

Identification of wheat flour allergens by means of 2-dimensional immunoblotting

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Background: Wheat flour proteins are allergens for 60% to 70% of bakers with workplace-related respiratory symptoms. **Objective:** The aim of the study was to investigate the variability of IgE antibody patterns of wheat flour-sensitized bakers and to identify the most frequently recognized allergens.

Methods: Water/salt-soluble wheat flour proteins from the cultivar Bussard were separated by using 2-dimensional gel electrophoresis with immobilized pH gradients. IgE-reactive proteins were identified by means of immunoblotting with sera of 10 subjects with baker's asthma. Mass spectrometric fingerprinting was used to identify the proteins most frequently recognized by IgE.

Results: The IgE immunoblots obtained with 10 different sera exhibited a remarkable heterogeneity. Each patient showed an individual IgE-binding pattern with 4 to 50 different allergen spots. Altogether, more than 100 IgE-binding protein spots were detected. Nine of the predominant IgE-binding protein spots were identified by using mass spectrometric fingerprinting. The obtained masses matched 2 different isoforms of glyceraldehyde-3-phosphate dehydrogenase from *Hordeum vulgare*, triosephosphate isomerase from *H. vulgare*, and serpin, a serine proteinase inhibitor from *Triticum aestivum*.

Conclusions: The results show a great interindividual variation of IgE-binding patterns of wheat flour proteins in baker's asthma. The clinical relevance of the identified 4 new allergens will be further investigated in the near future. (*J Allergy Clin Immunol* 2001;107:907-13.)

Key words: Baker's asthma, wheat flour allergens, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, serpin

Most bakers with workplace-related asthma or rhinitis are sensitized to wheat or rye flour allergens. Sixty percent to 70% of bakers claiming compensation have specific IgE to wheat or rye flour.^{1,2} Wheat seeds are composed of 4 protein classes: the water/salt-soluble albumins and globulins; the ethanol-soluble gliadins; and the urea, detergent, or KOH-soluble glutenins. Although studies by Walsh et al,³ Weiss et al,⁴ and Sandiford et al⁵ demonstrated IgE binding in the salt-insoluble fractions (gliadin and glutenin) of wheat flour and Maruyama et

Abbreviations used

CHAPS: 3-[(3-cholamidopropyl)dimethylammonio]propane-1-sulphonic acid

DTT: Dithiothreitol

EAST: Enzyme allergosorbent test

IPG: Immobilized pH gradient

pI: Isoelectric point

PVDF: Polyvinylidene difluoride

TBS: TRIS-buffered saline

al⁶ identified low-molecular-mass glutenin, α -gliadin, and γ -gliadin after expression in *Escherichia coli* as allergens for patients with clinical histories of allergies, the most relevant allergenic wheat fraction for baker's asthma consists of water/salt-soluble albumins and globulins exhibiting about 70% to 80% of the specific IgE-binding activity.⁴ Although up to 70 different IgE-binding protein spots⁷ could be identified by means of high-resolution 2-dimensional gel electrophoresis and immunoblotting of the water/salt-soluble wheat fraction, only a few of these allergens were identified and characterized on a molecular basis. To the latter belong various 14- to 17-kD proteins of the α -amylase/trypsin inhibitor family, including subunits of the tetrameric α -amylase inhibitor,^{8,9} 2 highly homologous dimeric α -amylase inhibitors,^{7,9-11} the monomeric α -amylase inhibitor,^{9,11,12} and a homologue to a barley trypsin inhibitor.¹¹ The highest in vivo allergenic activity showed the glycosylated subunit CM16* of the tetrameric α -amylase inhibitor with 14 positive skin prick test reactions of 31 tested patients allergic to wheat flour. Furthermore, Sánchez-Monge et al¹³ isolated a 36-kD wheat glycoprotein with peroxidase activity and similarity to a barley seed-specific peroxidase with a known primary structure. Six of 10 patients with wheat allergy and baker's asthma had specific IgE to the isolated enzyme. Other wheat allergens identified by using 2-dimensional gel electrophoresis, immunoblotting, and N-terminal sequencing or amino acid composition analysis are similar to the 27-kD acyl-CoA oxidase variants of rice and barley or to 39-kD fructose-bisphosphate-aldolase in maize and rice.^{7,14} Because the examinations were performed with pooled sera, no information about the frequency of these sensitizations was obtained.

The aim of our study was to examine the frequency of sensitizations to the numerous allergenic protein spots and to identify proteins most frequently bound by IgE antibodies with 2-dimensional gel electrophoresis and immunoblotting.

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METHODS

Baker's sera and specific IgE measurement

Sera were obtained from 10 bakers with bronchial asthma, rhinitis, or both caused by type I allergy to flour. Severity of asthma symptoms was classified according to the "Guidelines for the diagnosis and management of asthma."¹⁵ As controls, one serum from a baker without clinical symptoms and without specific IgE to any of the tested baker allergens and one serum of a patient who had never worked in a bakery were used. All patients were men.

