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Antigenic Homology between Buckwheat and Wheat Prolamins

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Abstract

Immunochemical relationships between buckwheat and wheat prolamins were evidenced by immunoblot using antiserum raised against buckwheat prolamin or an authentic gliadin.

The buckwheat prolamin antiserum strongly bound to the gliadin protein and vice versa. The same buckwheat antiserum did not bind to the homologous salt-soluble fractions and glutelin proteins, indicating its highly antigenic specifisity. SDS-PAGE analyses of the three prolamins revealed almost identical polypeptide profiles, which composed of roughly two subunits groups with respective molecular weights ranging from 30 to 38 kDa and 42 to 43 kDa. Little differences in the amino acid compositions were also observed.

Key words ; buckwheat, wheat, prolamins, immunoblot, antigenic crossreaction

Introduction

In our previous studies[1] we had noticed significant similarities in the amino acid compositions and polypeptide components judged by SDS-PAGE between the prolamins from buckwheat and wheat, in which both prolamins were rich in glutamate and proline, but poor in lysine and sulfuric amino acids, and consisted of two groups of the polypeptide bands with respective apparent molecular weights ranging from 30 to 38 kDa and 42 to 43 kDa. These findings raised a question of whether immunochemical relationships exist between the both prolamins, because antigenic crossreactions among cereal proteins are often caused by physicochemical similarities in the proteins.[2) 3) 4)]

At present paper, we describe some evidences that there are common antigen determinants or epitops between the prolamin proteins from buckwheat and wheat grains. Similarities in either amino acid compositions or in polypeptide subunits were also reported.

Materials and Methods

Buckwheat flours were purchased from Asahi Seifun Co. (Sakurai-shi, Nara 360, Japan).

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Authentic gliadin (wheat prolamin) was obtained from Sigma Chemicals (SAF Bulk Chemicals, Tokyo, Japan). All the flours were defatted with 2 volume of petroleum ether by stirring for 1 h at room temperature. After removing of the organic solvent by a Buchner filtrator, the flours were dried in air and stored at −20℃ until use. For preparation of prolamin, the defatted flour was previously extracted salt-soluble proteins stirred in 1 M NaCl for 1 h at room temperature and centrifuged at 21,000 g for 20 min at 4℃. The salt-insoluble residues were submitted to extraction of prolamin proteins by stirring for 1 h with 50% propanol and the alcohol soluble fractions were recovered by centrifugation. The alcoholic supernatants were then mixed with 2 volumes of 1 M NaCl and standed at 4℃ overnight. The precipitated prolamin was spun down by centrifugation and washed twice with cold acetone before dried in air. Prolamins from wheat, barlery and rye were also prepared by the procedures described above from the grain seeds obtained commercially.

Rabbit antisera against the authentic gliadin or buckwheat prolamin were raised by subcutaneous injection of the antigen homogenized with Freund's complete adjuvant as described previously.[5] Each 2 mg (dry weight) of the prolamin or gliadin antigens was administrated at two weeks intervals and 10 days after the last injection, the rabbits were bled.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) was done according to the method of Laemmli[6] with a 15% acrylamide gel.[1] Molecular weights of proteins were determined by the reference of low molecular mass protein kit (Pharmacia LKB biotechnol., Uppsala, Sweden). Immunoblotting was done according to the method of Towbin et al.[7] with a slight modification.[5] Sample proteins were submitted to SDS-PAGE and transferred to a nitrocellulose membrane by a mini cell apparatus (BioCraft-Model BE-300, Tokyo, Japan). The membrane was incubated for 60 min in T-PBS (0.5% Tween 20 in PBS, pH 7.2) containing rabbit antiserum diluted 1 : 100 with T-PBS followed with horseradish peroxidase conjugated IgG of antirabbit IgG got serum (Bio-Rad) diluted at 1 : 1,000 with T-PBS. Antigenic proteins were visualized by color development with 4-chloro-1-naphthol and peroxid hydrogen.

Amino acid determinations were made with a Hitachi KLA-5 amino acid analyzer. Hydrolysis of the sample proteins was carried out with 6 N HCl for 20 h at 110℃ in a sealed tube under vacuum.

