone (0.2%)-glucose (0.5%)-potassium phosphate (0.5%)} were sterilized at 121°C for 15 min. in an autoclave. The seed culture (100 ml) of *Bacillus* sp. IJ-3 strain, which had been grown in the same medium in 500 ml shaking flasks at 35°C for 2 days on a reciprocal shaker, was inoculated into the fermenter.

Preparation of 7S globulin

7S globulin was isolated by using the method of Thanh et al. (12). The defatted flour was treated with 63 mM Tris-HCl (pH 7.8) containing 0.2 M sodium chloride, 10 mM β-mercaptoethanol. The resultant crude 7S globulin solution was applied to the Sepharose 6B Column (2.5×100 cm) and 7S globulin fraction was obtained.

Reduction and alkylation of 7S globulin

The 7S globulin obtained above was reduced by the procedure described by Kim and Kinsella (13). Soybean 7S globulin was reduced with DTT, and the resulting SH-groups were prevented from the disulfide interchange reaction with iodoacetamide.

Isolation of α, α′ and β subunits from alkylated 7S globulin by using CM-Sephadex chromatography

Five hundred milligrams of a mixture of 7S globulin subunits were applied to a CM-Sephadex column (1.5× 90 cm) previously equilibrated with 32 mM potassium phosphate buffer solution (pH 6.0) containing 6 M urea and 10 mM β-mercaptoethanol. One liter linear gradient from 0 to 0.4 M sodium chloride in the same buffer solution eluted the 7S globulin subunits from the column.

Coagulation of 7S globulin by calcium ion, acid and IJ-3 enzyme

The coagulum of 7S globulin by an enzyme reaction was certified and compared with the calcium-induced and acid-induced coagulum by the SEM. Protein solution (5%) were made in 0.04 M potassium phosphate buffer at pH 6.0, and the Ca- and HCl-curds were made by the 1 M inorganic solution at 65°C for 15 min. Freeze dried curds, obtained by three different treatments, were examined under the electron microscope (JEOL-5200, Japan) at an acceleration voltage of 15 KV without fixation at a magnification of ×7,500.

Confidence of strands formation

The strands formation of 7S globulin by an enzyme reaction was observed under the Axiolab camera. Pro

