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Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity

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Summary

Background Several studies have investigated water/salt soluble proteins which comprise 50% of the proteins in wheat. The remaining 50% of wheat proteins, are water/salt insoluble proteins of which there is limited information on their role in cereal hypersensitivity.
Objectives To investigate the allergenicity of the water/salt insoluble gliadin and glutenin proteins (prolamins).
Methods RAST, electrophoresis and Western blotting were used to identify water/salt insoluble wheat allergens. Competitive RAST inhibition was conducted to investigate cross-reactivity between prolamins and water/salt soluble wheat proteins.
Results Specific IgE to α-gliadin and to total glutenins were detected in all sera. IgE to β-, γ-, fast ω-, and slow ω-gliadin were present in lower numbers of sera. Prolamin allergens of 90–11 kDa were identified by immunoblotting. Water/salt soluble proteins crossreacted with α-gliadin and total glutenins.
Conclusions Individuals who are hypersensitive to water/salt soluble wheat proteins produce specific IgE to water/salt insoluble wheat proteins. Western blotting has shown that gliadins, glutenins and proteins with similar molecular weights as the endogenous water/salt soluble wheat enzyme inhibitors are important allergens. Alpha and fast ω- are the most allergenic gliadins. The water/salt insoluble proteins share cross-reacting epitopes with water/salt soluble proteins. These data show that the numbers of proteins involved in the development of cereal hypersensitivity is greater than previously believed and that the development of specific IgE to α-gliadin may in part depend on the presence of cross-reacting antibodies to water/salt soluble flour allergens.

Keywords: cereal hypersensitivity, wheat flour, albumins, globulins, gliadins, glutenins, α-amylase inhibitors

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Introduction

Bakers' asthma [1–3] is an occupational hypersensitivity disease primarily caused by the inhalation of flour proteins, mainly derived from wheat but also from rye, barley or soya [4,5]. Wheat and other cereals are composed of four classes of proteins; the water/salt soluble albumins and globulins and the water/salt insoluble gliadins and glutenins which together are known as prolamins [6]. The role of water/salt soluble allergens, which comprise 50% of the proteins in wheat flour, in cereal hypersensitivity has been examined in several studies which have identified major allergens within the albumin and globulin classes of proteins. These proteins are associated with the development of cereal hypersensitivity in those exposed in an occupational setting [7–9] and also in those with atopic dermatitis [10].

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While there is a large literature on the role of water/salt soluble proteins in cereal hypersensitivity, there is more limited data on the water/salt insoluble proteins and their role in the induction of cereal hypersensitivity. Studies by Walsh [11] and Weiss [12] using limited numbers of sera, have indicated that the water/salt insoluble gliadin and glutenin proteins of wheat, are capable of inducing specific IgE. A study by Donovan *et al.* [13] which used sera from individuals with rye grass hypersensitivity has shown that the sera of these patients contained crossreacting IgE to both the water/salt soluble proteins of rye grass pollen and to water/salt insoluble proteins of wheat.

It is not known which of the different classes of water/salt insoluble proteins are the most important in the development of cereal hypersensitivity and whether these proteins share crossreacting epitopes with water/salt soluble wheat proteins.

We have investigated the role of purified water/salt insoluble gliadins and glutenins in wheat flour induced cereal hypersensitivity. RAST has been used to measure the IgE responses to the five different classes of gliadins and to total glutenins. Western blotting has been used to determine the molecular weights of the non-water/salt soluble proteins and to identify the most important water/salt insoluble allergens involved wheat hypersensitivity.

Competitive RAST inhibition has been used to determine whether water/salt insoluble cereal proteins (prolamins) share crossreacting epitopes with water/salt soluble proteins including the enzyme inhibitors which have previously been identified as major water/salt soluble allergens [13–15].

Materials and methods

Patient selection

Sera were obtained from 24 individuals who were diagnosed as having bakers' asthma and who were known from previous studies to have specific IgE to water/salt soluble proteins [9].

Extraction of water/salt soluble wheat flour proteins

Wheat flour (cv. Chinese Spring) (100 g) was defatted by stirring for 1 h at room temperature with 500 mL chloroform, filtered and the extraction repeated. The defatted flour was then air dried. Albumins and globulins were extracted by stirring the flour for 1 h with 1 L of 0.5 m NaCl at room temperature, centrifuged for 15 min at 15 000 ***g*** and the extraction then repeated. The pooled supernatants were retained and freeze-dried. The absence of gliadin and glutenin proteins from the albumin/globulin preparation was confirmed by probing an immunoblot of the water/salt soluble proteins using monoclonal antibodies to the different prolamins [16,17].

Purification of gliadins

The pelleted fraction obtained from the extraction of the albumin and globulin proteins, contained the gliadin and glutenin proteins. This fraction was resuspended in water and stirred for 1 h at room temperature to remove any residual salt and centrifuged for 10 min at 15 000 ***g***. The pellet fraction was stirred for 1 h with 1 L of 70% (v/v) aqueous ethanol at 4°C and centrifuged. This was repeated and the supernatants containing the gliadins pooled and dialysed against 1% (v/v) acetic acid for 60 h prior to being freeze-dried.