Human IgE antibodies to wheat flour, rye flour, soy flour, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and grass pollen mixtures (gx2 from Pharmacia, Uppsala, Sweden) were measured by using the Pharmacia CAP system, according to the manufacturer's recommendations.

Additionally, enzyme allergosorbent tests (EASTs; Allergopharma, Reinbek, Germany) were performed with allergen disks prepared in our laboratory with the following allergen couplings: xylanase (BASF, Ludwigshafen, Germany), cellulase (Sigma, Deisenhofen, Germany), and glucoamylase (Serva, Heidelberg, Germany), all of which were derived from *Aspergillus niger*, and α -amylase, which was developed from *Aspergillus oryzae* (Fungamyl 1600s, Novo, Denmark). For coupling, BrCN-activated paper disks were incubated in 0.1 mol/L NaHCO₃ (pH 8.5) containing 1 mg/mL protein for 5 hours at room temperature, saturated with ethanolamine, and washed according to the method described by Ceska and Lundkvist.¹⁶ The allergen-coated disks were incubated with patient sera, and bound IgE levels were determined, as described by the manufacturer. The EAST reference curve, always run in parallel, was used for calculation. EAST values of less than 0.35 kU/L represent absent or undetectable levels of allergen-specific IgE antibodies. Values between 0.35 and 0.7 kU/L correspond to RAST class 1, values between 0.7 and 3.5 kU/L to RAST class 2, values between 3.5 and 17.5 kU/L to RAST class 3, and values greater than 17.5 kU/L to RAST class 4.

Protein extraction and sample preparation for 2-dimensional gel electrophoresis with immobilized pH gradients

Wheat seeds of the cultivar Bussard were kindly provided by Lochow Petkus GmbH. Wheat seeds (480 mg) were crushed in a mortar with a pestle and extracted with 1 mL of TRIS-buffered saline (TBS; 50 mmol/L TRIS/HCl [pH 7.4] and 150 mmol/L NaCl) containing 0.8% wt/vol carrier ampholytes (pH 3-10; Pharmalyte, Pharmacia). After stirring at 4°C for 30 minutes, the sample was centrifuged for 1 hour at 40,000g. The clear supernatant containing the salt-soluble albumins and globulins was frozen and stored at -80°C. The protein concentration of the supernatant was determined according to the method of Bradford,¹⁷ with BSA as the standard.

Two-dimensional gel electrophoresis with immobilized pH gradients of wheat grain proteins

The supernatants described above were diluted with an identical volume of lysis buffer: 9 mol/L urea, 0.8% carrier ampholytes (pH 3-10), 1% dithiothreitol (DTT), and 2% 3-[(3-cholamidopropyl)dimethylammonio]propane-1-sulphonic acid (CHAPS). IPG gels with linear gradients (pH 4-8; 4% T and 4% C, size 250 × 180 × 0.5 mm³) were cut into 3-mm wide strips and, for analytical runs, rehydrated overnight with a solution of 8 mol/L urea, 0.2% carrier ampholytes (pH 3-10), 0.25% DTT, and 0.5% CHAPS. The rehydrated IPG strips were placed onto the cooling plate of a flatbed focusing chamber (Multiphor II electrophoresis chamber, Pharmacia), and a sample of 100 µg of protein was applied near the anode. Isoelectric focusing was performed for 35,000 volt-hours at 20°C.

For preparative runs, the IPG strip was hydrated overnight in the dry strip-reswelling tray from Pharmacia in a volume of 350 µL of rehydration solution (9 mol/L urea, 2% CHAPS, 0.3% DTT, 0.2% carrier ampholyte (pH 3-10), and 10% glycerol) containing about 500 µg of salt-soluble wheat flour proteins. Isoelectric focusing was performed for 60,000 volt-hours at 20°C.

Before the second dimension, the IPG strips were equilibrated for 10 minutes in equilibration solution (6 mol/L urea, 30% glycerol, 2% SDS, and 50 mmol/L TRIS/HCl [pH 8.8]) containing 1% DTT and then for 10 minutes in an equilibration solution containing 250 mmol/L iodoacetamide. SDS-PAGE (13% T and 2.6% C) was performed simultaneously on 4 slab gels (210 × 185 × 1 mm³) with the buffer system of Laemmli.¹⁸ The proteins on analytical gels were silver stained according to the method of Blum et al,¹⁹ whereas the proteins on micro-preparative gels were Coomassie stained²⁰ or used for immunoblots.

Electrophoretic transfer and immunostaining

The electrophoretically separated proteins were electroblotted onto an immobilizing polyvinylidene difluoride (PVDF) membrane (Millipore, Eschborn, Germany) in a semidry blotting apparatus with 50 mmol/L TRIS base, 50 mmol/L boric acid, and 10% methanol at 0.8 mA/cm² for 90 minutes at room temperature. For immunostaining, nonoccupied binding sites on the PVDF membrane were blocked with 1% BSA and 1% polyvinylpyrrolidone 40 in TBS for 6 hours. Sera from bakers were diluted with 2% BSA/TBS plus 0.1% Tween-20 (TBST; 1:10), incubated overnight with the blots at room temperature, and washed 3 times with TBST. Bound IgE was detected after incubation for 2 hours with anti-human IgE/alkaline phosphatase (Sigma) diluted 1:1000 in TBST with 2% BSA by using NBT/BCIP as a substrate (0.03% 4-nitroblue tetrazolium-chloride and 0.015% 5-bromo-4-chloro-3-indolyl-phosphate). The assignment of immunostained proteins to Coomassie- or silver-stained spots was carried out manually.

