

Detection of Antigens Reactive to IgE and IgA during Wheat Seed Maturation and in Different Wheat Cultivars

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Key Words

Wheat allergy • Coeliac disease • Antigen profile • Wheat cultivars • IgA-reactivity • IgE-reactivity

Abstract

Background: Dietary intake of wheat causes hypersensitivity reactions in patients suffering from IgE-mediated food allergy and coeliac disease. **Aim:** To study the expression of IgE- and IgA-reactive antigens during wheat seed maturation and in different wheat cultivars. **Methods:** Summer wheat was grown in a glasshouse and seeds were harvested at defined maturation stages. Mature seeds were obtained from 13 different defined cultivars. Protein extracts were prepared from different maturation stages and cultivars with a standardized procedure based on seed weight and analysed by IgE and IgA immunoblotting using sera from clinically defined patients suffering from wheat allergy or coeliac disease. **Results:** With a few exceptions the expression of IgE- and IgA-reactive wheat antigens increased during wheat seed maturation. Wheat cultivars could be identified in which the expression of certain IgE- and IgA-reactive components was strongly reduced or not detectable. **Conclusions:** The expression of IgE- and IgA-reactive antigens depends on wheat seed maturation and varies in different wheat cultivars.

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Introduction

Wheat (*Triticum aestivum*) is a major component of our diet but elicits 2 types of immunological hypersensitivity disease, IgE-mediated allergy and coeliac disease (CD) [1, 2].

IgE-mediated allergy to wheat can either cause food allergy upon ingestion of wheat or wheat-containing foods or respiratory allergy (e.g., baker's asthma) when wheat flour is processed [3–7]. Accordingly, wheat belongs to the major elicitors of food allergy, particularly in children, leading to various clinical manifestations, including gastrointestinal, cutaneous and respiratory symptoms of immediate or delayed onset. Respiratory sensitization to wheat flour frequently occurs as an occupational disease among bakers, affecting 4–10% of bakery workers in Europe [8].

In addition, wheat is an important elicitor of CD, affecting approximately 1% of the population [2, 9]. CD is mainly diagnosed in children who upon ingestion of wheat develop inflammatory reactions in the mucosa of the small intestine with typical clinical and histological alterations which improve under a wheat-free diet [9]. CD patients also develop an autoimmune enteropathy and frequently suffer from a variety of other autoimmune diseases [2].

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Avoidance of wheat and wheat products is currently the most effective treatment for patients suffering from wheat-induced food allergy, respiratory allergy and CD. Many plant-derived allergens belong to a group of pathogenesis-related proteins and show a varying expression profile in response to endogenous (e.g. plant hormones) or exogenous (e.g. pollution) factors [10–15]. The expression of several other plant allergens has been shown to depend on maturation processes and varies in different cultivars [16–22]. Each of the latter factors may therefore affect the allergenicity of plant allergen sources.

Preventative research has included silencing allergenic proteins in plants by anti-sense technology, which has been used to reduce the expression of certain allergens in rice, grass pollen, apples and tomatoes, with the aim of reducing their allergenic activity [23–29].

Here we studied the expression of IgE- and IgA-reactive antigens in wheat seeds during maturation and in different cultivars used for bread-making in Austria. The expression of certain wheat allergens depends on maturation processes and varies between different cultivars.

Patients and Methods

Patients

Sera were derived from 2 patients with food allergy (patient 1, a 26-year-old male, and patient 2, a 55-year-old male), 2 patients with baker's asthma (patient 3, a 35-year-old male, and patient 4, a 26-year-old male), and 2 patients with CD (patient 5, a 42-year-old male, and patient 6, a 70-year-old female). Serum from food allergic and baker's asthma patients had been analysed for total serum IgE levels and IgE specific for wheat and rye by CAP-FEIA System (Phadia, Uppsala, Sweden). Baker's asthma was diagnosed after specific inhalation challenge tests [30] and CD was diagnosed according to ESPGAN criteria [31]. All sera were stored at –20°C until use.