The gliadins were dissolved in 6 m urea, 1% (v/v) acetic acid and separated from low molecular weight glutenin subunits by size exclusion chromatography using a Sephacryl S300 column (2.5 × 60 cm) (Pharmacia Biotech UK). The glutenin proteins were extracted from the undissolved material as described below. The gliadin and glutenin peaks were dialysed against 1% (v/v) acetic acid for 48 h and freeze-dried. The gliadins were separated into α-, β-, γ-, fast ω- and slow ω-gliadins by ion-exchange chromatography using a carboxymethyl cellulose column (5 × 20 cm) (Pharmacia Biotech, UK) in 10 mm glycine-acetate buffer pH 4.6, containing 3 m urea and eluted with a linear gradient of 0–0.25 m NaCl. Gliadin peaks were dialysed against 1% (v/v) acetic acid for 60 h prior to being freeze-dried [16,17].

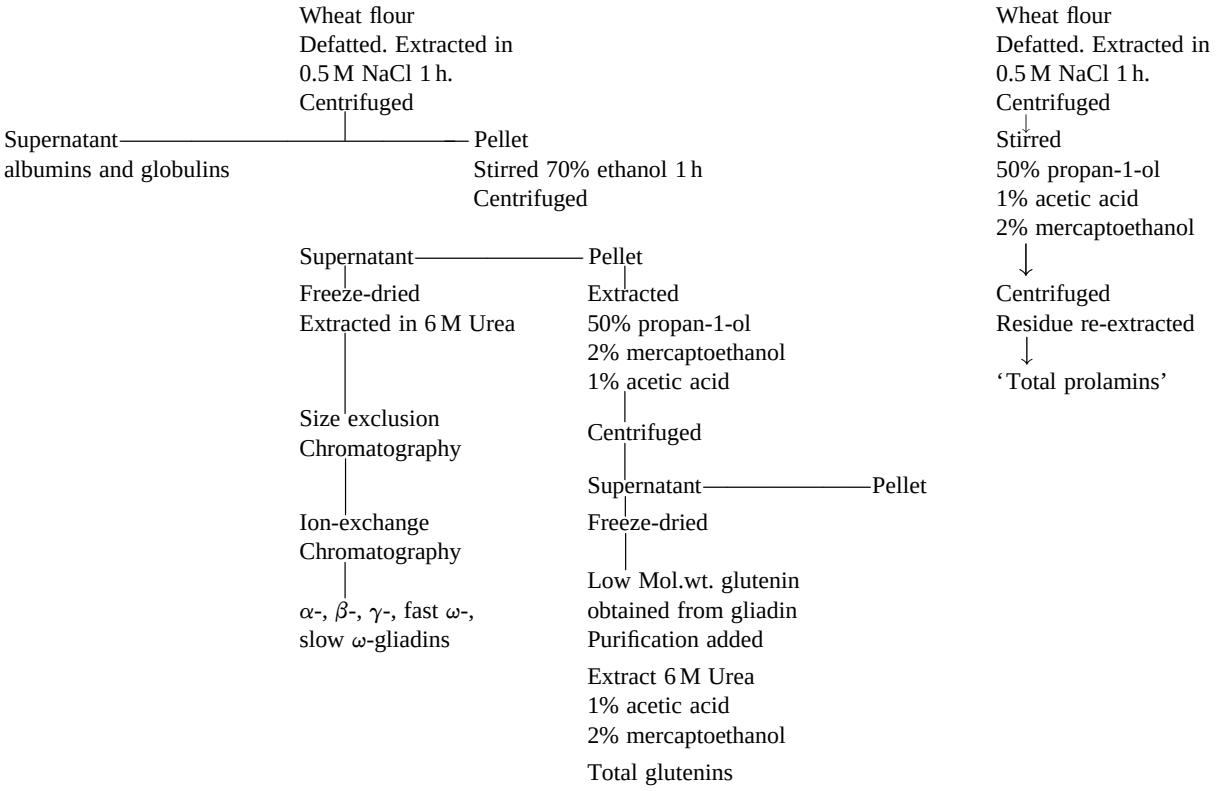
Preparation of total glutenins

The flour residue remaining from the extraction of the gliadin proteins was stirred with 50% (v/v) aqueous propan-1-ol, 1% (v/v) acetic acid and 2% (v/v) 2-mercaptoethanol for 1 h at room temperature and centrifuged at 15 000 ***g*** for 15 min. The extraction was repeated. The supernatants were pooled and dialysed against running water for 12 h and then against 1% (v/v) acetic acid for 40 h prior to being freeze-dried. The low molecular weight glutenin subunits obtained from the purification of the gliadins and the glutenin fraction were dissolved in 6 m urea, 1% (v/v) acetic acid and 2% (v/v) 2-mercaptoethanol to ensure complete mixing and dialysed against 1% (v/v) acetic acid for 60 h [16].

Preparation of total prolamins

Wheat flour was defatted and salt extracted and the supernatant then stirred for 1 h at room temperature with 50% (v/v) aqueous propan-1-ol, 1% (v/v) acetic acid and 2% (v/v) 2-mercaptoethanol, centrifuged for 10 min at 15 000 ***g*** and the residue re-extracted. The supernatants were pooled and dialysed against 1% (v/v) acetic acid. Table 1 briefly shows a scheme indicating the preparation of the different protein fractions [16,17].

Table 1. Flowchart indicating method of preparing different prolamin fractions [16,17]



Electrophoresis

The purity of the α-, β-, γ-, and ω-gliadin fractions was determined by acid PAGE which divided the gliadins into their different families [18,19]. The total gliadins and glutenins were analysed by SDS-PAGE on 12.5% acrylamide gel, using a modified Laemmli system which gave the location of the different prolamins [18,19].