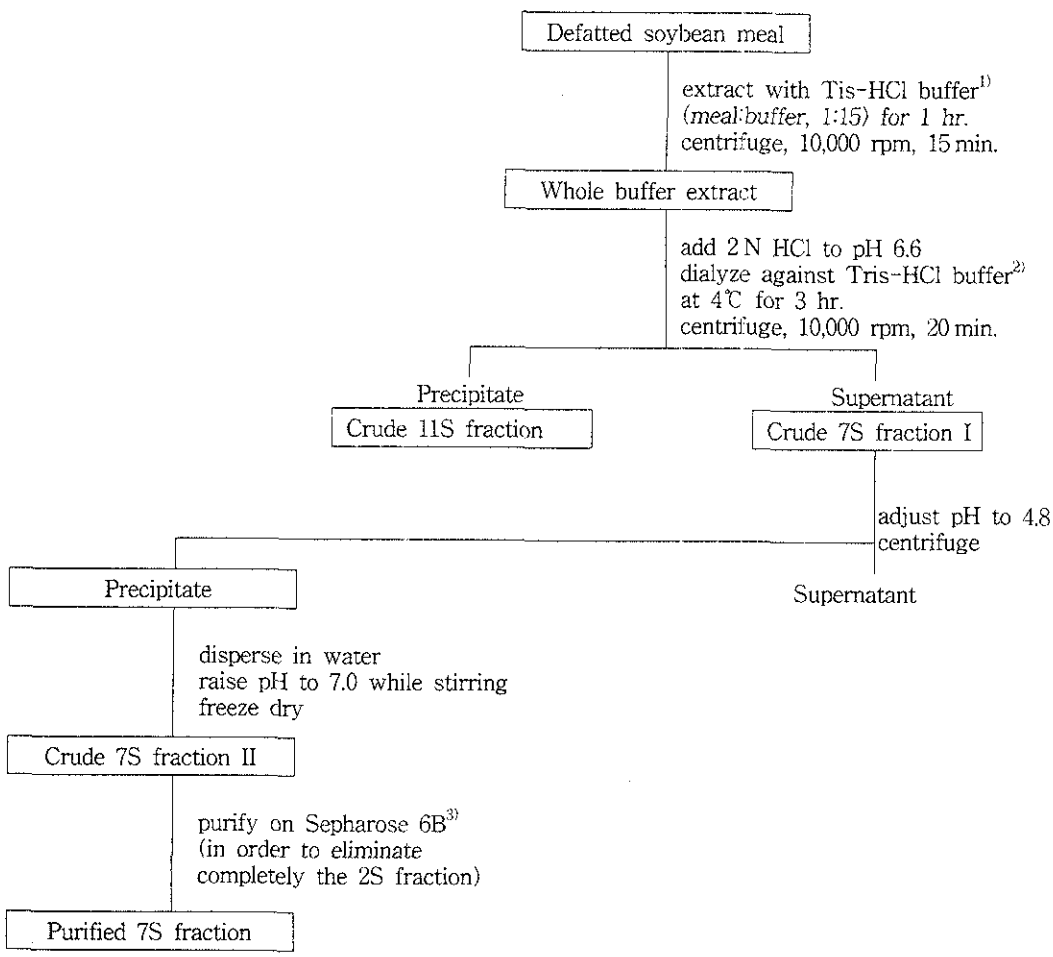

Fig. 1. Schematic outline of the simultaneous separation of the 7S and 11S fractions from defatted soybean meal.
[1)]63 mM Tris-HCl buffer containing 10 mM β-mercaptoethanol, pH 7.8.
[2)]63 mM Tris-HCl buffer containing 10 mM β-mercaptoethanol, pH 6.6.
[3)]35 mM KH_2PO_4, 26mM K_2HPO_4, 0.4 M NaCl containing 10 mM β-mercaptoethanol pH 7.6 at 4°C.

tein solution (5%) was made in 0.04 M potassium phosphate buffer (pH 6.1) and the enzyme reaction was carried out at 65°C for 30 min. The reaction mixtures were then collected at varing times and the strand formation was investigated at a magnification of ×100.

RESULTS AND DISCUSSION

Preparation of 7S globulin

As shown in Fig. 1, obtained crude 7S globulin was purified over 95% through the Sepharose 6B Column Chromatography adjusted to standard buffer solution (35 mM phosphate; 0.4 M NaCl; 10 mM β-mercaptoethanol; pH 7.6).

Network structure of the 7S globulin coagulum

Fig. 2 shows micrographs of the network structure of 7S globulin treated with the enzyme. In order to study the gel formation of 7S globulin between the enzyme, calcium and hydrochloric acid, the solution of 7S (5%) was incubated at 65°C for 30 min. Upon incubation, the 7S globulin fraction slowly aggregated at 65°C by enzyme treatment, whereas the Ca- and HCl-7S mixture showed immediate aggregation at this temperature. Unlike a calcium-induced and acid-induced coagulum, fragments forming the network structure were more compact and had a regular diameter.

This coagulum, however, was not self-supporting, and when shaken, it moved easily like weak-pudding or frozen yoghurt. These properties may come from this network structure with voids of the magnitude of flossy.

Action of IJ-3 strain enzyme on the 7S globulin

The coagulum formed at 5% 7S globulin was firm and retained moisture. These results indicate that IJ-3 strain enzyme might be suitable for producing frozen yoghurt-like texture useful in food product formulation and used for producing the coagula with varying rheological properties.

This study suggests that coagula with microbial enzyme might have a potential application for modification of proteins to improve their fuctional properties.