Protein Extracts

Wheat seeds from different wheat breeds (*Triticum aestivum* cv. Silvius, Romanus, Grandios, Dekan, Belmondo, Renan, Saturnus, Xenos, Josef, Granat, Edison, Capo, Atrium and Michael) were obtained from the Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH. Summer wheat Michael was planted in a glasshouse to obtain wheat seeds of defined maturation stages. Immature seeds developed after approximately 2 months. Day 0 was defined as the day on which pollination occurred. Seeds were continuously harvested directly into liquid nitrogen for a period of 35 days and stored at –80°C until analysis. We previously compared different extraction procedures for the preparation of wheat extracts containing IgE- and IgA-reactive antigens and found that the extraction of ground seeds in SDS-sample buffer followed by boiling allowed us to extract the majority of antigens. Accordingly, protein extracts were prepared by grinding exactly 3 g of seeds from different maturation steps or from dif-

ferent cultivars in a blender and stirring these in 32 ml of sample buffer [32] followed by boiling for 10 min. Extracts were then centrifuged at 10,000 g for 10 min at 4°C to remove insoluble particles. The supernatants were stored in aliquots at –20°C until use.

Immunoblot Experiments

Equal volumes (30 µl/cm preparative gel) of wheat seed protein extracts were separated by 12.5% preparative SDS-PAGE [33]. After electrophoresis, the gels were either stained with Coomassie brilliant blue or blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) [34]. The sera from food allergic and baker's asthma patients for IgE detection were diluted 1:10 and those for IgA detection 1:1,000 in buffer A (50 mM sodium phosphate buffer, pH 7.4, 0.5% w/v bovine serum albumin, 0.5% v/v Tween-20, 0.05% w/v NaN₃). The remaining free binding sites of the nitrocellulose membranes were blocked twice for 5 min and once for 30 min in buffer A at room temperature. The membranes were probed overnight with the patients' diluted sera at 4°C, washed as described above and then incubated overnight at 4°C either with mouse monoclonal anti-human IgA antibody (M1265; CLB, Amsterdam, The Netherlands) which had been ¹²⁵I-labeled using the chloramine-T method [35] or ¹²⁵I-labeled anti-human IgE antibodies (Phadia). After washing and drying, membranes were exposed to Kodak X-OMAT films using intensifying screens (Kodak, Heidelberg, Germany) at –70°C.

Results

Alteration of the Protein Expression Profile during Wheat Maturation

In order to investigate the expression of antigens during wheat seed maturation, summer wheat Michael was planted in a glasshouse and harvested on days 7, 10, 15, 20, 25, 30 and 35 after pollination had started. Figure 1a shows wheat spikes at typical time points of maturation. In the figure, pollination has occurred between stages 'b' and 'c', and stage 'm' shows a spike containing mature wheat seeds. The analysis of protein expression during wheat maturation is shown in figure 1b. In Coomassie-stained SDS-PAGE, several proteins were up-regulated during wheat maturation (fig. 1b, solid arrows). They included proteins with molecular weights of approximately 90, 50, 40, 35, 30, 12, 8 and 5 kDa. On the other hand the expression of certain proteins with molecular weights of approximately 47 and 16 kDa decreased during maturation (fig. 1b, dotted arrows).

The Expression of IgE- and IgA-Reactive Antigens Varies during Wheat Maturation

Serum IgE from the two patients with IgE-mediated food allergy to wheat reacted with antigens which were up-regulated during seed maturation (e.g., antigens with a molecular weight of approximately 90 kDa, 50–60 kDa