In situ gel digestion for mass spectrometry

Tryptic digestion was performed in the gel matrix according to the procedure of Gevaert et al,²¹ with certain modifications. The excised gel plugs were destained by several washing procedures with water and a solution of 50% acetonitrile. The gel pieces were shrunk by addition of acetonitrile. Subsequently, the acetonitrile was removed by means of vacuum evaporation, and the digestion was started by adding 0.1 µg of trypsin in 10 mmol/L ammonium hydrogencarbonate (pH 7.8) overnight at 37°C. The peptides were eluted by adding 10 µL of 10 mmol/L ammonium hydrogencarbonate (pH 7.8) and subsequent ultrasonic treatment. The supernatants were concentrated by using Zip-Tips (Millipore, Bedford, Mass), mixed with matrix (4-hydroxy- α -cinnamic acid), and prepared on a target for matrix-assisted laser desorption mass spectrometry (MALDI-TOF, Bruker Reflex III; Bruker, Bremen, Germany).

Protein identification

A peptide mass fingerprint database search was performed with the masses obtained by means of MALDI-TOF analysis with the program ProFound/www (<http://www.prowl.rockefeller.edu/cgi-bin/ProFound>) from Rockefeller University and ProteoMetrics. The nonredundant protein database NCBI nr (version 06/03/2000) was used, and the expected protein mass range was adjusted from 15 to 150 kD. Peptide masses were assumed to be monoisotopic, with a mass accuracy of 0.10 D after internal calibration.

RESULTS

Description of patients

Ten patients with work-related wheat flour allergy were recruited for the identification of wheat grain albu-

TABLE I. Case history and clinical data of bakers and control subjects

Patient No.	OET	Symptoms	Total IgE (kU/L)	Specific IgE (kU/L)							
				Wheat flour	Rye flour	Soy flour	<i>D pteron-yssinus</i>	<i>D farinae</i>	α -amylase <i>A oryzae</i>	Xylanase <i>A niger</i>	Grass pollen
1	42	A1, RC	431	14.40	10.50	3.44	<0.35	<0.35	0.78	<0.35	0.61
2	34	A3, R	81.6	5.19	5.50	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
3	27	A2, RC	1305	11.70	7.24	<0.35	13.10	11.10	<0.35	0.62	1.7
4	10	A2, RC	192	6.18	11.80	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
5	42	A3, R	99.9	27.70	21.10	<0.35	4.33	3.76	<0.35	<0.35	<0.35
6	7	A3, RC	175	6.34	17.50	0.40	0.89	<0.35	<0.35	<0.35	NT
7	12	A1, RC	978	8.95	6.52	4.36	<0.35	0.51	0.69	<0.35	90.7*
8	10	A4, R	61.8	7.70	4.82	3.22	1.64	1.35	<0.35	<0.35	<0.35
9	2	A4	1378	11.40	9.49	2.79	7.71	7.57	<0.35	<0.35	0.51
10	7	A1, R	212	5.90	3.92	0.79	<0.35	<0.35	<0.35	<0.35	NT
Control 1	0	None	567	<0.35	<0.35	<0.35	1.60	1.33	<0.35	<0.35	NT
Control 2	50	None	144	<0.35	<0.35	<0.35	NT	NT	<0.35	<0.35	NT

OET, Occupational exposure time; A, work-related asthma (A1, mild intermittent; A2, mild persistent; A3, moderate persistent; A4, severe persistent); R, work-related rhinitis; C, work-related conjunctivitis; NT, not tested.

*Diluted 1:2.

mins and globulins bound by IgE antibodies. All patients had workplace-related asthma and high levels of specific IgE to wheat and rye flour (Table I). Additional sensitizations to soy flour, house dust mites, enzymes used for the baking process, and grass pollens were found. The control patients had no specific IgE to wheat or rye flour.

Two-dimensional electrophoresis of wheat flour proteins

Water/salt-soluble wheat grain proteins of the cultivar Bussard were separated by means of 2-dimensional gel electrophoresis with immobilized pH gradients (IPGs) and SDS-PAGE. Fig 1 shows a typical 2-dimensional map of the water/salt-soluble wheat flour proteins. About 300 distinct spots with molecular masses from 100 kd to less than 6.5 kd were visible with silver staining. The albumin/globulin polypeptides were distributed more or less uniformly over the entire isoelectric point (pI) and molecular weight range. Most proteins had a pI above pH 4.5 and a molecular mass between 10 and 20 kd or between 24 and 80 kd.

