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Allergies to Wheat, Yeast and Royal Jelly: A Connection between Ingestion and Inhalation?

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Introduction

Identification of food allergens and their elimination from the diet remains the main objective in successful therapy but the need for allergen identification may extend beyond implication of a single food or even a single allergen source. For example, a subject allergic to wheat proteins may also react to other cereals such as rye, barley and oats but such allergenic cross-reactivity may not be restricted to anatomically similarly located proteins (such as grains in cereals) or even to taxonomically closely related species. There have been several reports of subjects who are hypersensitive to pollens exhibiting reactivity to a number of unrelated foods [see references in 1] and it has been suggested that, in some subjects, seasonal pollinosis is likely to be exacerbated by a diet of wheaten products which may provide continuous sensitization during the remainder of the year [1].

Given that a connection between allergen ingestion and inhalation probably exists in some food allergies, the question of allergenic cross-reactivity between taxonomically similar and dissimilar proteins arises. Progress in predicting and understanding such cross-reactivities at the serological and immunochemical levels depends on a knowledge of the composition and complexity of the allergen sources involved. With this in mind, three complex but quite different protein extracts ingested by man have been selected to illustrate the difficulties often involved in relating clinical findings to the identification, characterization and source of the sensitizing polypeptides.

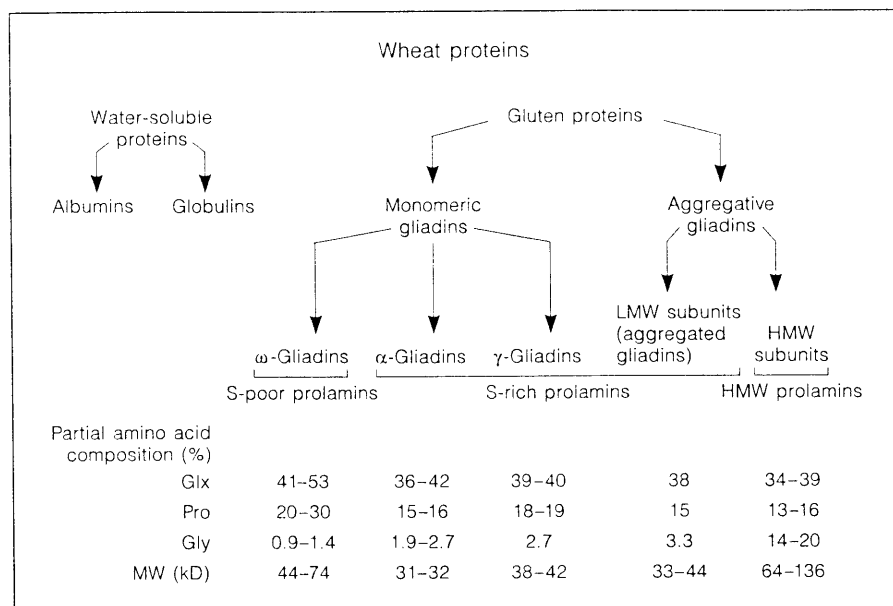


Fig. 1. Main groups of wheat proteins and some important physicochemical characteristics of the gluten proteins [adapted from 2].

Wheat

The complexity of the protein composition of wheat flour should not be underestimated but recent advances defining molecular relationships between wheat gluten proteins has led to a reassessment of the classification and nomenclature of these proteins [2]. Figure 1 summarizes the main groups of wheat proteins and lists some important characteristics of the gluten proteins. Although the albumins and globulins have been shown to be important inhalant allergens [3], physicochemical information on these proteins is still lacking. Wheat gliadins contain mainly single polypeptide chains that associate by H-bonding and hydrophobic interactions. Only three amino acids, glutamine, proline and phenylalanine, make up about 80% of the total amino acids in the ω -gliadins. The α -, β - and γ -gliadins have less of these amino acids but contain significant amounts of the S-containing amino acids cysteine and methionine which are at low levels or lacking in the ω -gliadins. Glutenin is made up of polymers of MWs extending into millions. SDS-PAGE of reduced glutenin produces two groups of separated proteins, the HMW and LMW subunits. The latter have amino acid composi-

