Generality and Diversity of Winged Bean (Psophocarpus tetragonolobus) Protein in Eight Various Lines

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Protein components from eight lines of winged bean (Psophocarpus tetragonolobus) seeds which were originally introduced from Papua New Guinea, Indonesia, Nigeria, and Ishigaki, and cultivated in Okinawa and Fukuoka, were investigated. Two major peaks which had sedimentation coefficients, $s_{20}$, of about 2.5S and about 6.5S (6.0 to 6.6 for the 8 lines), and no larger component were observed in all specimens with more than 90% extraction. Electrophoretic profiles of the "6.5S" component(s) which was separated with Sepharose 6B column chromatography showed a main broad band and a few minor bands which seemed to be essentially similar among the eight lines of winged bean. Thus the "6.5S" protein surely could be regarded as the common storage protein in winged bean seeds. The subunit structure of the "6.5S" component(s) in SDS solution consisted of four major bands. The "2.5S" components were mixtures and combinations of various proteins which were distinctly different from one selection to another.

Winged bean (Psophocarpus tetragonolobus) has been cultivated in a large area of southeast Asia for a long period as a domestic or small scale market vegetable. Since the National Academy of Science, U.S.A. organized a society for the study of the winged bean in 1975,1 experimental plantings of winged beans have been proceeding in some fifty countries. The unique excellent property of winged beans which attracted researchers' attention is that all parts of the plant are edible and protein rich and protein and fat contents of the seeds are very high among pulses and comparable to the soybean.2)

Variability in the protein and lipid contents of many sample selections of winged bean have been mentioned in publications on analyses of seed components and on nutrition.2) Protein extracted with an acidic buffer from a variety of lines in Papua New Guinea were reported by Blagrove and Gillespie.3)

In this paper, the protein components of eight lines of winged bean originally introduced from several countries to Okinawa and Kyushu in Japan were studied. The extraction of protein was with a neutral (pH 7.5) buffer containing NaCl and dithiothreitol which could extract more than 90% of the seed proteins. The conditions were chosen to be the same as in many other analyses,4) so that these results can be compared directly with the results of many other reports on other legume proteins, especially those of soybeans.

MATERIALS AND METHODS

Seven lines of winged bean seeds which were originally introduced from Papua New Guinea, Indonesia, Nigeria, and Ishigaki (Japan) and one line from Papua New Guinea, were supplied by the Okinawa Branch of the Tropical Agriculture Research Center (Ministry of Agriculture, Forestry and Fisheries) and Kyushu University, respectively (Table I).

Moisture content was determined by placing weighed and crushed seeds in a 110°C drying oven and weighing until the weight became constant. Nitrogen content was measured by the micro-Kjeldahl method.3)

Seeds were ground into flour with a coffee mill, defatted with n-hexane, sieved, and reground with a mortar to the fineness of 30 mesh. Four to five weight volume of

Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
Table I. Composition of Eight Lines of Winged Bean Seeds

<table>
<thead>
<tr>
<th>Line</th>
<th>Moisture</th>
<th>Protein</th>
<th>(Fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-001 Indonesia</td>
<td>7.6</td>
<td>32.6</td>
<td>17.6</td>
</tr>
<tr>
<td>O-002 Indonesia No. 902</td>
<td>8.6</td>
<td>32.8</td>
<td>16.5</td>
</tr>
<tr>
<td>O-003 Indonesia No. 909</td>
<td>8.3</td>
<td>35.1</td>
<td>17.3</td>
</tr>
<tr>
<td>O-004 Indonesia No. 1126 (1)</td>
<td>7.2</td>
<td>37.2</td>
<td>15.2</td>
</tr>
<tr>
<td>O-007 Nigeria Tpt 2</td>
<td>6.9</td>
<td>34.8</td>
<td>16.6</td>
</tr>
<tr>
<td>O-012 Papua New Guinea UPS 122</td>
<td>6.5</td>
<td>33.7</td>
<td>17.3</td>
</tr>
<tr>
<td>O-013 Ishigaki</td>
<td>6.7</td>
<td>32.3</td>
<td>15.8</td>
</tr>
<tr>
<td>F-UPS 99d Papua New Guinea</td>
<td>12.2</td>
<td>38.7</td>
<td>15.9</td>
</tr>
</tbody>
</table>

*Percentage of dry weight, calculated by multiplication of 6.25 to total nitrogen.

Estimation from the weight difference of the whole flour and defatted flour.

*Numbered and cultivated at the Okinawa branch of the Tropical Agriculture Research Center, Ministry of Agriculture, Forestry and Fisheries.

*Cultivated at Fukuoka, Kyushu University.

RESULTS

Compositions of the eight lines of winged bean seeds were listed in Table I. Seeds from Okinawa contained only 6.5 to 8.6% moisture, while the seeds from Fukuoka contained 12.2% moisture, probably reflecting the climate during cultivation or storage conditions after harvest. Protein contents of the eight selections of seeds were 32.3 to 38.7% of dry weight. These values were rather even, considerably higher than various pulse protein contents, and comparable to those of soybeans. Lipid contents, which were estimated by the weight differences before and after n-hexane extraction, were also very close to those of soybeans.