Immunoprobings with sera from bakers with wheat flour allergy

Micropreparative 2-dimensional electrophoresis gels were blotted and incubated with the sera of the 10 symptomatic bakers described in Table I. The IgE-stained patterns of the water/salt-soluble wheat proteins obtained with the 10 sera differed remarkably; 8 of these immunoblots are shown in Fig 2. Immunoblot results are summarized in Table II. Control experiments with the serum of one baker without sensitization to wheat flour and one patient who had never worked in a bakery indicate the specificity of IgE binding, with the exception of spots 1a, 1b, and 2. The latter were detected more or less by all sera, including the control sera, as well as with the anti-human-IgE antibody alone (data not shown).

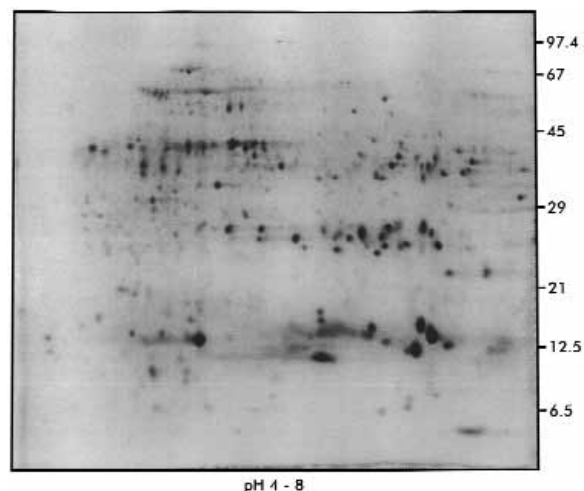


FIG 1. Water/salt-soluble wheat flour proteins separated by means of 2-dimensional electrophoresis and stained with silver.

Although the immunoblots with sera No. 1 (Fig 2, A), No. 2 (Fig 2, B), No. 5 (Fig 2, E), No. 6, and No. 9 showed less than a dozen different IgE-binding protein spots, the blots with sera No. 3 (Fig 2, C), No. 4 (Fig 2, D), No. 7 (Fig 2, F), No. 8 (Fig 2, G), and No. 10 (Fig 2, H) revealed up to 50 different immunostained spots. Most IgE-binding spots were unique for one patient's serum, and only 40 spots were detected by more than one serum. The most abundant IgE-binding spots were numbers 3a and 4a, with 80% of reactions; 3b and 4b, with 60% of reactions; and 5a, 5b, and 3c, with 50% of reactions.

Identification of IgE-binding proteins by means of mass fingerprinting

Nine Coomassie-stained protein spots (3a, 3b, 3c, 4a, 4b, 5a, 5b, 6a, and 6b) were analyzed after tryptic digestion by means of mass spectrometry. The mass fingerprints could

