

Characterization of Buckwheat 19-kD Allergen and Its Application for Diagnosing Clinical Reactivity

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Key Words

Buckwheat allergen · Major allergen · 19-kD allergen ·
Clinical reactivity

Abstract

Background: The 19-kD protein of buckwheat (BW) has been suggested to be a major allergen, but its characteristics and clinical significance are poorly defined. **Methods:** cDNA of the 19-kD BW allergen was cloned and expressed in *Escherichia coli*. Allergenicity and cross-allergenicity were confirmed by inhibition immunoblotting or by ELISA inhibition. The recombinant (r19-kD) protein was assessed for clinical utility in the diagnosis of BW reactivity in 18 BW-allergic and 19 BW-asymptomatic sensitized subjects using receiver operating characteristic analysis. **Results:** The 19-kD BW allergen, which is composed of 135 amino acids, has a weak homology to the vicilin-like allergens of cashew (*Ana o 1*), English walnut (*Jug r 2*) and 7 S globulin from *Sesamum indicum*. The r19-kD protein can inhibit sIgE binding to native 19-kD BW allergen. The maximum percentage inhibition of sIgE binding to crude BW extract was 56%. About 83.3% of the BW allergy patients had sIgE bound to r19-kD protein, compared to only 1 of the 19 BW-asymptomatic sensitized subjects. The areas under the receiver operating characteristic curves for the skin prick tests [0.925 (95% confidence interval: 0.839–1.012), $p < 0.001$] as well as r19-kD protein sIgE ELISAs [0.860 (95% confidence interval: 0.725–0.995),

$p < 0.001$] were higher than that of BW sIgE coated allergen particle test results [0.803 (95% confidence interval: 0.661–0.945), $p = 0.002$]. **Conclusions:** The 19-kD BW allergen may be the major allergen from BW. For the diagnosis of clinical reactivity to BW, the r19-kD protein sIgE ELISA test was more discriminative than the coated allergen particle sIgE measurement using whole BW extract.

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Introduction

Buckwheat (BW; *Fagopyrum esculentum*) belongs to the Polygonaceae group of weeds, and its fruit can be used as food. In recent years, BW has become more popular in many countries as a health food [1, 2]. Many cases of BW-allergic subjects have been reported in Korea, Japan, the USA and other western countries [3–8].

The 24-, 19-, 16-, 10- and 9-kD proteins of BW are recognized as major allergens. The 24-kD BW protein was identified as the β -subunit of 11 S globulin [8–10], the 9-kD BW allergen as vicilin [11–13] and the 10-kD BW protein as 2 S albumin [14]. Specific IgE (sIgE) antibodies to the 24- and 9-kD allergens can be found in both BW-allergic and asymptomatic subjects, making these allergens poor candidate biomarkers for diagnosis of BW reactivity [13, 15]. We have reported that the 19-kD protein is one of the major allergens and have determined the N-

Table 1. Clinical features of patients allergic to BW

Patient	Age years	Sex	Provocation	CAP IgE kU/l	SPT wheal mm	r19-kD protein sIgE AU/ml	Other food	Urti.	Resp.	GI
1	25	M	–	9.86	8	150	peach	+	+	+
2	28	M	D	9.97	19	152	–	+	+	+
3	19	M	–	>100	12	1,200	crab	+	+	–
4	40	F	–	22.4	17	410	–	+	+	–
5	32	M	–	2.78	15	0	–	+	–	–
6	19	M	–	12.20	38	170	–	+	+	–
7	23	M	D	6.40	19	200	–	+	+	–
8	22	M	–	4.84	10	6	–	+	–	–
9	22	M	D	3.80	29	1	–	+	+	–
10	20	M	–	17.4	16	1,220	–	+	+	–
11	11	M	–	3.80	33	2,000	peach	+	+	–
12	44	M	–	0.53	14	0	–	+	–	+
13	19	M	–	0.60	11	16	–	+	+	–
14	25	M	D	1.66	30	40	–	+	–	–
15	20	M	–	>100	50	1,800	–	+	+	–
16	29	M	–	0.48	14	16	–	+	+	–
17	15	M	–	76.60	28	980	–	+	+	–
18	19	M	–	20.8	15	360	–	+	+	–

AU = Arbitrary unit; D = positive result to DBPCFC; Urti. = urticaria; Resp. = respiratory; GI = gastrointestinal.

terminal amino acid sequences of this protein [13]. Interestingly, sIgE antibodies to the 19-kD allergens are found exclusively in patients who complain of allergic symptoms after ingestion of BW, but not in asymptomatic subjects with BW sensitization [13, 15]. These findings suggest that measurement of sIgE for the 19-kD allergen may be particularly useful for the diagnosis of the clinical reactivity of BW.