Electrophoretic patterns of the whole seed protein from seven selections of winged bean lines are shown in Fig. 1. The main protein components with small mobilities [S area] were similar among all lines, but several major components with large mobilities [L area] were...
clearly different from each other. Thus, as the common storage protein of winged bean seeds the protein bands in S area were noteworthy. 

Sepharose 6B column chromatography gave the clearest separation of these protein components among Sepharose, Sephadex and Toyopearl. The chromatography patterns of eight lines of whole seed extract from winged bean on Sepharose 6B were essentially similar although the relative amounts of peak A and peak B fractions varied. A typical elution pattern is given in Fig. 2, and the fraction numbers of peak A and B of each of the eight lines are noted at the bottom.

Further separation of the components in peak A and B by gel electrophoreses are shown in Fig. 3-a and -b. Peak A on Sepharose 6B consisted of no large mobility protein bands, and was concentrated in the S area of Fig. 1. Distinct differences between the eight selections were not observed at this step. However the main band was somewhat broad and was supposed to consist of three or four components at least which might have some small

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**Fig. 2.** Elution Pattern of Winged bean Whole Seed Extract through Sepharose 6B Column Chromatography. Column volume was 500 ml and fraction volume was 6 ml.

**Fig. 3.** Polyacrylamide Gel Electrophoresis of Winged Bean.

a: Peak A of Sepharose 6B column chromatography “6.5S” components.
b: Peak B of Sepharose 6B column chromatography “2.5S” components.

O, origin; F, front; BSA, bovine serum albumin. Electrophoresis was carried out in the absence of SDS.
variations between the winged bean lines. Peak B consisted of many protein components which showed various electrophoretic mobilities. This B fraction included almost all the L area bands and some parts of the S area too of Fig. 1. From these results, peak A of Sepharose 6B chromatography was suitable to study the general storage protein of winged beans, and peak B was good for examining the protein variation among winged bean varieties.

On the other hand schlieren patterns by analytical ultracentrifugation showed that the void peak of Sepharose 6B chromatography consisted of only very high molecular weight compounds and that peaks A and B corresponded to about 6.5S and about 2.5S components, respectively.4) The "6.5S" components, peak A of Sepharose 6B chromatography, of winged beans, therefore, seemed to have rather uniform s values among varieties.

Ultracentrifugal sedimentation profiles of whole protein extracts from eight lines of winged bean seeds are shown in Fig. 4. Two main peaks were observed in every sample, and the relative heights of the two peaks were diverse among the lines used. Sedimentation coefficients, $s_{20}$, of these two peaks were around 2.5S and 6.5S. The sedimentation coefficient, $s_{20}$, and extrapolated $s_{20,w}$ of each of
very low ionic strength. No other larger component, like soybean 11S protein, was observed in any specimen of the different lines.

The subunit composition of the “6.5S” protein component from eight lines of winged bean is shown in Fig. 5. Four major bands were seen in all eight selections, though some differences were observed in relative amounts of those major subunits and in the appearance of minor bands.

DISCUSSION

The generally accepted classifications of legume proteins were apt to divide them into two groups, one of them “legumin” (with a sedimentation coefficient of 10 to 15S), and the other, “vicilin” (6 to 9S). Also, the majority of legume seeds were supposed to contain both legumin-like and vicilin-like proteins. However, the conditions of reported analyses were distributed in a wide range, and we knew that some typical edible pulses did not contain “legumin” in neutral pH solutions. Therefore, the conditions for the extraction of seed proteins here were carefully chosen to take out as much protein, and be as mild as possible, and to be comparable with other important publications about legume proteins.

Protein contents of the eight various lines of winged bean seeds were quite high, comparable to soybeans. However winged bean did not have any traces of the “US” group protein even when the extraction efficiency was more than 90%. Thus protein rich legume seeds do not necessarily contain a “legumine” type of high molecular weight protein.

Analyses with ultracentrifugation and electrophoresis showed that the “6.5S” component(s) was one of the main protein groups and that its diversity was not wide. The reasons for diversity could not be sought merely in the difference of winged bean lines, because the precise sedimentation coefficient of legume proteins changes considerably during seed development. So the “6.5S” component(s) had to be studied cautiously as a typical winged bean seed protein.

Other components of the “2.5S” peak were mixtures of many proteins. The components with large electrophoretic mobilities were all included in the “2.5S” group, and they seemed to be a combination of several proteins. The “2.5S” components seemed to be very interesting in defining the varieties of winged bean.

The subunit composition of the “6.5S” protein from 8 lines in SDS solution was almost the same as the description of Sathe and Salunkhe, and seemed to have no drastic variation between the winged bean lines. The detail of differences, however, should be discussed with the results from more purified preparations.

The seven selections from the Okinawa branch of the Tropical Agriculture Research Center were tried in cultivation at the National Food Research Institute (Tsukuba, Ibaraki). All the lines could flower, but seed development was not observed in any lines.

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