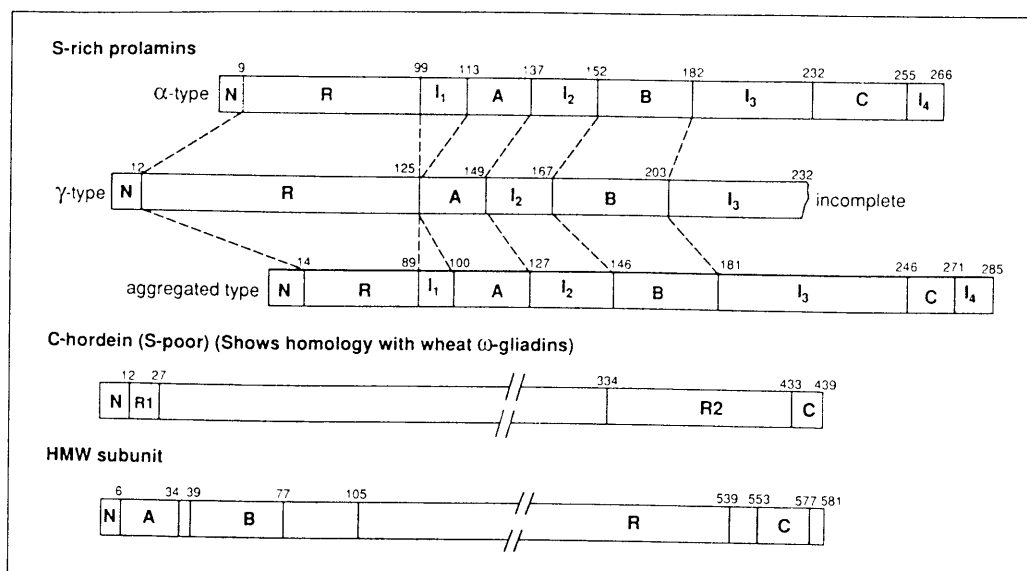


Fig. 2. Comparison of the structures of the S-rich and HMW prolamins of wheat and S-poor C-hordein of barley which shows homology with wheat ω -gliadins. N, N-terminal domains; R, repeated sequences; A, B and C, three homologous subdomains of the proline-poor C-terminal domain; I₁–I₄, four unique intermediate regions of the proline-poor C-terminal domain. The HMW subunits have regions homologous with A, B and C of the S-rich prolamins [reproduced from 2, with permission].

tions similar to the α -, β - and γ -gliadins while the HMW subunits differ from the gliadins in their high content of glycine and low content of proline.

Extensive amino acid sequences are now available from a number of S-rich prolamins and HMW subunits of glutenin. Sequence comparisons between α -, γ - and aggregated gliadins and HMW subunit and C-hordein of barley, selected because of homology with wheat gliadins, reveal regions of homology. The basic structural organization of these proteins is depicted in figure 2. The S-rich prolamins have two distinct structural domains, a small, unique N-terminal region (N) and a domain of proline-rich repeats (R). Repeat motifs present in the R domains of the different prolamins and the HMW subunits are shown in table 1. Three conserved subdomains, A, B and C, separated by divergent intervening regions I₁, I₂, I₃ and I₄ have been recognized in the nonrepetitive domains of S-rich prolamins [2]. The C-hordeins of barley (homologous with wheat ω -gliadins) have an extensive region of repeats with two types of repeat motifs (table 1). Both peptides feature the ProGlnGlnProX motif which is present in the repetitive domains of S-rich prolamins and this indicates an evolutionary