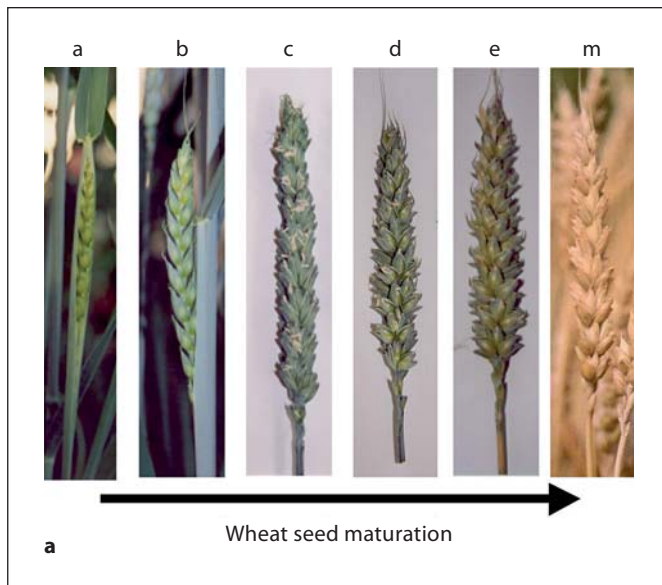
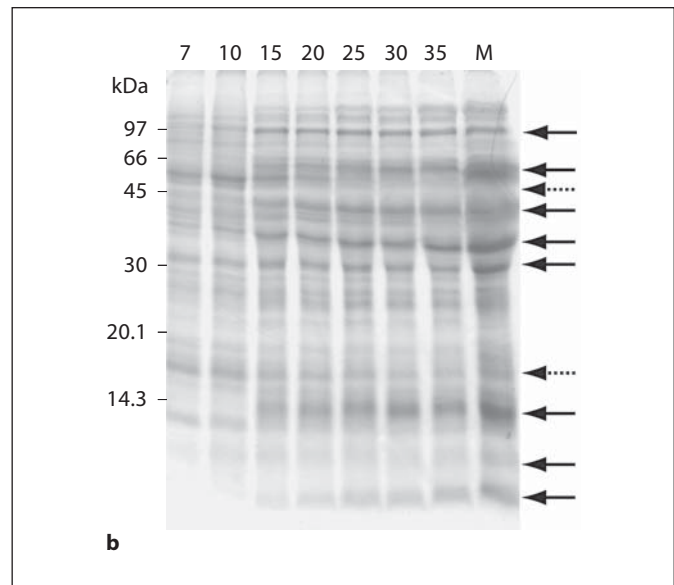


Fig. 1. Protein expression during wheat seed maturation. Wheat (summer wheat Michael) was planted in a glass house to obtain wheat seeds of defined maturation stages. Day 0 is defined as the day on which pollination occurred. Seeds were continuously harvested directly into liquid nitrogen for a period of 35 days. **a** Pictures of wheat spikes during maturation. Pollination has occurred between stage 'b' and 'c'. Stage 'm' shows a spike containing ma-



ture seeds. **b** Protein extracts of wheat seeds of different maturation stages (days 7, 10, 15, 20, 25, 30, 35 and mature, M) were separated by SDS-PAGE and stained with Coomassie blue. Molecular weights, in kDa, are indicated on the left. Dotted arrows point to bands which increase during maturation and solid arrows point to bands which decrease during wheat seed maturation.

detected by serum No. 1 and a 16 kDa antigen detected by serum No. 2). Other IgE-reactive antigens were down-regulated during maturation (e.g., antigens of 8 kDa detected by serum No. 1 and 43–44 kDa detected by serum No. 2; fig. 2a).

Similar results were obtained with IgE antibodies from patients with respiratory wheat allergy (fig. 2b). Certain IgE-reactive antigens increased during maturation (e.g., 10 kDa, serum No. 3; 30 kDa, serum No. 4), others decreased (e.g., around 40 kDa, serum No. 3) and antigens with a varying expression pattern (e.g., 47 kDa and 19 kDa, serum No. 4) were observed. Other IgE-reactive antigens showed a transient expression (e.g., a 43 kDa-antigen, serum No. 4).

As reported earlier, coeliac disease patients recognized antigens different from those recognized by patients with IgE-mediated allergy (fig. 2c). Certain IgA-reactive antigens showed increases of expression during wheat seed maturation (e.g., 14 kDa and 40 kDa, serum No. 5, and a 18 kDa-antigen, serum No. 6) whereas several showed an almost constant expression during seed development (e.g., 25 kDa and 60 kDa, serum No. 5, and a 30 kDa-antigen, serum No. 6; fig. 2c).

Variability of the Expression of IgE- and IgA-Reactive Antigens in Different Wheat Cultivars

The analysis of IgE-reactive antigens in different wheat cultivars with sera from patients suffering from food allergy showed that certain antigens were not detectable or were expressed at low levels in certain wheat cultivars (e.g. 15- to 17-kDa antigens in Renan, Saturnus, Granat and Capo-serum No. 1, 19- to 25-kDa antigens in Romanus, Dekan, Renan, Xenos and Capo-serum No. 2; fig. 3a). Similar observations were made for IgE-reactive antigens detected by sera from patients with respiratory allergy (fig. 3b). No or little amounts of 8-kDa antigens (Romanus, Grandios, Belmondo, Renan, Josef, Granat, Capo), 14-kDa antigens (Belmondo, Saturnus, Capo) and 35-kDa antigens (Belmondo, Xenos, Edison) were detected by serum No. 3. Serum No. 4 reacted weakly to 14-kDa (Capo) and 19-kDa (Granat, Capo) antigens (fig. 3b).