Specific IgE and total measurements

RAST was undertaken using nitrocellulose discs to which the allergen had been coupled using a modification of the method of the nitrocellulose polydisc method of Walsh *et al.* [20,21]. The water/salt soluble Chinese Spring wheat proteins were coupled to 0.2 μm nitrocellulose discs (Schleicher and Schuell, Germany) by shaking gently overnight at 4°C in PBS at a concentration of 1 mg ml^{-1}. The remaining protein binding sites on the discs were blocked by three 1 h incubations in PBS with RAST washing substance (Pharmacia and Upjohn UK) which contains detergent [21] at 20°C.

The five different gliadin fractions: α-, β-, γ-, fast ω- and slow ω- were dissolved at a concentration of 1 mg ml^{-1} in 50% (v/v) propanol, 1% (v/v) acetic acid. The gliadins were coupled to 0.2 μm nitrocellulose discs by shaking gently overnight at 4°C. The unused protein binding sites were blocked by incubating the discs with RAST washing buffer (Pharmacia and Upjohn UK) at room temperature. The total glutenins were dissolved in 50% (v/v) propan-1-ol, 1% (v/v) acetic acid, 2% (v/v) 2-mercaptoethanol and coupled to nitrocellulose discs as described above [20,21].

The levels of specific IgE to each allergen in the 24 sera were measured by RAST. In the absence of known positive and negative sera for each allergen, cord sera were used to determine the level of nonspecific IgE binding to the different allergens. The levels of total IgE were measured using total IgE CAPS (Pharmacia and Upjohn) according to the manufacturers' instructions.

Western blotting

The individual water/salt insoluble proteins were separated by SDS-PAGE using a 12.5% acrylamide gel and were then transferred to nitrocellulose membranes (Bio-Rad, UK) using the method of Towbin *et al.* [22]. The membranes

were cut into 0.5 cm wide strips and probed with fresh aliquots of 22 sera for 16 h at room temperature with shaking. Sera with a radioisotope uptake $\geq 2\%$ to α-gliadin were used, as this level of isotope uptake would allow the molecular weights of allergens to be determined with greater accuracy. A strip was incubated with cord sera as a negative control. The strips were given three 10 min washes with PBS-RAST wash (Pharmacia and Upjohn UK) and were probed overnight with ^{125}I anti-IgE (Pharmacia and Upjohn UK) diluted 1:4 with PBS for 16 h at room temperature. The strips were given three further washes, dried and exposed to Hyperfilm MP (Amersham UK) at -70°C for 48 h. The autoradiographs were over-exposed to enable visualization of proteins that had weakly bound IgE [9].

Inhibition of IgE binding to prolamins using water/salt soluble proteins

Competitive RAST inhibition was conducted as previously described [9]. Briefly semipurified water/salt soluble albumin/globulin proteins were used to inhibit the binding of IgE to the different gliadins and the total glutenins. Each inhibition was conducted using pooled sera with optimal binding for the allergen under study [21]. Inhibition of IgE binding to the five different gliadins and total glutenins was carried out using fourfold dilutions of the inhibiting albumin/globulin proteins (with values from 2000 mg ml^{-1}–0.015 mg ml^{-1}) in phosphate buffered saline (PBS). Self-inhibitions of the different water/salt insoluble proteins and inhibition of IgE binding to the water/salt soluble albumin/globulin by the prolamins, could not be conducted due to the prolamin solubilization buffer causing a large degree of interference in the assay.

Inhibition of IgE binding to α-gliadin using purified 0.19 wheat α-amylase inhibitor

Inhibition of IgE binding to α-gliadin was carried out using purified 0.19 wheat α-amylase inhibitor. Inhibition was undertaken using doubling dilutions (with values from 10 mg mL^{-1}–1 mg mL^{-1}) in PBS as above.

Results

Electrophoresis

Figure 1(a) shows the SDS-PAGE profiles of the water/salt insoluble prolamin and albumin/globulin fractions of wheat. Total prolamins consist of a heterogeneous mixture of proteins of a molecular weight 90–30 kDa (track 2). The albumin/globulin fraction contains proteins of lower molecular weight than those of the prolamin fraction by $\approx$15 kDa