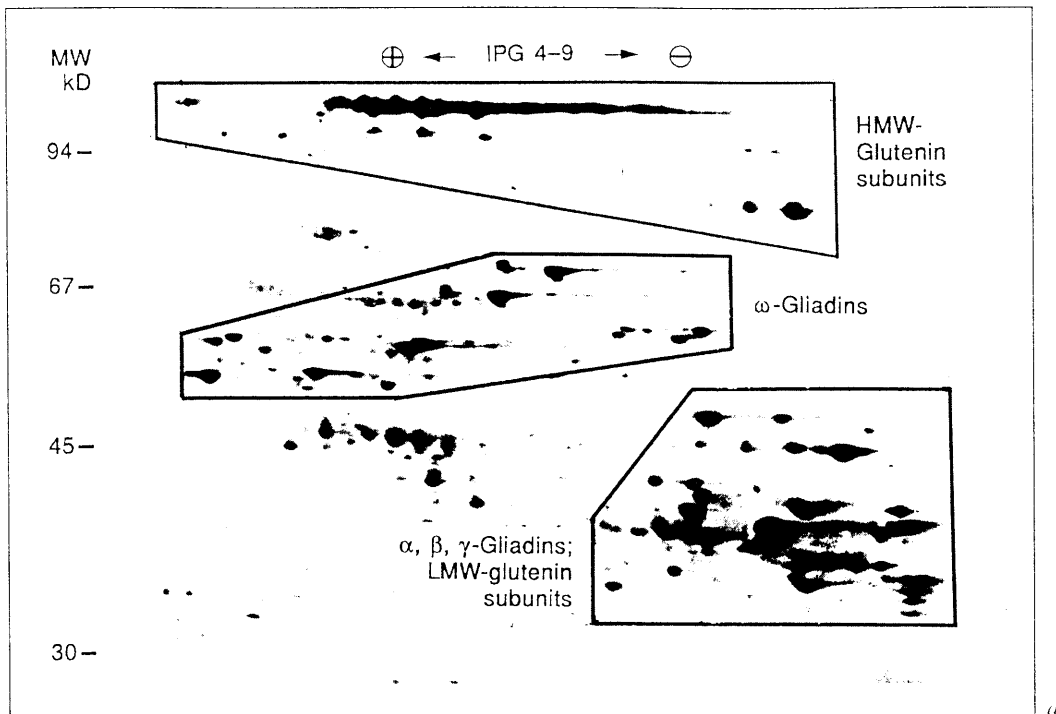
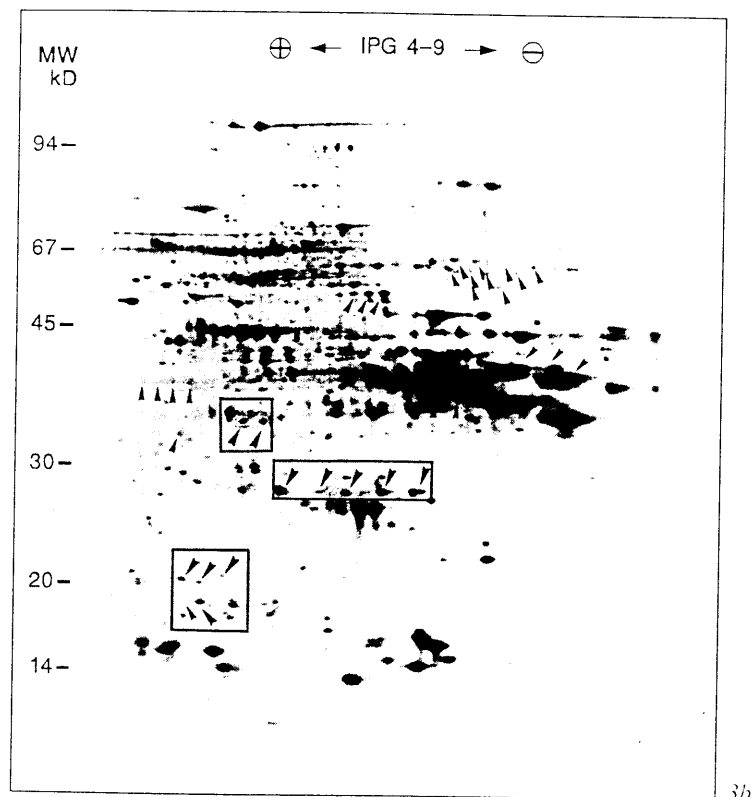


Fig. 3. *a* 2-D electrophoretic separation of wheat gliadin and glutenin proteins. *b* Location of wheat allergens in a 2-D map. The IgE antibody-binding proteins (allergens) are labelled with arrows [reproduced from 6, with permission].