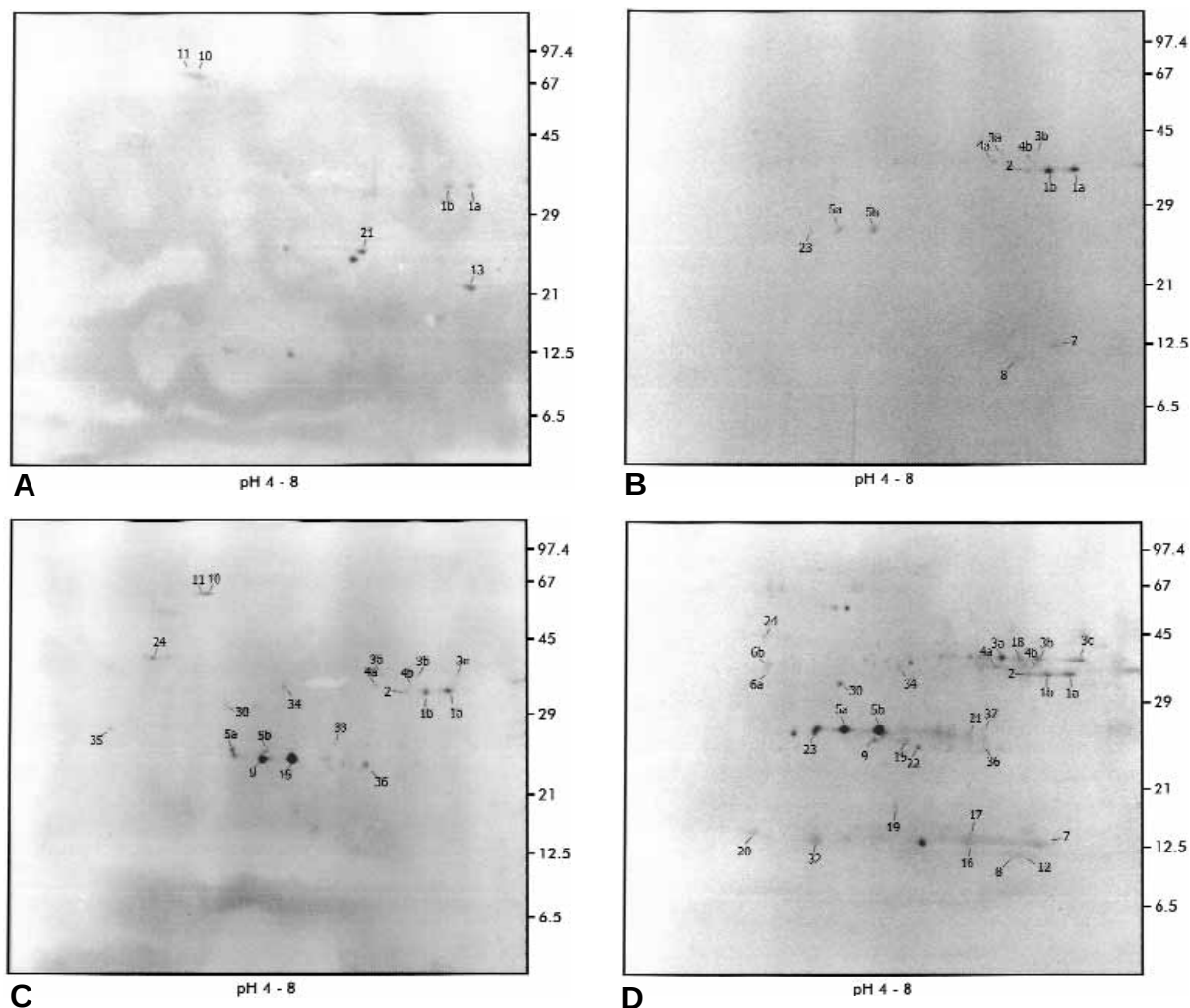


FIG 2. Water/salt-soluble wheat flour proteins separated by means of 2-dimensional electrophoresis, electroblotted onto a PVDF membrane, and immunostained with patient sera (A, No. 1; B, No. 2; C, No. 3; D, No. 4; E, No. 5; F, No. 7; G, No. 8; and H, No. 10) and alkaline phosphatase-labeled anti-human IgE. IgE-binding spots with the same position in the 2-dimensional electrophoresis are in principle numbered according to the frequency of detection with different sera.

be matched significantly with known cereal proteins (Table III). Five IgE-reactive proteins represent not-yet-described wheat flour allergens similar to 2 different isoforms of glyceraldehyde-3-phosphate dehydrogenases from *Hordeum vulgare*. Spots 3a, 3b, and 3c corresponded to a 37-kD protein with a theoretical pI of 6.7, and spots 4a and 4b corresponded to a 33-kD variant with a theoretical pI of 6.2. Spots 5a and 5b matched to triosephosphate isomerase from *H vulgare*, and spots 6a and 6b were shown to be serpin, a serine proteinase inhibitor from *Triticum aestivum*. A spot with the same position as our spot 5b was identified by Posch et al⁷ as IgE binding with pooled sera of sensitized bakers. N-terminal sequencing obtained the sequence KRKFIVXXN, which could not be matched unambiguously with a protein in the databases. Comparing the first 9 amino acids of triosephosphate isomerase from *H vulgare* (GRKFFVGGN) with this N-terminal sequence shows enough similarity to prove that identification by means of

mass fingerprinting is reliable and, additionally, that 2-dimensional electrophoresis with IPGs reveals highly reproducible protein positions, even if different cultivars (Bussard and Fregatt⁷) were used. Using the N-terminal sequence information from Posch et al⁷ for our investigation, further allergen spots can tentatively be identified: spot 15 is identified as a protein similar to acyl-CoA oxidase (spot 2 of Posch et al), and spot 32 is identified as wheat dimeric α -amylase inhibitor (spot 7 of Posch et al).

DISCUSSION

Water/salt-soluble wheat flour proteins of the cultivar Bussard were analyzed by using high-resolution 2-dimensional electrophoresis with IPGs. Similar to the results of Posch et al,⁷ who investigated the cultivar Fregatt with the same method, we obtained a complex but highly reproducible pattern of several hundred silver-stained albumin