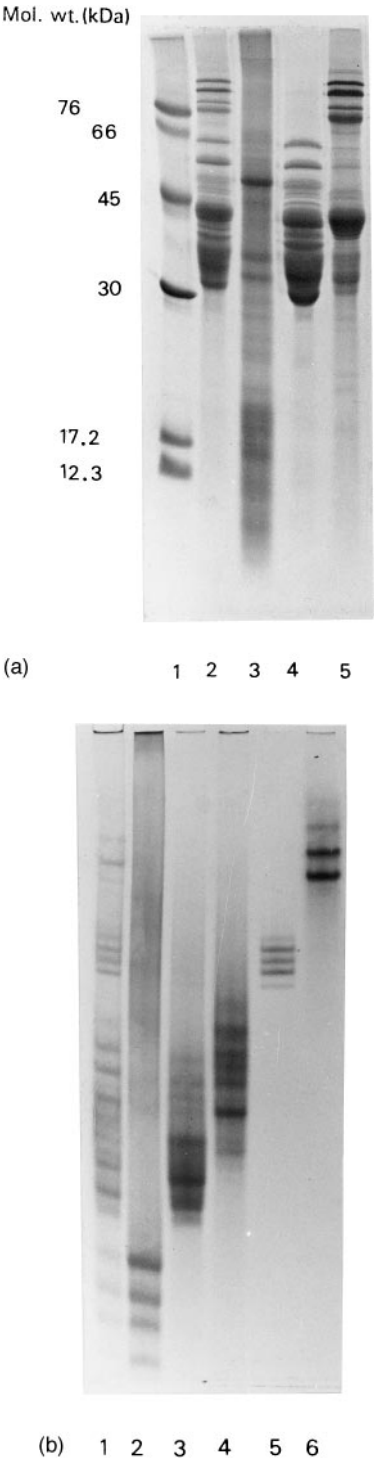


Fig. 1. (a) SDS-PAGE gel; track 1, Mol.wt markers in order of decreasing weight 76, 66, 45,. 30, 17.2 and 12.3 kDa; track 2, total prolamins; track 3, water/salt soluble albumins and globulins; track 4, total gliadins; track 5, total glutenins; (b) Acid-PAGE gel of gliadin fractions; track 1, total gliadins; track 2, α-gliadin; track 3, β-gliadin; track 4, γ-gliadin; track 5, fast ω-gliadin, track 6, slow ω-gliadin.

Table 2. Results of RAST analysis

Sera	Total IgE (kU/L)	Percentage anti-IgE ^{125}I-radioisotope uptake						
		Albumins/globulins	α-gliadin	β-gliadin	γ-gliadin	Fast ω-gliadin	Slow ω-gliadin	Total glutenins
1	533	38.00	5.63	1.16	0.99	2.09	1.07	7.97
2	650	22.68	4.52	1.12	1.28	1.75	1.11	2.76
3	404	12.84	25.88	2.04	0.70	6.28	0.97	11.86
4	725	39.57	33.28	6.72	4.53	15.80	7.62	31.26
5	270	29.76	6.72	1.67	0.75	3.34	0.55	8.76
6	7524	7.57	9.23	7.34	5.84	9.54	5.69	6.27
7	875	1.17	1.37	0.88	0.75	0.85	0.76	0.76
8	635	48.20	31.75	3.22	1.32	12.62	2.49	24.08
9	350	28.59	4.06	0.85	0.51	0.94	0.55	6.12
10	450	25.21	1.98	1.01	0.81	1.09	0.80	1.70
11	275	17.43	3.40	0.94	0.67	1.38	0.93	5.55
12	100	6.16	7.26	0.73	0.38	1.95	0.60	2.69
13	150	5.74	7.82	0.59	0.34	1.25	0.31	3.09
14	750	6.34	2.98	1.54	1.65	1.60	1.40	1.42
15	3239	25.29	30.81	19.08	18.94	30.29	13.18	29.23
16	5070	17.55	9.51	12.12	9.45	8.51	4.40	7.68
17	612	3.31	1.25	0.44	0.36	0.70	0.43	1.02
18	361	39.83	25.73	6.39	5.08	12.15	4.99	20.83
19	4058	45.14	51.82	29.77	23.44	41.41	7.76	10.41
20	712	18.55	5.83	1.27	0.86	3.78	6.56	4.80
21	733	26.21	12.35	1.44	0.84	2.65	1.06	4.81
22	3250	29.15	11.55	3.76	2.64	9.04	3.76	30.59
23	750	1.80	1.86	1.14	1.10	1.26	1.02	1.43
24	1000	11.27	6.34	2.34	2.28	2.79	2.26	7.34

(track 3). The total gliadins have molecular weights between 65 and 30 kDa, with the α-, β- and γ -gliadin having molecular weights of 45–30 kDa and the ω-gliadins 65–45 kDa (track 4). Total glutenins, comprising high molecular weight subunits 90–75 kDa and low molecular subunits of gluten 45–30 kDa, are shown in track 5. Figure 1(b) shows the acid-PAGE separation of individually purified members of the α-, β-, γ-, fast ω- and slow ω-gliadins.

Table 3. Correlation analysis of specific IgE RAST data

	α-gliadin	β-gliadin	γ-gliadin	Fast ω gliadin	Slow ω gliadin	Total glutenins	Total IgE
Chinese spring (water/salt soluble proteins)	0.624▲	0.535◇	0.438	0.636▲	0.465	0.771*	−0.01
α-gliadin		0.750*	0.538◇	0.895*	0.611▲	0.846*	0.202
β-gliadin			0.907*	0.917*	0.862*	0.743*	0.641
γ-gliadin				0.774*	0.915	0.528▲	0.769
Fast ω-gliadin					0.839*	0.843*	0.414
Slow ω-gliadin						0.574▲	0.680
Total glutenins							0.125