Several wheat cultivars were identified that lacked detectable or contained low amounts of IgA-reactive antigens, among them Romanus, Dekan, Renan, Saturnus, Edison, Capo and especially Xenos (e.g. 18- and 40-kDa antigens, serum No. 5; 14-, 20- and 50-kDa antigens, serum No. 6; fig. 3c).

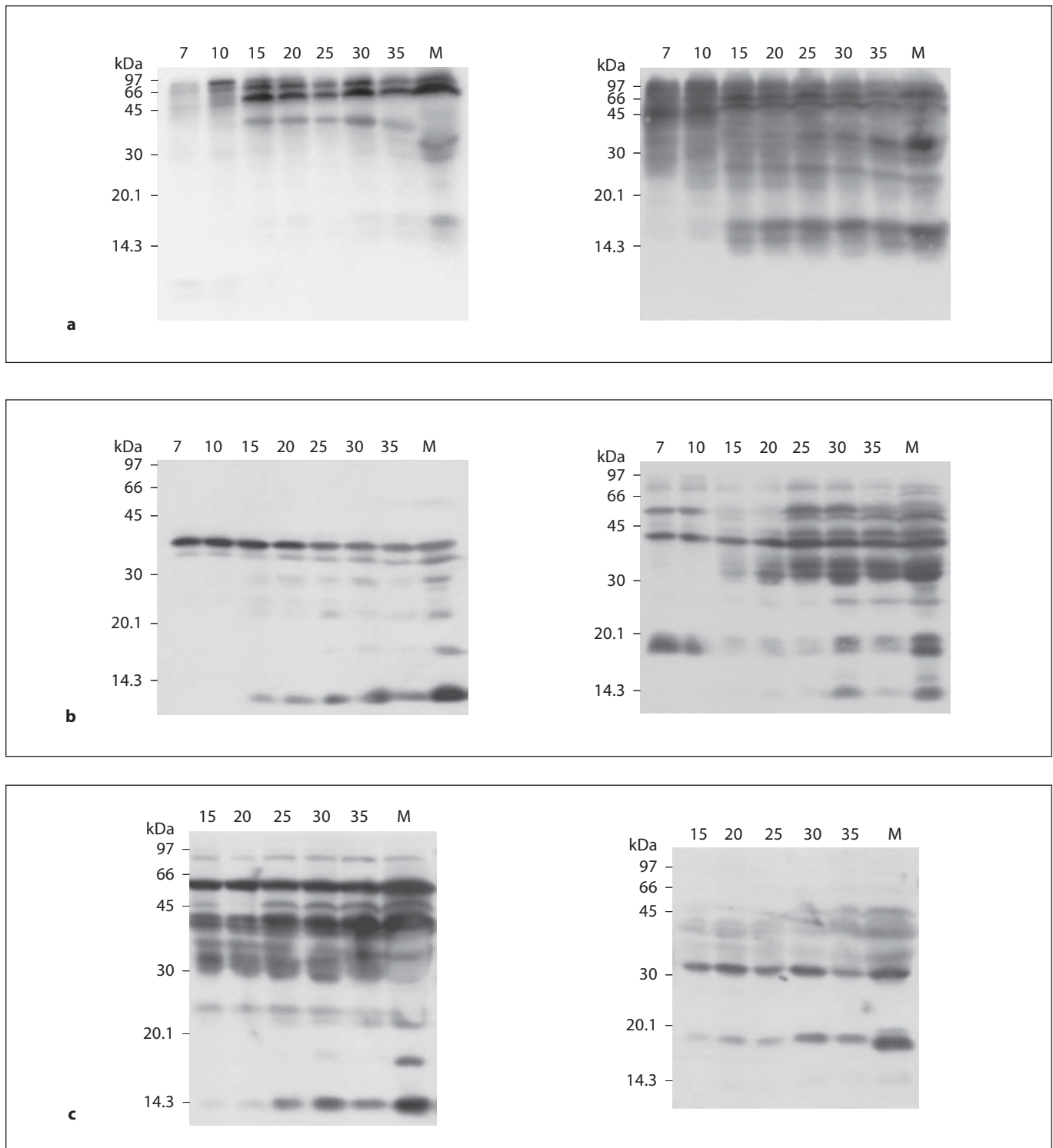


Fig. 2. Developmental regulation of allergen expression in wheat. Three groups of patients were tested for antibody reactivity to nitrocellulose-blotted wheat extract from immature (days 7, 10, 15, 20, 25, 30, 35) and mature (M) wheat seeds. Molecular weight markers, in kDa, are shown on the left. **a** Two patients (patient 1 and 2) with gastrointestinal symptoms – IgE-mediated food al-

lergy (serum 1: left; serum 2: right), tested for IgE reactivity. **b** Two patients (patient 3 and 4) with respiratory symptoms – IgE-mediated baker's asthma (serum 3: left; serum 4: right), tested for IgE reactivity. **c** Two patients (patient 5 and 6) with gastrointestinal symptoms – coeliac disease (serum 5: left; serum 6: right), tested for IgA reactivity.