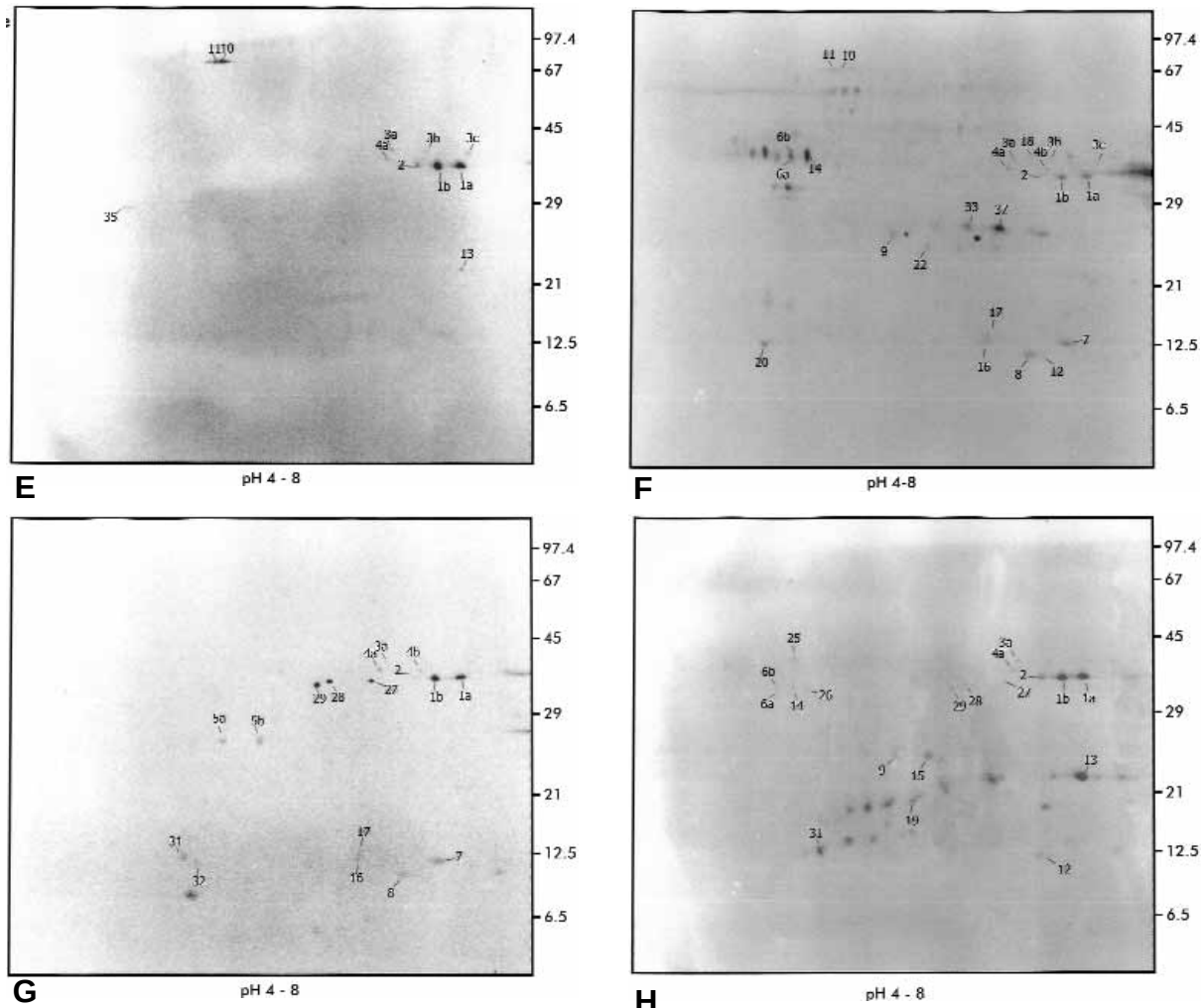


FIG 2. Continued.

and globulin spots. The high reproducibility of spot positions in 2-dimensional electrophoresis with IPGs offers the possibility of producing a series of nearly identical wheat protein blots for immunoprobings with the sera of many patients. The IgE immunoblots obtained with 10 different sera showed a remarkable heterogeneity in recognized allergens. Each patient showed an individual sensitization pattern with 5 to 50 different allergen spots. To our knowledge, a similar diversity of IgE-binding patterns to other allergen sources (eg, grass pollen, tree pollen, and house dust mites) has not been observed yet. With most allergen sources, the IgE-binding patterns established for different individuals were quite similar on the basis of the presence of common or major allergens, with occasional rare or minor allergens. In contrast to pollen and house dust mite allergy preferentially caused by exposure to surface or secreted proteins, flour as an allergen source additionally bears the risk of contacting intracellular proteins.

Our investigation is the first one performed with 2-dimensional fractionation of wheat flour allergens, allowing the detection of possible variations between individu-

als. Our results confirm and strengthen observations from Blands et al²² and Sutton et al²³ obtained with crossed radioimmuno-electrophoresis, ion-exchange chromatography, or preparative isoelectric focusing. These authors found that there were many flour allergens and that they differed from one individual to another. Whereas a possible limitation of interpreting their data is the poor resolution of these fractionation techniques, this is surely not the case for 2-dimensional electrophoresis. Even isoallergens with small differences in pI or molecular mass corresponding to sequence isoforms or different posttranslationally modified (eg, phosphorylated) protein variants could be resolved and distinguished from each other (spots 3a, 3b, 3c, 4a, 4b, 5a, 5b, 6a, and 6b; Table III).