In our study, we first deduced the full amino acid sequence of the 19-kD allergen from its cDNA sequence, evaluated the allergenicity of a recombinant 19-kD (r19-kD) allergen, and then applied this information to diagnose BW reactivity in 18 BW-allergic and 19 BW-asymptomatic sensitized subjects.

Material and Methods

Subjects

Eighteen BW allergy patients and 19 BW-asymptomatic sensitized subjects were enrolled in our study, which had been approved by Yonsei University's Medical Ethics Committee (No. 4-2006-0204). Four allergy patients were diagnosed as having BW allergies by double-blind placebo-controlled food challenge (DB-PCFC), and the other 14 patients complained of allergy symptoms

such as urticaria, angioedema, dyspnea and abdominal pain, immediately after eating BW noodles or jellies. The 14 patients did not undergo DBPCFC because their symptoms were too serious to be justified for the test. Clinical features of the BW allergy patients are summarized in table 1. BW-asymptomatic sensitized subjects did not complain of any symptoms after ingestion of BW even though they had a positive skin prick test (SPT) response (Bencard Co., Worthing, UK). The mean wheal diameter for all subjects was larger than 4 mm. Two of the asymptomatic sensitized subjects were remitted from BW allergy. Patient serum was kindly donated with permission from each subject. These samples were frozen at -70°C until further use.

cDNA Cloning

One hundred milligrams of immature BW seeds were ground to a powder after being frozen in liquid nitrogen. Total RNA was isolated using RNeasy plant mini kit (Qiagen, Germantown, Md., USA) according to the manufacturer's instructions. To obtain the complete 3' end of the sequence, we used rapid amplification of cDNA ends polymerase chain reaction (RACE-PCR), and cDNA was generated from total RNA of BW following the manufacturer's recommendations. Degenerated sense primer was synthesized based on the N-terminal amino acid sequence of the 19-kD BW protein [13], 5'-GGN GAY TAY CCN YTN GA-3', and oligo(dT)-conjugated adaptor (5'-CTGATCTAGAGGTACCG-GATCCTTTTTTTTTTTT-3') was used as antisense primer. RACE-PCR was carried out in a 20- μl reaction mixture containing 2 μl of cDNA, 50 pmol of the degenerated sense primer, 2 pmol

of the adaptor-conjugated antisense primer, 2.5 mM of each dNTP, PCR buffer and 1 U *i-Pfu* DNA polymerase (Intron Biotechnology, Seongnam, Korea). Reaction condition was 94°C for 5 min, followed by 35 cycles of 94°C for 20 s, 44°C for 15 s and 72°C for 2 min, and a final extension step of 72°C for 7 min. The amplified products were cloned with a Zero Blunt TOPO PCR cloning kit (Invitrogen, Carlsbad, Calif., USA) and nucleotide sequences were determined with the BigDye Terminator Cyclic Sequencing reaction system (ABI 3100 Genetic Analyzer; Applied Biosystems, Foster City, Calif., USA). The clone representing the correct nucleotide sequence for the 19-kD BW protein was selected and used as a template for expression cloning.

Expression of cDNA Clones

We performed PCR using the following primers: forward primer 5'-CAA CCA TAT GGG GGA TTA TCC GTT TGA ACC-3' and reverse primer 5'-CGC AAG CTT CCG TTC GCA AAA AAG CGC GCA AA-3'. The forward and reverse primers contained the NdeI and Hind III restriction sequences, respectively, for subcloning into pET-21a. PCR was carried out in 50 µl of PCR buffer containing 2.5 mM of each dNTP, 50 pmol of the forward primer, 10 pmol of the reverse primer, 1.25 U of *i-Pfu* DNA polymerase and 1 µl of the cDNA for 19-kD BW protein selected by 3' RACE-PCR. Five annealing precycles at 40°C were followed by 30 cycles of amplification (94°C for 20 s, 44°C for 15 sec, 72°C for 2 min).