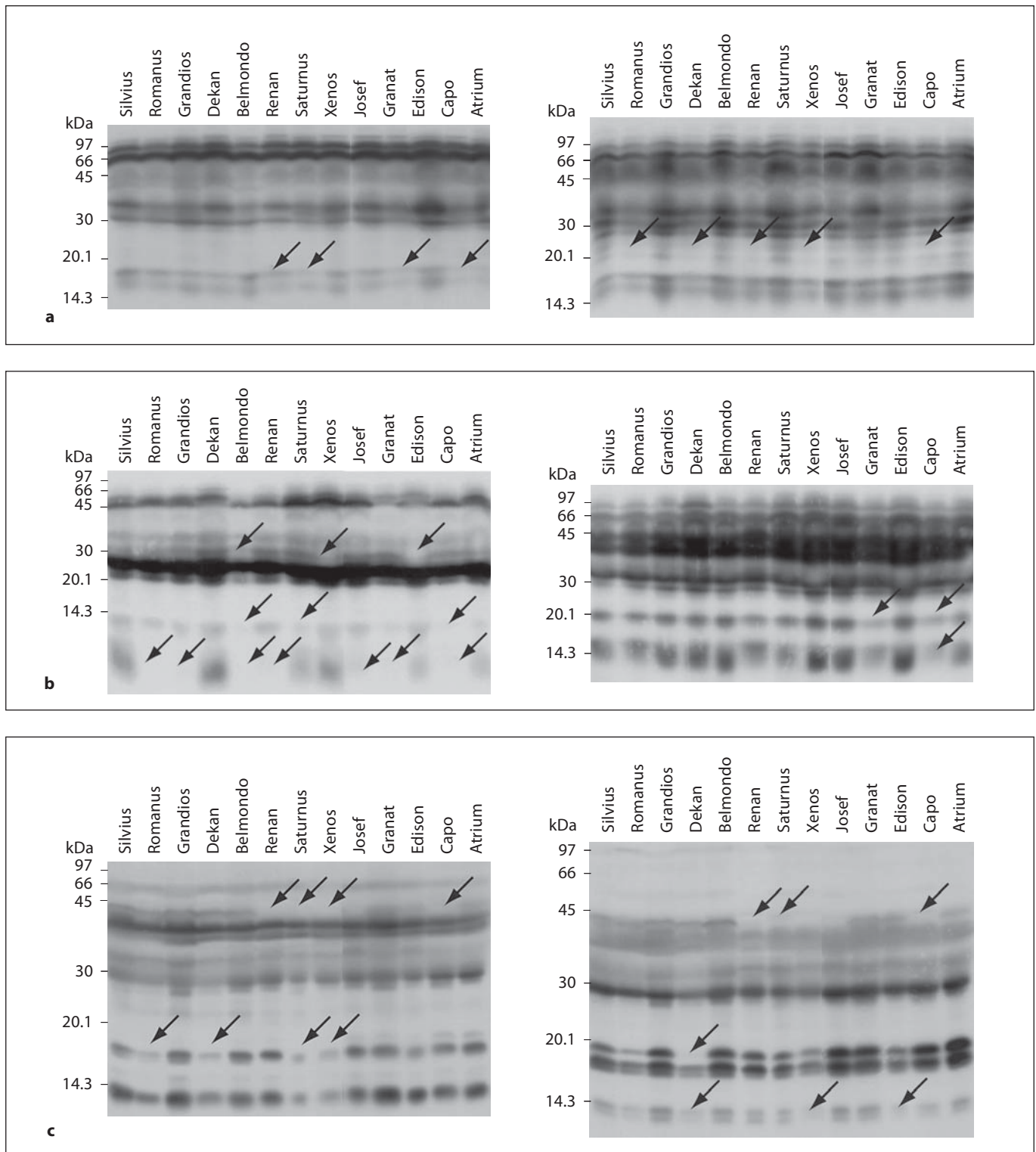


Fig. 3. Variability of IgE- and IgA-reactive wheat antigens from different diet cultivars. Sera from 2 representative food allergic patients (patients 1 and 2; **a**), patients suffering from baker's asthma (patients 3 and 4; **b**) and CD patients (patients 5 and 6; **c**) were probed for IgE (**a**, **b**) and IgA (**c**) reactivity to wheat extracts from

winter wheat Silvius, Romanus, Grandios, Dekan, Belmondo, Renan, Saturnus, Xenos, Josef, Granat, Edison, Capo and Atrium. Arrows indicate antigens with reduced expression. Molecular weights, in kDa, are indicated on the left.

Discussion

We have investigated the expression of IgE- and IgA-reactive antigens during wheat seed maturation and analysed their expression in 14 wheat cultivars most frequently used for milling in Austria. Our results demonstrated variations in protein expression during seed maturation. The expression of IgA-reactive antigens detected by sera from CD patients increased constantly during seed maturation. Among the IgA-reactive protein fractions, α -, β - and γ -gliadins are reported to migrate between 30 and 45 kDa, Ω -gliadins between 50 and 70 kDa, low molecular weight glutenins between 35 and 45 kDa and high molecular weight glutenins between 80 and 110 kDa. Variations in expression of IgA-reactive bands were observed between 45 and 10 kDa and may correspond to gliadins and/or glutenins. Many of the IgE-reactive antigens also increased during seed maturation but several others were prominently expressed in the early stages of maturation and disappeared in mature seeds. The increased expression during maturation has been recently described for a serine proteinase inhibitor-like wheat allergen [36]. The different expression profiles of IgE- and IgA-reactive antigens may be explained by different biological functions of these antigens of which the early expressed proteins may be required for endosperm (i.e. starch body) development whereas other proteins (e.g. storage proteins, the major constituents in the mature wheat seed) are increasingly