The reason for the strong diversity of reactions among bakers is not known. One explanation may be that individuals who have allergies to inhaled flour have generally been exposed to a much greater antigen load than patients with pollen or house dust mite allergy. Under conditions of an extremely high inhalational exposure level, even proteins without a high allergenic potential may become aller-

TABLE II. Summary of IgE-binding protein spots in 2-dimensional immunoblots

Spot	MM	pI	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Positive (%)
1a*	40	7.2	++	+++	++	++	+++	++	++	+++	+++	+++	100
1b*	40	7.0	++	+++	++	++	+++	++	++	+++	+++	+++	100
2*	40	6.8		+	+	+	++		+	+	+	++	80
3a	42	6.7		+	+	+++	+		+	+	+	+	80
4a	40	6.6		+	+	+	+		+	+	+	+	80
3b	42	6.9		+	+	++	+		+		+		60
4b	40	6.9		+	+	+			+	+	+		60
3c	42	7.2			+	++	+		+		+		50
5a	26	5.5		++	++	+++				++	+		50
5b	26	5.8		++	+	+++				++	+		50
6a	40	5.0				+		+	++			+	40
6b	40	5.0				+		+	++			+	40
7	12	7.0		+		+			+	+			40
8	11	6.7		+		+			+	+			40
9	24	5.7			+++	+			+			+	40
10	75	5.3	++		++		+++		+				40
11	75	5.3	++		++		+++		+				40
12	11	6.8				+			+			+	30
13	19	7.2	+++				+					+++	30
14	40	5.1						+	+++			+	30
15	24	6.0			+++	+						++	30
16	12	6.5				+			+	+			30
17	13	6.5				+			+	+			30
18	42	6.8				+			+				20
19	17	5.9				+						++	20
20	12	4.9				+			++				20
21	26	6.4	+++			+							20
22	23	6.1				++			+				20
23	25	5.3		+		+++							20
24	49	5.0			++	+							20
25	47	5.1						+				+	20
26	40	5.2						+				+	20
27	40	6.6								+++		+	20
28	37	6.3								+++		+	20
29	36	6.2								+++		+	20
30	35	5.5			+	++							20
31	12	5.3								+		++	20
32	12	5.3				+				+			20
33	26	6.4			+				++				20
34	40	5.9			+	+							20
35	29	4.6			+		+						20
36	23	6.5			++	+							20
37	26	6.6				+			+++				20

Molecular mass, pI, and relative intensity of immunostained spots in 2-dimensional blots with the 10 patients' sera (Fig 2). Spots detected with at least 2 of the patients' sera are numbered according to the frequency of detection in the 10 immunoblots (positive percentage).

MM, Molecular mass.

*Also stained with the secondary antibody alone.

gens. The interindividual variation of the antibody response could be due to the patients' predisposition or to variable exposure levels of single allergens. Weiss et al⁴ could show considerable cultivar-specific differences in IgE-binding patterns of wheat allergen fractions.

Another aspect of our investigation is the identification of 4 hitherto unknown wheat flour allergens similar to 2 isoforms of glyceraldehyde-3-phosphate dehydrogenases from *H vulgare*, triosephosphate isomerase from *H vulgare*, and serpin from *Triticum aestivum*. According to our immunoblotting results, these allergens belong to the most frequently detected ones. This seems to differ from the findings of other investigators, who predominantly found members of the α -amylase inhibitor family, a peroxidase homologue, or proteins

similar to acyl-CoA oxidase. However, taking into consideration the great interindividual variations in sensitization patterns, cultivar- and method-dependent results of the investigations performed in different European countries are not surprising.

In conclusion, our results demonstrate the interindividual variation of wheat flour sensitization in baker's asthma and the applicability of 2-dimensional electrophoresis with IgE immunoprobings and mass spectrometric fingerprinting to identify allergens. The role of the novel IgE-binding proteins has to be assessed when these allergens are isolated and purified for in vitro and in vivo studies.

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TABLE III. Profound search for mass fingerprint results

Spot No.	Protein description	Matched peptides	Coverage of protein sequence	Residue numbers of matched regions	MM and pI of matched proteins: Theoretical/found
3a	Glyceraldehyde-3-phosphate dehydrogenase, isoform I, cytosolic, <i>Hordeum vulgare</i> , P26517	14 (37)	40%	6-13, 49-57, 68-109, 203-217, 230-250, 263-271, 274-325	37/42 kd, 6.7/6.7
3b	Glyceraldehyde-3-phosphate dehydrogenase, isoform I, cytosolic, <i>Hordeum vulgare</i> , P26517	14 (62)	42%	6-13, 49-57, 68-109, 203-217, 230-250, 263-271, 274-331	37/42 kd, 6.7/6.9
3c	Glyceraldehyde-3-phosphate dehydrogenase, isoform I, cytosolic, <i>Hordeum vulgare</i> , P26517	13 (70)	42%	6-13, 68-109, 129-141, 203-217, 230-250, 263-271, 274-325	37/42 kd, 6.7/7.2
4a	Glyceraldehyde-3-phosphate dehydrogenase, isoform II, cytosolic, <i>Hordeum vulgare</i> , P08477	17 (41)	52%	17-25, 36-77, 97-108, 157-167, 171-185, 198-218, 230-241, 262-299	33/40 kd, 6.2/6.6
4b	Glyceraldehyde-3-phosphate dehydrogenase, isoform II, cytosolic, <i>Hordeum vulgare</i> , P08477	14 (47)	46%	17-25, 43-77, 97-108, 157-167, 171-185, 198-218, 262-299	33/40 kd, 6.2/6.9
5a	Triosephosphate isomerase, cytosolic, <i>Hordeum vulgare</i> , P34937	8 (24)	35%	5-12, 56-70, 114-135, 176-219	27/26 kd, 5.4/5.5
5b	Triosephosphate isomerase, cytosolic, <i>Hordeum vulgare</i> , P34937	9 (36)	41%	5-12, 56-70, 114-149, 175-219	27/26 kd, 5.4/5.8
6a	Serpin, <i>Triticum aestivum</i> , AJ245879	9 (20)	25%	11-18, 62-75, 125-137, 159-171, 182-191, 261-270, 289-300, 335-353	43/40 kd, 5.5/5.0
6b	Serpin, <i>Triticum aestivum</i> , AJ245879	13 (30)	39%	11-18, 62-98, 125-137, 159-191, 261-270, 289-300, 328-353, 379-396	43/40 kd, 5.5/5.0