We used the pET-21a vector (Novagen, Madison, Wisc., USA) for protein expression. The amplified cDNA was digested with NdeI and Hind III (TAKARA Korea Biomedical Inc., Seoul, Korea), then ligated into pET-21a vector which was digested with the same restriction enzymes. The construct harboring cDNA insert with correct pET orientation was selected by nucleotide sequencing. The pET construct was transformed into BL21 cells, then the r19-kD BW protein was expressed by induction with 1 mM isopropyl β-D-1-thiogalactopyranoside (Sigma, St. Louis, Mo., USA) at 30°C for 4 h.

Purification of r19-kD BW Allergen

The induced and control flasks were placed on ice for 5 min before the cells were harvested by centrifugation at 5,000 g for 5 min at 4°C. The pellets were thawed in ice for 15 min and then resuspended in lysis buffer (100 mM NaH₂PO₄; 10 mM Tris-Cl; 8 M urea, pH 8.0) under denaturing conditions at 5 ml/g wet weight. For complete lysis, cells were stirred for 30–60 min at room temperature and centrifuged at 10,000 g for 20–30 min in order to pellet cellular debris.

Based on the Ni-NTA affinity purification kit (Qiagen), 1 ml of the 50% Ni-NTA slurry was added to 4 ml cleared lysate and gently mixed by shaking for 1 h at room temperature. The lysate-resin mixture was carefully loaded into an empty column and the flow through collected for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. After washing twice with wash buffer (5-fold resin volume; 100 mM NaH₂PO₄, 10 mM Tris-Cl, 8 M urea, pH 6.3), the recombinant protein was eluted by the subsequent use of 2 elution buffers (pH 5.9 and pH 4.5). The eluates were dialyzed with PBS before use. The protein concentrations of the recombinant allergen or the BW crude extract were assayed with a protein assay kit (Bio-Rad, Hercules, Calif., USA).

SDS-PAGE and IgE/Inhibition IgE Immunoblotting

Antigens were dissolved in reducing sample buffer (12.5 mM Tris-HCl; pH 6.8, 5% glycerol, 0.4% SDS, 1% mercaptoethanol and 0.02% bromophenol blue) and boiled at 100°C for 5 min. Allergens were separated by electrophoresis on 13.5% polyacrylamide gels (Hoeffer, San Francisco, Calif., USA) at 50 V for 30 min followed by 180 V for 2 h. Proteins were transferred onto nitrocellulose membranes (pore size 0.45 µm; Amersham, Little Chalfont, UK) on a transfer unit (Hoeffer) in buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3) at 350 mA for 2 h. The membranes were blocked by 5% nonfat dried milk in TBS-T (50 mM Tris, 0.1% Tween 20, pH 7.5), incubated overnight at room temperature with 1:10 diluted atopic sera, and then washed with TBS-T. To inhibit immunoblotting, the atopic pooled sera were preincubated with the appropriate concentration of r19-kD BW allergen at room temperature for 2 h. This process bound up any reactive antibody. Membranes were incubated with 1:1,000 diluted alkaline phosphatase-conjugated goat anti-human IgE (Sigma) for 60 min. After washing, the colorimetric reaction was developed with the BCIP/NBT system (Promega, Madison, Wisc., USA).

ELISA sIgE Measurement and Inhibition ELISAs

r19-kD protein sIgE was assayed by ELISA. A 96-well polystyrene plate was coated with 50 µl of 10 µg/ml of allergen in 0.05 M carbonate buffer (pH 9.6) overnight at 4°C. After washing with PBS-T (137 mM NaCl, 1.8 mM KH₂PO₄, 10 mM Na₂HPO₄, 27 mM KCl, 0.1% Tween 20, pH 7.4), each well was blocked with 1% bovine serum albumin in PBS-T. sIgE were detected by incubating the plate with 50 µl of BW-atopic sera for 1 h at room temperature. After washing, 1:1,000 biotin-labeled goat anti-human IgE (Vector, Burlingame, Calif., USA) was added to each well and incubated with 1:1,000 streptavidin peroxidase (Sigma) for 30 min before another washing step. The colorimetric reaction was developed with an ABTS solution (25 mg of 2,2-azino-bis-3-ethylbenzthiazoline-sulfonic acid in 50 mM citrate phosphate buffer, 50 µl of 30% H₂O₂). The reaction was stopped by adding 100 µl of 2 mM NaN₃ and afterwards we determined the optical density reading at the 405-nm ultraviolet wavelength by an automated microplate spectrophotometer (Dynatec, Alexandria, Calif., USA). The r19-kD protein sIgE levels were expressed in arbitrary units (AU). The pooled sera (n = 5) with high BW sIgE were assumed to have 1,000 AU/ml. The threshold level for the presence of r19-kD protein sIgE was 5.5 AU/ml, which was determined by the mean ± 2 standard deviations of 15 nonatopic control subjects.