* $P = 0.0001$
▲ $P \leq 0.005$
◇ $P \leq 0.01$

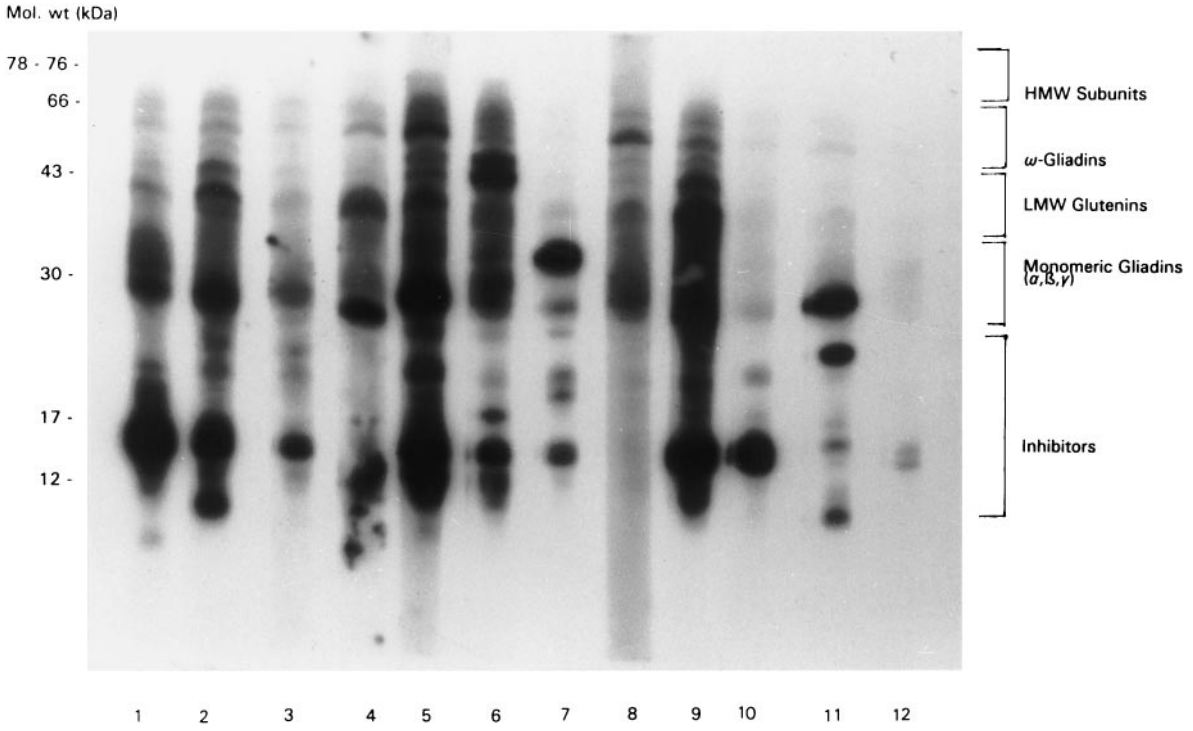


Fig. 2. Representative Western blot of 'total prolamin' allergens (12 sera).

Specific and total IgE measurements

Table 2 shows the results of the RAST analysis of IgE binding to the different allergens and to total IgE. All sera used in this study had specific IgE to the water/salt soluble proteins (binding $\geq 1\%$, three times the level of binding of the cord sera). All sera had specific IgE antibodies binding to α-gliadin, 21 of 24 sera (88%) had specific IgE to fast ω-gliadin, 18 of 24 sera (75%) had specific IgE to β-gliadin, 15 of 24 sera (63%) had specific IgE to slow ω-gliadin and 12 of 24 (50%) sera had specific IgE to γ-gliadin. A moderate correlation was observed between the levels of specific IgE to water/salt soluble proteins with α-gliadin $r = 0.624$ ($P = 0.0011$) and fast ω-gliadin $r = 0.636$ ($P = 0.0008$) and between specific IgE to water/salt soluble proteins and β-gliadin $r = 0.535$ ($P = 0.07$). Weaker (non-significant) correlations were observed between specific IgE to water/salt proteins and the other gliadins. Twenty-three of the 24 sera (96%) had specific IgE to the total glutenins. A moderate correlation of 0.771 ($P = 0.0001$) was observed between the levels of specific IgE to water/salt soluble proteins and total glutenins.

The levels of total IgE varied between 100 and 7524 kU/L. Correlations of 0.641 ($P = 0.0007$), 0.769 ($P = 0.0001$) and 0.68 ($P = 0.0003$) were observed between the levels of total IgE and the levels of specific IgE to β-, γ- and slow ω-gliadin. The results of the correlation analysis are given in Table 3.

Western blotting

Figure 2 shows the results of immunoblotting the total prolamins (gliadins and glutenins) of wheat using 12 different sera and is representative of the data obtained from Western blotting using 22 sera. A large number of allergens were detected by the different sera with molecular weights between 90 and 11 kDa. Strong IgE binding was specifically seen with the total glutenins, α-, β- and γ-gliadins and proteins with molecular weights similar to those of water/salt soluble wheat enzyme inhibitors. No IgE binding was observed to the prolamins by cord sera. Figure 3 shows an allergogram giving the rate of detection of the different prolamin allergens. Proteins of 29, 15 and 14 kDa were detected by 13 (59.09%), 11 (50%) and 12 (54.54%) of the 22 sera, respectively. Proteins of 79, 76 and 21 kDa were detected by 10 (45.45%) sera with a protein of 66 kDa detected by nine (40.90%) sera.