Result and Discussion

Polypeptide components judged by SDS-PAGE

Fig. 1 shows SDS-PAGE profiles of buckwheat (lane 1) and wheat prolamins (lane 2), and the authentic gliadin (lane 3). All three prolamin proteins revealed basically similar polypeptide subunit patterns, which were com-

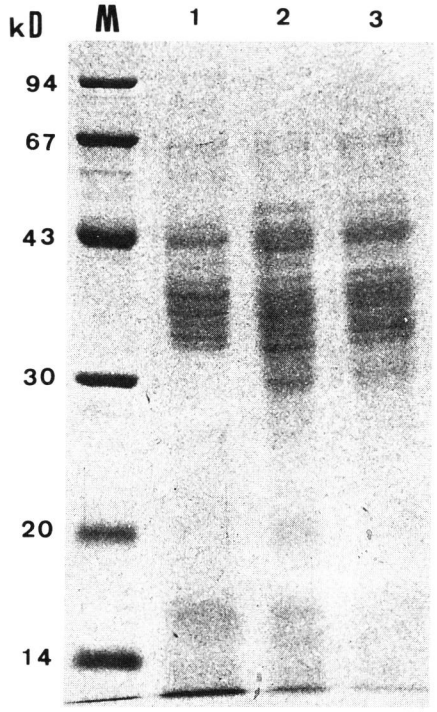

Fig. 1. SDS-PAGE profiles of the prolamins from buckwheat (lane 1) and wheat (lane 3), and sigma's authentic gliadin (lane 2). Each the lanes contains 20 μg of protein. Lane M contains low molecular weight calibration kit proteins: phosphorylaseb (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactoalbumin (14,000).

posed of roughly two groups of a thin-layered like structure of the subunits: one contained several thin layers with molecular weights (MW) ranging from 43 to 44 kDa and the other over ten layers with MWs ranging from 33 to 38 kDa, but both the wheat prolamin and gliadin had two satellite miner subunits with MWs of about 30 and 45 kDa.

This SDS-PAGE patterns, however, did not consist with that of the buckwheat prolamin reported by Skerritt,[8] who had showed that the prolamin composed of numerous faint polypeptide bands which were broadly scattered on a gel with MWs ranging from about 10 to 28 kDa. We have no idea that explain precisely this distinction, but it is probably caused by the difference in variety of the grain.

Amino acid composition

The amino acid compositions of the prolamins from buckwheat, wheat and authentic gliadin were compared in Table. 1. All three proteins shared almost identical chemical compositions in which the contents of glutamate and proline, which were generally rich in cereal prolamins, fell into high ranges of 33 to 35 mol%, and conversely those of sulfuric amino acids and lysine were very low. These chemical profiles of the buckwheat prolamin consisted with the report of Taira,[9] but not with those of Pomerantz,[10] in which glutamate and proline were poor, but lysine was rich. This is presumably caused by a contamination with other protein fractions, which resulted from a lack of prior salt solution extraction.

Tle 1. Amino acid compositions of buckwheat and wheat prolamins[a]

Amino acid	Mol%		
	Buckwheat[b]	Gliadin[c]	Wheat[d]
Asp/Asn[e]	2.4	2.2	2.2
Thr	3.4	2.3	2.6
Ser	5.5	5.1	6.0
Glu/Gln[f]	38.4	38.9	39.1
Pro	17.5	16.6	16.8
Gly	3.7	3.4	4.0
Ala	3.2	3.0	3.2
Cys	1.2	1.3	1.5
Val	4.4	4.8	5.0
Met	0.4	0.8	0.7
Ile	4.2	4.6	4.3
Leu	6.8	7.3	6.6
Tyr	2.0	1.9	2.2
Phe	5.8	5.1	6.0
Lys	0.6	0.7	0.7
His	1.5	1.7	1.5
Arg	2.0	1.9	2.1

[a] Data are the mean of duplicate determinations.
[b] Buckwheat prolamins were prepared from 70% ethanol extract.
[c] Sigma gliadin.
[d] Wheat prolamins were prepared from 70% ethanol extract.
[e] Aspartate plus asparagine.
[f] Glutaminate plus glutamine.

Immunoblot analysis

Before the immunoblot analysis, antigenic specificity of the buckwheat prolamin antiserum

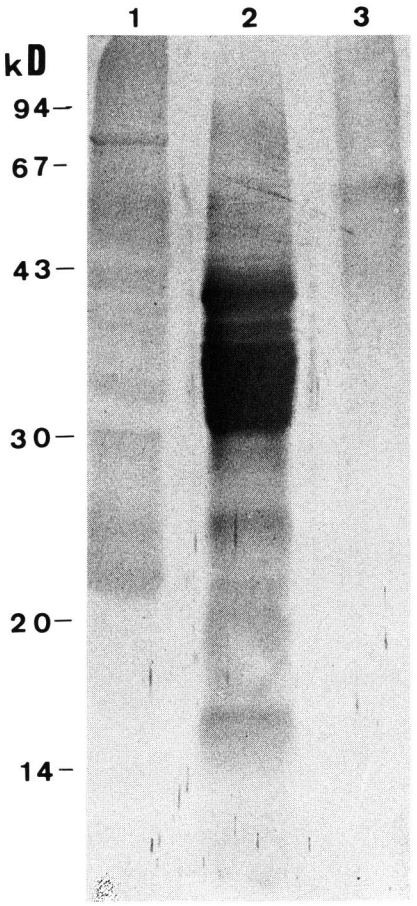

Fig. 2. Immunoblot showing the specificity of buckwheat prolamin antibodies. Each lanes contains 30 μg of the salt-soluble fraction (1), prolamin (2), and glutelin (3) from buckwheat.