For ELISA inhibition tests, atopic sera were preincubated for 2 h with each allergen concentration at room temperature before being measured in the ELISAs described above.

Statistical Analysis

Statistical analysis was performed using SPSS 9.0 (SPSS Inc., Chicago, Ill., USA). We analyzed the differences between groups with regards to mean wheal diameter as well as sIgE levels as determined by ELISA or coated allergen particle (CAP) tests. Any group differences were analyzed with the Wilcoxon signed-ranks test. The relationship between r19-kD protein sIgE and CAP IgE was evaluated using the Pearson correlation analysis. Clinical validation of r19-kD protein sIgE and CAP IgE measurements were performed using a receiver operating characteristic (ROC) curve analysis. $p < 0.05$ were considered significantly different.

<i>Ana o 1</i>	31	----- E LKQC KHQCKVQRQ YDE QQK EQCV KECEKYKKEKKG RE R E HE E E E E	75
<i>Jug r 2</i>	11	RGRDDDEEN PRDPREQYRQCQ EY CRRQGQ GQR QQQQ-- CQ I RCE E -----RLE ED Q R S Q E E R ER	68
7 S globulin	85	----- P I E RLRE CS RGCEQQHG EQ R E-----E--C L RRCQE -----E--- YQ R E KG RQ DDD	125
19-kD BW	1	----- DY PFE PRRQ I E HCRQR CQDRS Q G F R E T R E--C L ETCEK----- RAG FD D E P S KE V DS	50
<i>Ana o 1</i>	76	W----- GT GGVDE PST HEPAEKH LSQCMRQCERQE---- GGQKQ L--CRFR CQERYKKE RGQHNYKRE	133
<i>Jug r 2</i>	69	RRG RD VD-----DQNPRDP--EQRYEQC QQQCERQRR-- GQ EQT L---CRRRCEQR---RQQE E-----	117
7 S globulin	126	N-----PT ----- D -----P-- EKQYQQCRLQCRRQEGGG FS REH CER RREE KYREQQG -----	168
19-kD BW	51	Y-----TT -----DTRSRDP---QQEF RQCRHRCQTQE EGG RQQRK--CEQRCEKQ LERQQGY-----	98
<i>Ana o 1</i>	134	DDEDE DEDEA EEEDEN----- PY V F E DEDFT T KVKTEQGKVV LLPKFTQKSKLLHALEKYRLAVLVA-----	195
<i>Jug r 2</i>	118	RE RQRGRDRQ D-----PQQQYHRCQRR C-----	143
7 S globulin	168	----REGGRGEM YEGRER EEEQE EQG RGRI P YVFEDQHF I TGFR-----	207
19-kD BW	99	DDEEF GQGRS NI GAPV RNYDDCTEMCGSPLCALFCE -----	135

Fig. 1. Homology of r19-kD BW allergen to vicilin-like allergens of English walnut (*Jug r 2*), cashew (*Ana o 1*) and 7 S albumin of *Sesamum indicum*.