Inhibition of IgE binding to prolamins using water/salt soluble proteins

Water/salt soluble wheat flour albumin/globulin proteins significantly inhibited (80% at 1.5 mg ml^{-1}) the IgE binding to the water/salt insoluble α-gliadin (Fig. 4). Binding of specific IgE to γ-gliadin was also inhibited to a lesser extent (35% at 1 mg mL^{-1}) by the water/salt soluble proteins (Fig. 5). The albumins/globulins did not inhibit IgE-binding to either

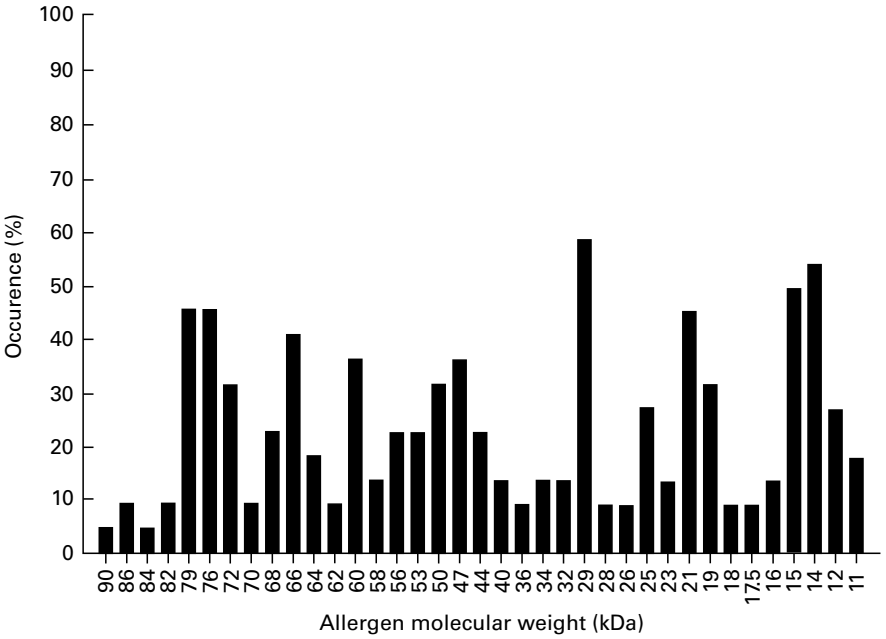


Fig. 3. Allergogram of 'total prolamin' allergens (22 sera).

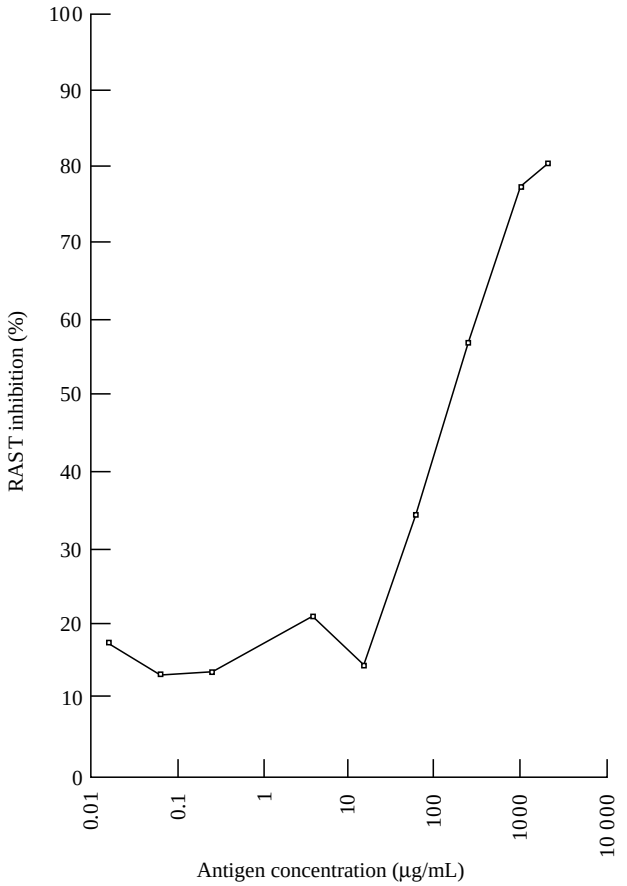


Fig. 4. Competitive RAST inhibition of IgE binding to α-gliadin by water/salt soluble. albumins/globulins.

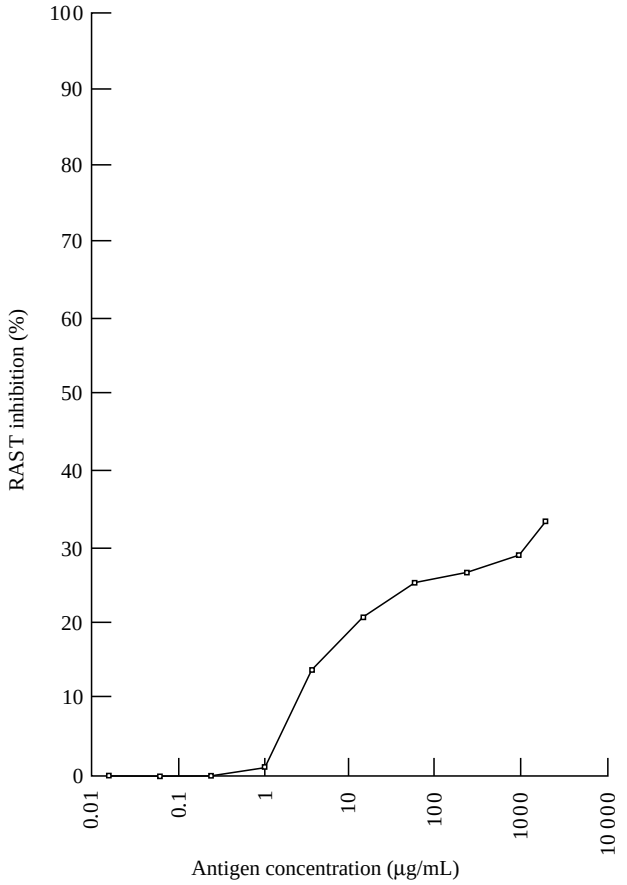


Fig. 5. Competitive RAST inhibition of IgE binding to γ-gliadin by water/salt soluble. albumins/globulins.