Table 1. Consensus repeat motifs present in the repetitive domains of S-rich and HMW prolamins of wheat and S-poor hordein of barley (shows homology with wheat ω -gliadins) [modified and reproduced from 2, with permission]

S-rich prolamins	
α -type	Pro Gln Gln Gln Pro (Phe Pro) + Pro Gln Gln Pro Tyr
γ -type	Pro Gln Gln Pro Phe Pro Gln (Gln)
Aggregated type	Pro Gln Gln Pro Pro Phe Ser + (Gln) Gln Gln Gln Gln (Ile/Val) Leu
C-hordein (S-poor)	Pro Gln Gln Pro Tyr + Pro Gln Gln Pro Phe Pro Gln Gln
HMW subunits	Pro Gly Gln Gly Gln Gln + Gly Tyr Tyr Pro Thr Ser Leu Gln Gln

relationship between S-rich and S-poor prolamins. Repeat motifs in the HMW subunits of glutenin are not related to the repeat motifs of the S-rich and S-poor prolamins but, overall, there is evidence that the HMW subunits are structurally and evolutionarily related to the S-rich, and therefore, also the S-poor prolamins.



Wheat Proteins as Allergens

Early studies generally showed that the water-soluble proteins are the most relevant to IgE-mediated food allergy but later modifications of the testing procedure made it possible to test the gluten proteins for allergenicity [3, 4]. Gluten proteins were shown to be at least as allergenic as albumins and globulins. The highest RAST scores were obtained with the globulin fraction of wheat flour followed by the glutenins while gliadins, and a peptic-tryptic digest of gluten which is toxic to celiac patients, gave the lowest scores [5].

Although these studies indicate the general types of proteins involved in ingested wheat allergies, they do not identify the number and exact nature of the polypeptides involved. Clearly, this will only be possible by utilizing the type of background information provided above and by application of analytical separation techniques powerful enough to distinguish the relevant allergenic proteins. At present, the best definition of allergenic wheat proteins has been provided by studies on bakers' asthma [6, 7] and while this work is concerned with inhalant allergies, the definition of allergenic peptides produced (fig. 3) may prove to be

relevant to allergies caused by ingestion of wheat. The excellent two-dimensional (2-D) electrophoretic separations of wheat proteins already achieved can be utilized in studies of ingested cereal allergies.

Yeast

Adverse reactions to yeast, particularly urticaria, atopic dermatitis and asthma accompanied by positive skin test to *Saccharomyces cerevisiae* and foods containing yeast have been reported [see references in 8], but, as with wheat proteins, the source of the sensitization is not always clear. For example, in some of our patients with perennial rhinitis and/or asthma diagnosed as allergic to inhaled fungi and with positive skin and RASTs to *S. cerevisiae* and some other fungi (particularly *Alternaria* and *Candida*), challenge with bakers' yeast after being placed on a yeast-free diet led to falls in peak flow volumes, disturbed sleep, wheeze and cough. When a yeast-free diet was reinstituted, symptoms improved greatly or disappeared [Baker and Baldo, unpubl. data].

Studies with sera from subjects with inhalant allergies to *S. cerevisiae* [9] and with atopic dermatitis [8, 10] have shown that enolase is an important IgE-binding component of *S. cerevisiae* but the 'complete' spectrum of allergens recognized in subjects with inhaled and/or ingested allergies to yeast has not yet been defined. Studies on the inhalant allergies [9, Baldo and Baker, unpubl. data] revealed up to 14 serum IgE-binding components (fig. 4) but, as with wheat proteins, 2-D electrophoretic separations [11] offers the best means of precisely defining the full spectrum of allergens.