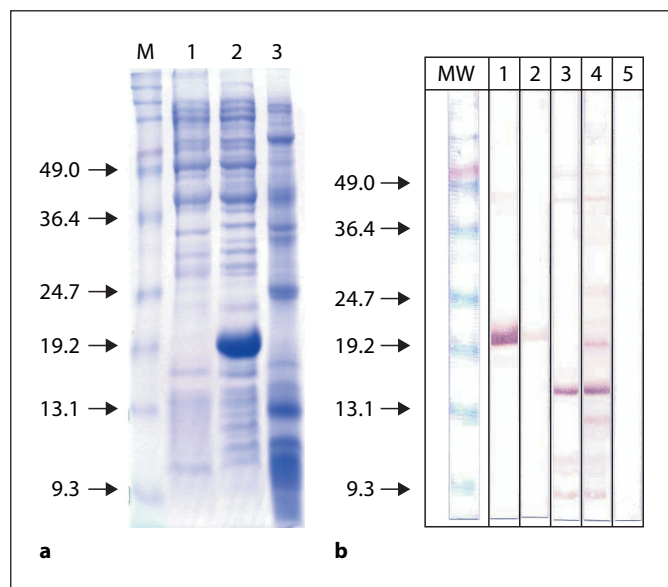


Fig. 2. SDS-PAGE (**a**) and IgE immunoblots (**b**) of the BW crude extract. **a** Lane 1 = Extract from unexpressed *E. coli*; lane 2 = r19-kD BW protein expressed in *E. coli*; lane 3 = crude BW extract. **b** Lane 1 = sIgE binding to r19-kD BW protein; lane 2 = specific inhibition of sIgE binding to r19-kD BW protein by itself (10 µg/ml); lane 3 = inhibition of sIgE binding to BW crude extract by binding to r19-kD BW protein (10 µg/ml); lane 4 = sIgE binding to BW crude extract; lane 5 = nonatopic control.

Results

Full Amino Acid Sequence of 19-kD BW Allergen and Its Homology to Other Food Allergens

Based on the reported N-terminal sequence of the 19-kD BW allergen [13, 16], we cloned the 19-kD BW allergen cDNA and deduced its full amino acid sequence (Genbank access No. EF532322). The 19-kD BW allergen consisted of 135 amino acids and had an estimated molecular weight of 16.05 kD (fig. 1). Homology tests to other food allergens were done through the National Center for Biological Information program. The 19-kD BW allergen has a 35% homology to the vicilin-like allergen of English walnut (*Jug r 2*; Genbank access No. AAF18269), a 25% homology to the vicilin-like allergen of cashew (*Ana o 1*; Genbank access No. AAM73730) and a 32% homology to 7 S globulin in *Sesamum indicum* (Genbank accession No. AAK15089). No homology was found between 19-kD and other BW allergens.

Allergenicity of r19-kD BW Allergen

The 19-kD BW allergen was expressed in *Escherichia coli* and its allergenicity evaluated with pooled sera from patients with allergies to BW (n = 5; patients No. 3, 10, 11, 15 and 17 in table 1). The r19-kD allergen inhibited the binding of sIgE to the native 19-kD BW protein when IgE was used for immunoblotting (fig. 2). The BW crude extract and r19-kD BW protein completely and dose-de-