used here was confermed by the cross-reactions between the antiserum and non-prolamin proteins (the 1 M NaCl soluble fraction and glutelin described in the materials and methods). As shown in Fig. 2, the prolamin antiserum dominantly bound to the homologous antigen protein (lane 2), but not to such non-prolamin fractions as the salt-soluble fraction (lane 1) or glutelin protein (lane 3), demonstrating a highly antigenic specificity of the antiserum.

Antigenic homologies between buckwheat prolamin and the gliadin was determined by immunoblot analysis (Fig. 3). The buckwheat prolamin antiserum (panel A) strongly bound to the gliadin polypeptide subunits (lane 2), as well as the homologous antigens (panel A, lane 1). Similarly, the gliadin antiserum (panel B) significantly cross-reacted with the buckwheat prolamin polypeptides (lane 2) as well as the homologous antigen (lane 1), indicating antigenic similarities between buckwheat and wheat prolamin proteins.

On the other hand, it is known that there are antigenic relationships among the prolamins of the Triticeae (comprising barley, wheat an rye).[3) 11)] Thus it was expected to cross-reactions between the prolamins of buckwheat and the Triticeae. Fig. 4 shows that it is true in this case, where the prolamins of barley (lane 3) and rye (lane 4), as well as the homologous prolamin or the gliadin, clearly cross-reacted with the buckwheat prolamin antiserum.

Consequently, these findings present some evidences on the existing of common antigen determinants or epitops between buckwheat and wheat prolamins.

These findings, however, were disaggree with the work of Skerritt,[8)] who reported that no antigenic crossreactions exist between the both prolamins. This inconsistency requires further investigation on the molecular biogenetcs as well

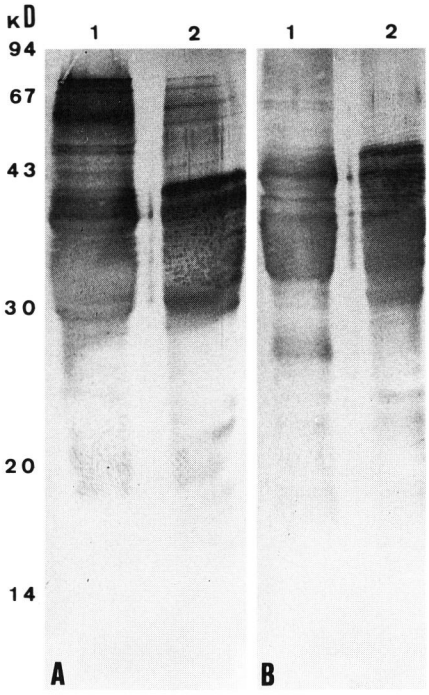

Fig. 3. Immunoblot analysis of buckwheat prolamin (lane 1) and the authentic gliadin (lane 2) with antisera against buckwheat prolamin (panel A) and the authentic gliadin (panel B). Aliquots (30 μg) of sample proteins were subjected to 15% SDS-PAGE, transferred to a nitrocellulose membrane, and immunostained with respective antisera cited above.

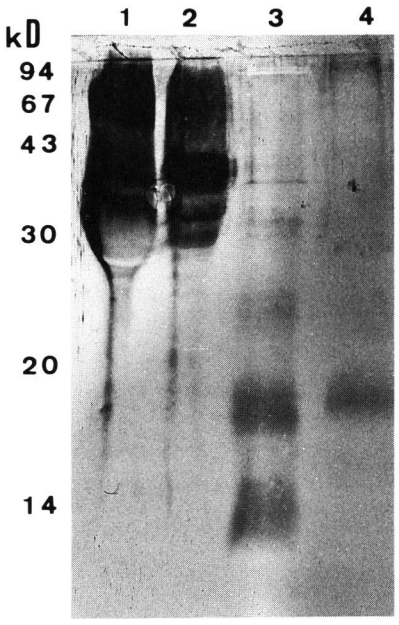

Fig. 4. Immunoblot showing the antigenic crossreactions among prolamins from buckwheat (lane 1), wheat (lane 2), barley (lane 3) and rye (lane 4). The prolamins were prepared with 70% aqueous ethanol from the salt-insoluble residue. Each lanes contains 30 μg of prolamins from buckwheat (1), wheat (2), barley (3), and rye (4).

as biochemical characteristics of the both buckwheat and wheat proteins.

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