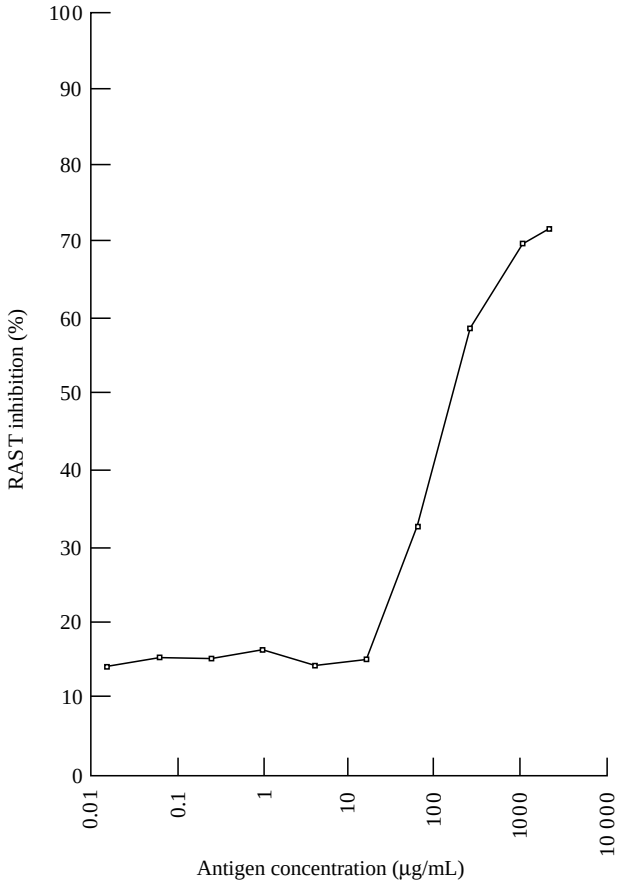


Fig. 6. Competitive RAST inhibition of IgE binding to total glutenins by water/salt soluble albumins/globulins.

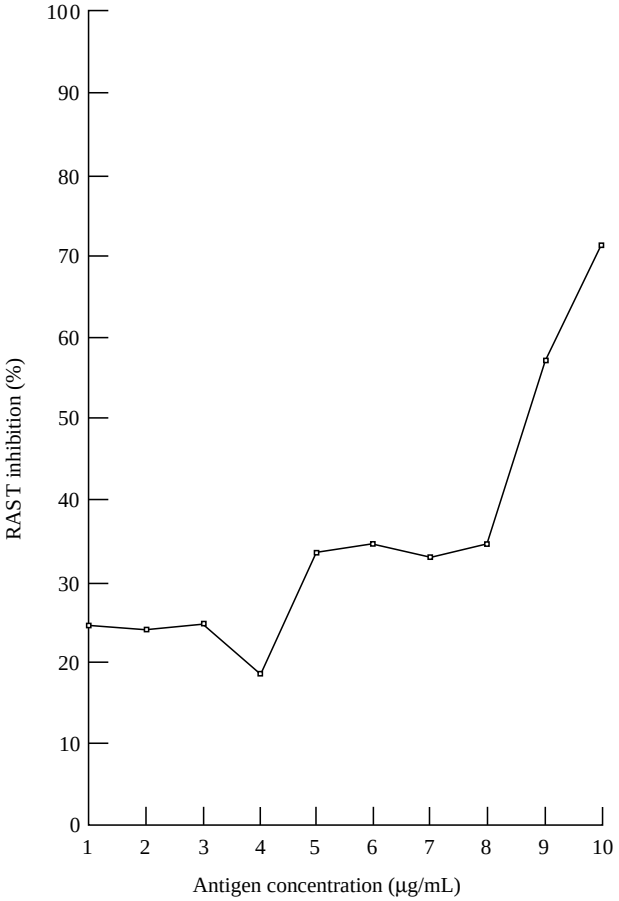


Fig. 7. Competitive RAST inhibition of IgE binding to α-gliadin by purified water/salt soluble 0.19 wheat α-amylase inhibitor.

β-, fast ω-or the slow ω-gliadin. Figure 6 shows that the albumin/globulin proteins significantly inhibited IgE binding to the total glutenins (73% at 1.5 mg ml^{-1}).

Inhibition of IgE binding to a-gliadin using purified 0.19 wheat α-amylase inhibitor

Binding of specific IgE to α-gliadin was significantly inhibited by the purified water/salt soluble 0.19 wheat α-amylase inhibitor (Fig. 7).

Discussion

Cereal hypersensitivity in both the occupational and non-occupational setting, has previously been shown to be induced by the water/salt soluble proteins of wheat and other flours [4,5,6,9,14]. Studies by Walsh *et al.* [11] using sera primarily from individuals with rye grass hypersensitivity and by Weiss *et al.* [12] using four sera from individuals with bakers' asthma, have suggested that the water/salt insoluble gliadins and glutenins (prolamins) of wheat may be allergens. In this study we have identified the proteins involved in the induction of specific IgE to water/salt insoluble prolamins using sera from individuals who have previously been diagnosed as suffering from occupational cereal hypersensitivity due to exposure to flour by inhalation [9].