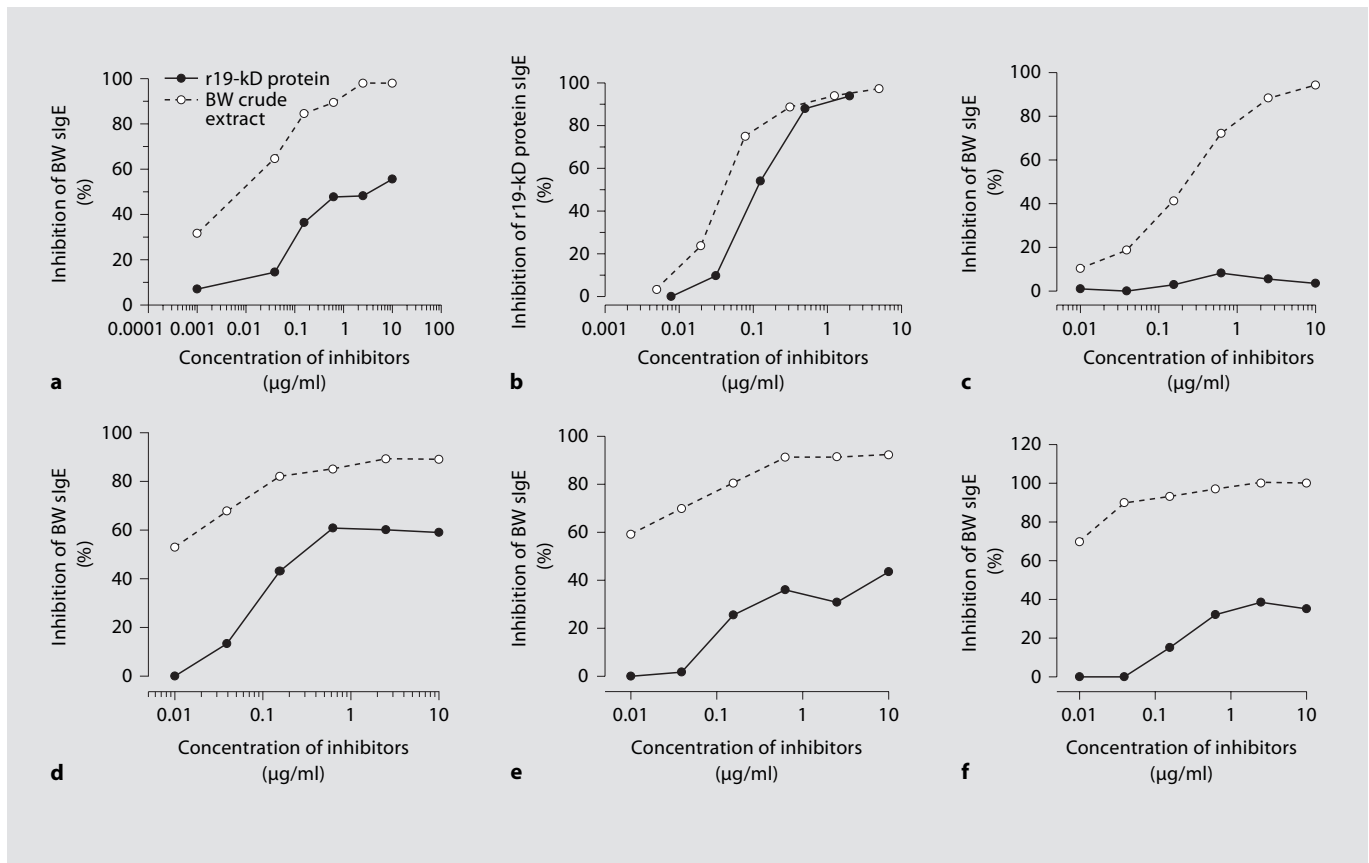


Fig. 3. ELISA inhibition of sIgE binding to BW crude extract (a) or ELISA inhibition of sIgE binding to r19-kD BW protein (b) with pooled atopic sera. ELISA inhibitions of sIgE to BW crude extract were done with individual BW allergy sera. Patients No. 4 (c), 11 (d), 15 (e) and 18 (f) in table 1.

pendently inhibited sIgE binding to the r19-kD protein in the ELISA inhibition assay. The r19-kD BW protein also inhibited sIgE binding to BW crude extract in a dose-dependent manner. For example, 10 µg/ml of the r19-kD protein inhibited sIgE binding to BW crude extract by 56% (fig. 3a). Individual variations for inhibition of crude BW extract sIgE with the r19-kD protein were found (fig. 3c–f).

Clinical Application of SPT, CAP sIgE from Whole Extract and sIgE from r19-kD Allergen for BW Reactivity Diagnosis

BW allergy patients had stronger skin reactivity in SPT ($p < 0.001$), higher CAP sIgE in BW crude extract ($P < 0.001$) and anti-r19-kD allergen sIgE ($p < 0.001$) compared to patients that were asymptomatic to the BW sensitizer. The optimal cutoff values for the SPT, CAP sIgE for the BW crude extract and r19-kD protein sIgE mea-

sured by ELISA were determined by ROC curve analysis. These cutoff values were: wheal size of skin prick test = 11.1 mm, BW CAP sIgE = 3.4 kU/l and r19-kD protein sIgE by ELISA = 5.5 AU/ml. For the diagnosis of BW-allergic subjects, the presence of anti-r19-kD allergen sIgE that were measured by ELISA were more discriminative than the CAP sIgE measured by crude BW extract. Thirteen of 18 (72.2%) BW allergy patients and 4 BW-asymptomatic sensitized subjects had values higher than the cutoff value of CAP sIgE, while 83.3% of BW allergy patients and just 1 asymptomatic subject had sIgE against the r19-kD allergen (fig. 4). The levels of r19-kD protein sIgE were correlated with CAP sIgE in BW SPT-positive subjects ($n = 37$, $r = 0.682$, $p < 0.001$) and BW-allergic subjects ($n = 18$, $r = 0.634$, $p = 0.005$), but not in BW-asymptomatic sensitized subjects ($n = 19$, $r = 0.420$, $p = 0.863$; fig. 4). The areas under the ROC curve for the SPT [0.925 (95% confidence interval: 0.839–1.012), $p < 0.001$]

and anti-r19-kD protein sIgE ELISAs [0.860 (95% confidence interval: 0.725–0.995), $p < 0.001$] were higher than that of a BW sIgE CAP test [0.803 (95% confidence interval: 0.661–0.945), $p = 0.002$] (fig. 5).