Royal Jelly

Skin prick tests, immunoassays and protein blotting studies confirmed that symptoms of asthma and anaphylaxis seen in subjects following ingestion of royal jelly were true IgE-antibody-mediated immediate hypersensitivity reactions [12]. A total of 18 different IgE-binding components were detected on blots following electrophoretic separation of royal jelly under dissociating conditions. IgE antibody binding of blotted royal jelly proteins was most marked in the MW region 25–55 kD and one component of MW ~ 55 kD was detected by all of the sera examined. This component may be the so-called major royal jelly protein recently described. Examination of 63 sera from subjects allergic to bee venom showed that there is no direct relationship between IgE antibody reactivity to the bee venom allergens and to royal jelly proteins although 38% of the sera reacted with a royal jelly solid phase. IgE antibody reactivity to royal jelly proteins was

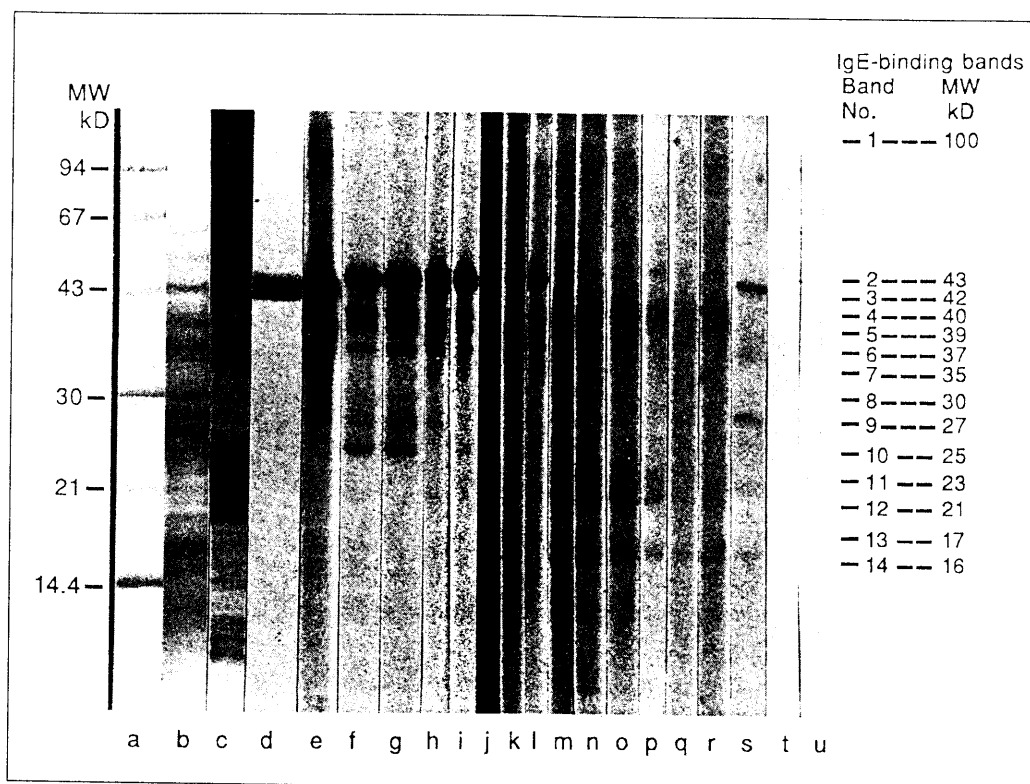


Fig. 4. Identification by autoradiography of fourteen IgE-binding components on nitrocellulose (NC) after SDS-PAGE of a *S. cerevisiae* extract. a = MW markers; b, c = *S. cerevisiae*-stained proteins in gel; d = *S. cerevisiae* enolase protein-stained; e-s = NC transfers incubated with individual sera from *S. cerevisiae*-allergic subjects followed by ^{125}I -anti-human IgE; t, u = NC strips incubated with serum from house dust mite and pollen-allergic subject/ ^{125}I -anti-IgE and cord serum/ ^{125}I -anti-IgE respectively.

also detected in 52% of 75 subjects with allergies to inhalant and/or food allergens. All of the positive control sera reacted with the ~55 kD protein. The high incidence of royal jelly-reactive IgE antibodies in the allergic control subjects may be due to cross-reactivity between royal jelly proteins and other commonly encountered ingested or inhaled allergen sources.

Once again therefore, both the route of sensitization and the nature of the sensitizing source(s) is not entirely clear-cut and with all three allergen source materials discussed here these uncertainties must be borne in mind for both diagnosis, and especially management, of the allergic subjects.

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