REFERENCES

- Baur X, Posch A. Characterized allergens causing bakers' asthma. *Allergy* 1998;53:562-6.
- Sander I, Merget R, Leonhardt L, Chen Z, Raulf-Heimsoth M, Baur X. Prevalence of allergen-specific IgE in baker's asthma. *Allergy* 1999;54(Suppl 52):50-1.
- Walsh BJ, Wrigley CW, Musk AW, Baldo BA. A comparison of the binding of IgE in the sera of patients with bakers' asthma to soluble and insoluble wheat-grain proteins. *J Allergy Clin Immunol* 1985;76:23-8.
- Weiss W, Vogelmeier C, Görg A. Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis* 1993;14:805-16.
- Sandiford CP, Tatham AS, Fido R, Welch JA, Jones MG, Tee RD, et al. Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. *Clin Exp Allergy* 1997;27:1120-9.
- Maruyama N, Ichise K, Katsube T, Kishimoto T, Kawase S, Matsumura Y, et al. Identification of major wheat allergens by means of the *Escherichia coli* expression system. *Eur J Biochem* 1998;255:739-45.
- Posch A, Weiss W, Wheeler C, Dunn MJ, Görg A. Sequence analysis of wheat grain allergens separated by two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 1995;16:1115-9.
- Sánchez-Monge R, Gomez L, Barber D, Lopez-Otin C, Armentia A, Salcedo G. Wheat and barley allergens associated with baker's asthma. Glycosylated subunits of the alpha-amylase-inhibitor family have enhanced IgE-binding capacity. *Biochem J* 1992;281:401-5.
- Armentia A, Sánchez-Monge R, Gomez L, Barber D, Salcedo G. In vivo allergenic activities of eleven purified members of a major allergen family from wheat and barley flour. *Clin Exp Allergy* 1993;23:410-5.
- Fränken J, Stephan U, Meyer HE, König W. Identification of alpha-amylase inhibitor as a major allergen of wheat flour. *Int Arch Allergy Immunol* 1994;104:171-4.
- Amano M, Ogawa H, Kojima K, Kamidaira T, Suetsugu S, Yoshihama M, et al. Identification of the major allergens in wheat flour responsible for baker's asthma. *Biochem J* 1998;330:1229-34.
- Walsh BJ, Howden ME. A method for the detection of IgE binding sequences of allergens based on a modification of epitope mapping. *J Immunol Methods* 1989;121:275-80.
- Sanchez-Monge R, Garcia-Casado G, Lopez-Otin C, Armentia A, Salcedo G. Wheat flour peroxidase is a prominent allergen associated with baker's asthma. *Clin Exp Allergy* 1997;27:1130-7.
- Weiss W, Huber G, Engel KH, Pethran A, Dunn MJ, Gooley AA, et al. Identification and characterization of wheat grain albumin/globulin allergens. *Electrophoresis* 1997;18:826-33.
- National Asthma Education and Prevention Program Expert Panel Report 2. Guidelines for the Diagnosis and Management of Asthma. Bethesda (MD): National Institutes of Health; 1997. National Institutes of Health publication No. 97-4051.
- Ceska M, Lundkvist U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972;9:1021-30.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- Blum H, Beier H, Gross HJ. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* 1987;8:93-9.
- Eckerskorn C, Lottspeich F. Internal amino acid sequence analysis of proteins separated by gel electrophoresis after tryptic digestion in polyacrylamide matrix. *Chromatographia* 1989;28:92-4.
- Gevaert K, Verschelde JL, Puype M, Van Damme J, Goethals M, De Boeck S, et al. Structural analysis and identification of gel-purified proteins, available in the femtomole range, using a novel computer program for peptide sequence assignment, by matrix-assisted laser desorption ionization-reflectron time-of-flight mass spectrometry. *Electrophoresis* 1996;17:918-24.
- Blands J, Diamant B, Kallos P, Kallos-Deffner L, Lowenstein H. Flour allergy in bakers. I. Identification of allergenic fractions in flour and comparison of diagnostic methods. *Int Arch Allergy Appl Immunol* 1976;52:392-406.
- Sutton R, Skerritt JH, Baldo BA, Wrigley CW. The diversity of allergens involved in bakers' asthma. *Clin Allergy* 1984;14:93-107.