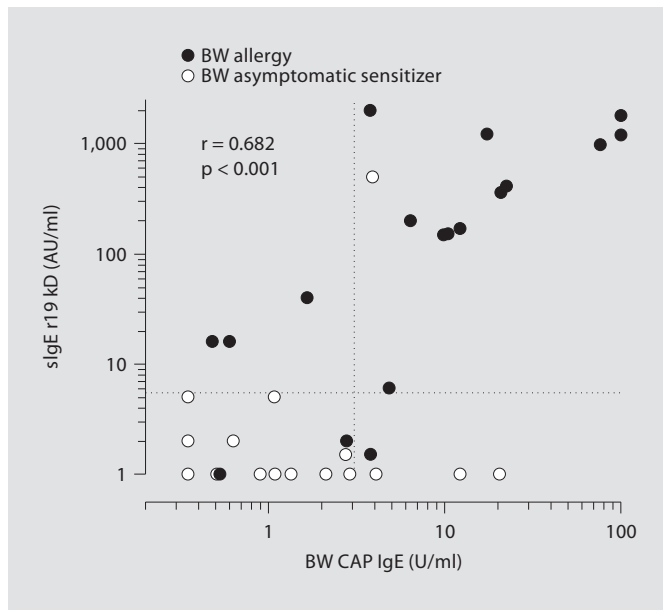


Fig. 4. Levels of sIgE in BW crude extract as determined by CAP and anti-r19-kD allergen sIgE in BW-allergic patients ($n = 18$) and BW-asymptomatic sensitized subjects ($n = 19$). Dotted lines represent the optimal cut-off values of BW CAP IgE and sIgE r19 kD for the diagnosis of BW allergy determined from the ROC analysis.

Discussion

Several BW proteins have been implicated as major allergens, but no consensus has been reached regarding which proteins are the major allergens. Recently, the 19-kD protein has been identified as a major BW allergen candidate [13, 15]. Studies have shown that sIgE affinity to whole BW extract has different patterns in BW allergy patients compared to asymptomatic sensitized subjects. sIgE to the 19-kD BW allergens were found specifically in symptomatic subjects, while the sIgE to the 11 S globulin protein (Mw 22–24 kD) was found nonspecifically in BW-allergic patients and asymptomatic BW-sensitized subjects. In our study, we are the first to identify the full amino acid sequence of the 19-kD BW allergen. We also confirmed allergenicity by immunoblotting and ELISA inhibition. As 83% of BW allergy patients have sIgE that bind to the 19-kD BW protein and also had significant allergenicity as measured by ELISA inhibition, we consider the 19-kD allergen to be the major allergen produced by *F. esculentum*.

Our results revealed weak amino acid sequence homology between the BW 19-kD allergen and the 7 S globulin from sesame, as well as the vicilin-like major allergens of cashew [17] and English walnut [18]. These homology results suggest that the 19-kD BW protein may belong to the vicilin storage protein family. However, cross-allergenicity is unlikely because the homologies are weak and we did not find sesame, cashew or walnut allergies in BW-allergic patients. In this study we could not find homology between the 19-kD allergen and other