expressed during seed maturation. Since the majority of IgE- and IgA-reactive antigens were highly expressed in late stages of seed maturation it can be assumed that late-stage seeds will be a suitable source for the construction of expression cDNA libraries which can be screened with serum IgE and IgA for cDNA clones coding for wheat antigens. Using cDNA cloning techniques it will be possible to reveal the molecular nature of the IgE- and IgA-reactive wheat antigens, as has been done for respiratory allergens, and to produce recombinant antigens for diagnostic and therapeutic purposes [37].

We have also investigated different wheat cultivars regarding the expression of IgE- and IgA-reactive wheat antigens. The wheat cultivars Granat and Capo lacked certain IgE-reactive wheat antigens whereas Romanus, Dekan, Renan, Saturnus, Xenos, Josef and Capo contained fewer IgA-reactive wheat antigens. These results demonstrate that cultivars containing fewer wheat allergens than others can be identified based on serological screening and may eventually open possibilities for a natural breeding of hypoallergenic wheat cultivars. Natural

breeding and breeding in combination with random mutation strategies [38–40] accompanied by serological analysis may allow us to foster effectively viable hypoallergenic cultivars. Several attempts have been made so far to silence the expression of certain allergens in rice, grass pollen, apple and tomato by anti-sense technologies [23–29]. Using this technology it has been possible to down-regulate the expression of certain allergens but it may affect growth and viability of the engineered plants. Alternatively it may be considered to replace potent allergens with hypoallergenic isoforms or homologous proteins from other plants with low or no allergenic activity [41, 42].

Our results demonstrate that frequently used wheat cultivars lack or express certain allergens at very low levels, suggesting that it may be possible to breed these cultivars further towards hypoallergenic forms.

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