One to five units of IJ-3 enzyme were found to give a clotting time of 5 minutes. As shown in Fig. 3, the network structure was an orientation on the molecular level in the form of strands. Enzyme yielded a degradation product having a low molecular weight, and this was observed not only at the early stage of proteolysis but also after a long time of incubation (data not shown).

Thickness of the coagulum in 7S globulin was larger than those reported on calcium- and acid-induced coagulun of 7S globulin.

In the case of 7S globulin, it was obvious from Fig. 2 and 3 that coagulum consisted of associated subunits in a rearrangement by an enzyme reaction. There are alternative mechanisms for the formation of coagulum. 7S globulin does not dissociate hydrolysis but enter the coagulum with its quaternary structure intact. Kamata et al. (14) demonstrated that digestive materials of 7S globulin treated with pepsin was only a little smaller than that of an intact protein, therefore, the digestion intermediates held its quarternary structure even after fragmentation.

It can be said that the coagulum of the 7S globulin has more complex mode of aggregation than the "string of beads" mechanism (3).

Generally, a gel structure can be expected to show the

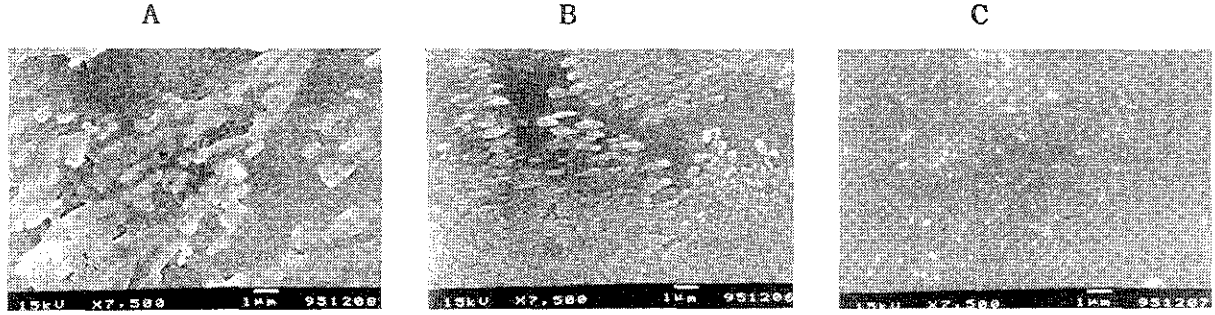

Fig. 2. The feature of scanning electron micrographs on the 7S globulin coagulum.
A: Coagulum by IJ-3 strain enzyme
B: Coagulum by calcium chlorides solution
C: Coagulum by hydrochloric acid solution