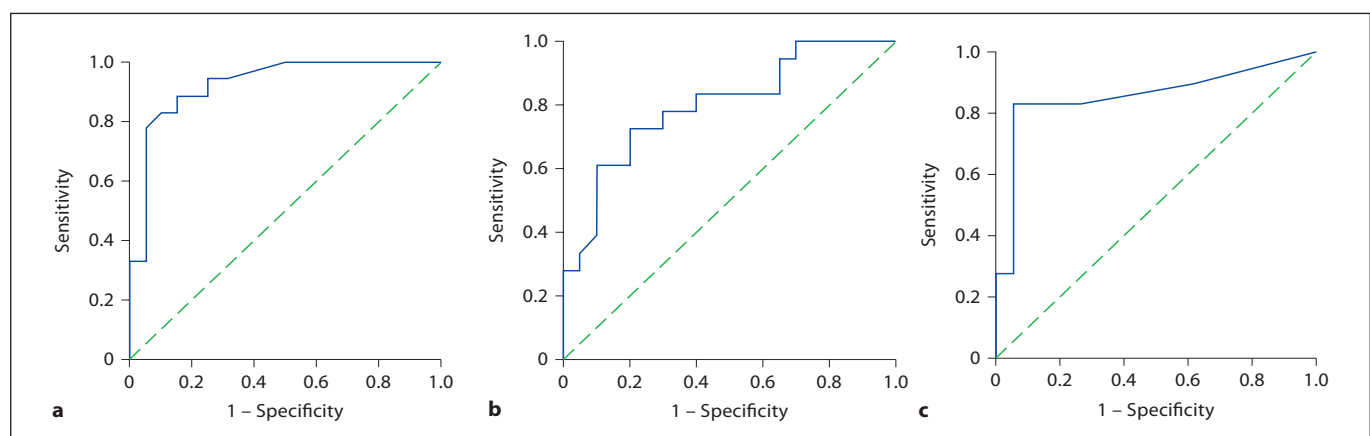


Fig. 5. ROC curves generated from SPTs (a), CAP IgE in BW crude extracts (b) and anti-r19-kD protein IgE ELISA (c).

known BW allergens. However, the inhibition immunoblotting with r19-kD protein showed that 13-kD BW allergen may share common IgE epitopes, but not the 9-kD allergen, which has been reported to be a vicilin family protein [11–13]. Shared IgE epitopes are not unique to 19-kD allergen. Marked homology and shared IgE epitopes between 16- and 10-kD BW allergens, which belong to the 2 S storage protein, were also found [14].

For food allergies, SPT and sIgE measurements using whole extract are well known to have poor positive predictive values. DBPCFC is the gold standard for diagnosing food allergies. Because DBPCFC is difficult and dangerous, the application of discriminating sIgE levels by using the CAP test or wheal size in SPT has been successfully evaluated for peanut, egg and milk allergies [19, 20]. We found that all BW allergy patients had strong SPT responses, and the optimal differentiating mean wheal size between BW-allergic subjects and asymptomatic sensitizers was 11.1 mm. A large wheal size (>8 mm) in SPTs was also predictive of clinical reactivity in milk, egg and peanut allergies with 95% accuracy [21–23]. We also found that r19-kD protein sIgE were more discriminative for the diagnosis of BW clinical reactivity than the CAP sIgE measurement. The measurement of r19-kD protein sIgE is especially useful for BW allergy patients with low CAP IgE levels in BW crude extract. The concentration of CAP IgE for an 85% positive predictive level in our study group was 4.5 kU/l. Therefore, 6 of 18 BW allergy patients were below the diagnostic decision level. Among these patients, 4 BW allergy patients had r19-kD protein sIgE.

We do not know why the 19-kD allergen has more specific clinical reactivity to BW than the 24-kD 11 S globulin or 9-kD vicilin family BW allergens. Many food allergens have both conformational and sequential epitopes. Individuals who possess sIgE to conformational epitopes may tolerate foods denatured by cooking or digestive enzymes,

while individuals allergic to sequential epitopes may react to the food in any form [24, 25]. Subjects with allergies to conformational epitopes may tend to develop clinical tolerance [26]. Little information is available about the 19-kD allergen epitopes. Previously, we showed that the 19-kD allergen does not have carbohydrate epitopes by periodate oxidation of carbohydrates. Furthermore, the r19-kD BW protein in our study was expressed in *E. coli*, which produces nonglycosylated proteins. Thus, we can exclude the presence of anticarbohydrate sIgE to the 19-kD BW protein. sIgE to carbohydrate epitopes are usually cross-reactive in many plant-derived molecules. Frequently these epitopes do not have any clinical reactivity [27–29]. The 19-kD allergen has been reported to be easily digested by pepsin [15]. However, the allergenicity of the digested fragments of 19-kD BW protein has not been evaluated. Some investigators have suggested that in vivo or in vitro tests using pepsin-digested food extracts may be a useful tool to identify the food-allergic patient against potentially dangerous stable allergens [30]. The digestion of protein with pepsin might destruct epitopes of the allergen, but it can also convert the protein from nonsensitizing to sensitizing forms. Moreover, the fragments from the digested protein may maintain their allergenicity [31]. Further studies are required to determine the IgE epitope structures of the 19-kD BW allergen.

In conclusion, we demonstrated that the 19-kD BW allergen is a major allergen of BW. The r19-kD BW protein might be particularly useful in the diagnosis of the clinical reactivity to BW.

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