Electrophoresis of the total prolamins has shown that there are a large number of water/salt insoluble proteins with a similar molecular weight range to that observed for the water/salt soluble albumin/globulin proteins. Acid-PAGE has shown that several proteins exist within each of the different gliadin families and that they are distinct from members of the other families of prolamins. The data obtained has shown that the prolamins are considerably more complex in nature than the water/salt soluble albumin/globulin proteins.

RAST has shown that α- and fast ω-gliadin are the most allergenic members of the gliadin family of proteins. The linear correlation data show that the α- and fast ω-gliadins and the total glutenins appear to have a closer allergenic relationship to the water/salt soluble wheat allergens than to the other gliadin proteins. These data suggest that the

different prolamins may share regions of amino acid sequence homology with each other and with some of the water/salt soluble albumin/globulin proteins [23].

Alpha, β-, γ-, fast ω- and slow ω-gliadin were detected by Western blotting using sera from individuals with Baker's asthma. In contrast to the study of Weiss *et al.* [12], we detected IgE binding to both fast ω- and slow ω-gliadins. This may be due to our use of a greater number of sera. Low molecular weight glutenin subunits were identified. The detection of these subunits is of interest as glutenins usually occur in wheat as polymers with molecular weights of up to 1000 kDa held together by disulphide bonds [24,25]. The detection of glutenin subunits suggests that either the polymers are capable of stimulating an immune response in their native state, or that the subunits share common epitopes either with members of the gliadin families and/or with water/salt soluble proteins. Water/salt insoluble proteins (21–10 kDa), which have molecular weights similar to chloroform/methanol soluble inhibitors of wheat α-amylase were detected by immunoblotting [23]. This finding is of major interest as these enzyme inhibitors have been shown to have amino acid sequence homology with several water/salt soluble wheat α-amylase/trypsin inhibitors which have previously been identified as major water/salt soluble allergens involved in cereal hypersensitivity [6,8,26].

Competitive RAST inhibition using albumin/globulin proteins (which include the water/salt soluble enzyme inhibitors) showed that water/salt soluble proteins and α-gliadin share a large number of crossreacting epitopes. Inhibition of γ-gliadin indicated that this protein shared a smaller number of epitopes that crossreacted with albumins/globulins. The minimal inhibition of IgE binding to β-, fast ω- and slow ω-gliadin indicates that these proteins contain either no or low numbers of epitopes that crossreact with water/salt soluble proteins.

Inhibition of the total glutenins by the albumins/globulins has shown that the glutenins contain epitopes that crossreact with those present in water/salt soluble proteins. It is, however, uncertain to what extent the production of specific IgE to the different glutenins is stimulated by epitopes that crossreact with those present in the different gliadins or with those present in water/salt soluble albumins/globulins.

The purified 24 kDa 0.19 wheat α-amylase/trypsin inhibitor significantly inhibited IgE binding to α-gliadin, indicating that α-gliadin shares crossreacting epitopes with a major water/salt soluble allergen [14,15,26]. The ability of the 0.19 inhibitor to inhibit IgE-binding is of interest as the 0.19 inhibitor shares amino acid sequence homology with the monomeric 12 kDa 0.28 and the dimeric 24 kDa 0.53 wheat α-amylase/trypsin inhibitors, which constitute a large proportion of the native and polymeric water/salt soluble proteins of wheat [27,28]. These data indicate that wheat α-amylase/trypsin inhibitors may play a major role in the development of cereal hypersensitivity as they occur as water/salt soluble, chloroform/methanol soluble and water/salt insoluble proteins, and that they may play a large role in the development of hypersensitivity to α-gliadin due to the presence of crossreacting epitopes.

Our results show that water/salt insoluble proteins stimulate cereal hypersensitivity in subjects exposed to wheat flour. The data obtained from RAST and competitive RAST inhibition indicate that the water/salt insoluble prolamins stimulate the production of at least two populations of IgE molecules. A proportion of the IgE produced may be directed towards domains, common between the different gliadins and glutenins and located in the proline rich repetitive amino acid sequence regions of the prolamins [25]. These domains do not share amino acid sequence homology with water/salt soluble proteins. There is also a population of IgE antibodies which may bind to domains located in the non-repetitive regions of the prolamins and which share amino acid sequence homology with water/salt soluble albumins/globulins, and in particular enzyme inhibitors, and which appear to be present in the α- and fast ω-gliadins and also in the glutenin fraction of wheat.

The data we have presented show that the water/salt insoluble protein fraction of wheat contains a large number of allergens with molecular weights ranging from 90 to 11 kDa. The α- and fast ω- are the most immunoreactive gliadins, with the total glutenins also being highly immunoreactive. The development of specific IgE to α-gliadin and total glutenins is moderately associated with the development of specific IgE to water/salt soluble wheat allergens and may be due to the presence of crossreacting epitopes in the prolamins and the water/salt soluble albumins/globulins and in particular, enzyme inhibitors. The development of specific IgE to the majority of gliadins is independent of both the levels of total IgE and the development of specific IgE to water/salt soluble proteins suggesting that two or more populations of IgE may be involved hypersensitivity to cereal prolamins; those which bind to epitopes homologous to those found in water/salt soluble proteins, and those which bind to the proline rich repetitive domains of prolamins [25]. These data have shown that water/salt insoluble allergens may play as large a role in the development of specific IgE and cereal hypersensitivity as the previously studied water/salt soluble wheat allergens.

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