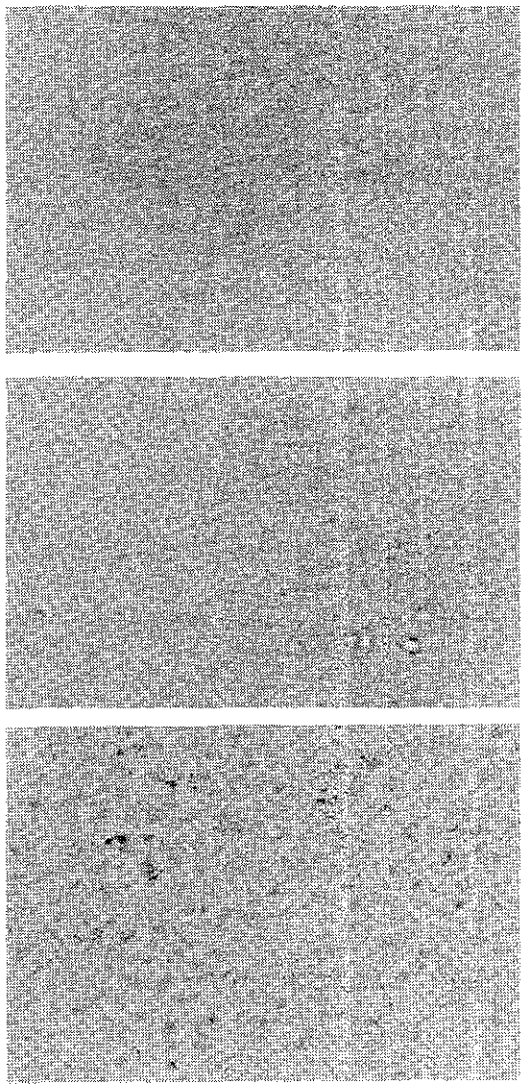

Fig. 3. Coagulum formation of 7S globulin by IJ-3 enzyme treatment at 65°C. (magnification, ×100)
A: 2 min incubation, B: 5 min incubation
C: 10 min incubation

highest degree of order at the point where the gel forms and any factor such as enzyme treatment causing a further increase in the degree of random aggregation will increase the tendency for phase separation and cause a gel with a more aggregated structure.

The micrographs showed a more crosslinked and denser network of 7S globulin coagulum than those of calcium- and acid-coagulum. This was because of that the weaker intermolecular bonds between coagulum of calcium and acid could not form during shearing, or there was a lower degree of crosslinking than in enzyme treatment gel.

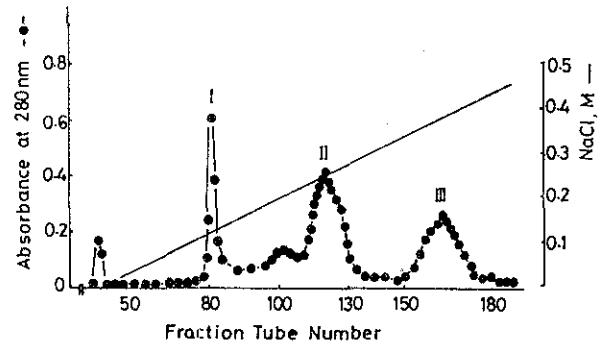

Fig. 4. CM-Sephadex C-50 column chromatography. The reduced, S-carboxyamidomethylated sample (500 mg) was applied to a 1.5×90 cm column previously equilibrated with 32 mM phosphate buffer containing 6 M urea (pH 6.0).
I: β subunit, II: α subunit, III: α′ subunit

Isolation of α, α′ and β subunits from 7S globulin

As shown in Fig. 4, α, α′ and β subunits of 7S globulin were isolated by using CM-Sephadex column chromatography. β subunit was eluted first from the column, which was followed by α and α′ subunits, as shown in Fig. 4.

These data show that under appropriate conditions of pH, temperature and protein concentration, 7S globulin can form a fairly firm gel with a smooth texture and fine structure. This is unique consideration in that we do not adopt the high thermal condition of the protein.

Gelation can occur at 90~100°C well below the denaturation temperature (≈110°C). German et al. (2) reported that heat may cause association/dissociation of the oligomer which would induce aggregation, and the disruption of the quaternary structure alone could lead to coagulation/gelation.

It is well known that disulfide linkage and thiol-disulfide exchange reaction may play a role in the gelation process. 7S globulin, however, has a very low sulfur content and there are no intermolecular SS-bonds between subunits.

An enzyme treatment affected 7S globulin gelation to varing extents, and the data suggest that formation of gel network depends on the balance between attractive hydrophillic-hydrophobic interactions.

At present, 7S globulin has limited use as heat-induced coagulum, metal ion-induced coagulum and acid-induced coagulum in food stuffs, but the enzyme treatment on the 7S globulin is able to normally encounter in foods near neutral pH.

This fact indicated that the enzyme treating 7S globulin may enhance the use of soy protein in food applications requiring new rheological food items.

In order to elucidate the coagulation mechanism of 7S globulin, it is necessary to investigate the process which occurs during the course of enzyme-induced gel formation.

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