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Properties of Tree and Grass Pollen Allergens: Reinvestigation of the Linkage between Solubility and Allergenicity

Abstract

In this study we reinvestigated the kinetics of allergen release from birch pollen (*Betula verrucosa*) and timothy grass pollen (*Phleum pratense*) using different protein extraction procedures, immunoblotting with specific antibodies and immune electron microscopy. Pollen allergens such as the major birch pollen allergen, *Bet v* I, the major timothy grass pollen allergens, *Phl p* I and *Phl p* V, group-II/III allergens from timothy grass and profilins were released rapidly and in large amounts from hydrated pollen. Within a few minutes pollen allergens could be detected in aqueous supernatants prepared from birch and grass pollen with serum IgE or specific antibodies. In parallel the allergen content in the pollen pellet fractions decreased. A nonallergenic protein such as heat shock protein 70 can be extracted in sufficient amounts only with harsh extraction procedures. Immune electron microscopy of dry and rehydrated birch pollens showed that after short hydration, the major birch pollen allergen, *Bet v* I, migrated into the exine and to the surface of intact pollen grains, whereas profilin, against which a lower percentage of patients is sensitized, was retained in the pollen grain. Comparing the amino acid composition and hydrophilicity of the tested allergens with a nonallergenic protein such as heat shock protein 70, no significant difference was noted. In agreement with earlier observations we conclude that the allergenic properties of proteins are rather linked to the amount and speed of solubility from airborne particles than to intrinsic properties.

Key Words

Tree and grass pollen allergens
Bet v I
Profilin
Phl p I
Phl p V
Allergens, group II/III
Immunoblotting
Electron microscopy, immune

Introduction

A number of allergens have been characterized by immunochemical and molecular biological techniques. Based on the sequence information of allergens, a first understanding of the biological function of these molecules

is possible. In the case of the major birch pollen allergen *Bet v* I, sequence homology was found with pathogenesis-related proteins [1] which are involved in plant defense against microorganisms. The family of profilins [2-5] constitutes plant cytoskeletal proteins which bind to actin [6] and participate in the phosphoinositol pathway [7]. De-

spite the increasing knowledge about the structure and possible functions of allergens, a clear relationship between the properties of allergens and their allergenic potency could not be established. Type-I allergies could be induced experimentally in animals by low-dose application of several different unrelated antigens in combination with aluminium hydroxide [8, 9], which also argues against a relationship between the intrinsic property of a protein and its potency to elicit type-I allergy. The clinical observation that exposure to mite antigens during early childhood is correlated with the development of house dust mite allergy [10] indicates that exposure to readily available proteins via the mucosa of the respiratory tract might sensitize predisposed individuals during early childhood to develop type-I allergy. We have characterized well-defined pollen allergens from trees, such as *Bet v 1* [1] and birch profilin [2], and relevant timothy grass pollen allergens, such as *Phl p 1* (unpublished data), *Phl p 5* (unpublished data), and timothy grass profilin regarding their aqueous solubility. Aqueous protein extraction procedures were compared with harsh extraction methods, immune electron microscopy of pollen grains before and after hydration was performed and the kinetics of allergen release from hydrated pollens was investigated. Important pollen allergens are readily available in large amounts as compared to other pollen proteins within minutes after hydration, but no correlation between the intrinsic properties (biological functions, distribution in the pollen grain, amino acid composition and hydrophilicity) of the allergens and their allergenicity could be established.

Materials and Methods

Pollen

Pollen from timothy grass (*Phleum pratense*) and birch (*Betula verrucosa*) that were >95% pure as determined by light microscopy were purchased from Allergon, AB, Valinge, Sweden. The purchased pollen was not defatted or treated with solvents. The birch pollen used for immune electron microscopy was harvested freshly from flowering male catkins of birch, air dried and stored at 4 °C until use.

Monoclonal and Polyclonal Antibodies

The mouse monoclonal anti-*Bet v 1* antibodies Bip1 and Bip4 [11] and their epitopes are described [12]. As control a mouse monoclonal antibody with no specificity for birch pollen proteins was used. The rabbit anti-recombinant birch profilin antiserum which cross-reacts with profilin from timothy grass was obtained by immunizing rabbits with purified recombinant birch profilin [6]. In addition an antibody raised against tomato heat shock protein 70 [13], a rabbit antibody raised against *Lol p 1* [14] and a rabbit antibody which binds to group-V and group-II/III grass pollen allergens [15] were used. Negative controls were performed with a normal rabbit nonimmune serum

or mouse antibodies with different specificity. The antibodies which were used for immune electron microscopy were purified by protein G affinity chromatography using monoclonal antibody traps from Pharmacia, Uppsala, Sweden. All antibodies were tested for reactivity with well-characterized recombinant tree and grass pollen allergens [1, 3, 16, 17].

Sera from Allergic Patients

Patients with allergy to grass or birch pollen were characterized according to case history, skin prick tests, RAST and by testing with recombinant tree and grass pollen allergens as described [16, 17]. Sera which had been tested for specificity with defined recombinant tree and grass pollen allergens were used to detect nitrocellulose-blotted allergens. Sera from nonallergic individuals according to the above criteria were used as negative controls.

Pollen-Protein Extraction Methods

1 g pollen from timothy grass and birch was shaken either in 30 ml distilled water, in 30 ml 10 mM potassium phosphate buffer (pH 7) with protease inhibitors [11] or in 30 ml PHEM-Tx buffer with protease inhibitors [18] for 2 h at 4 °C. The protein extracts were centrifuged for 1 h at 40,000 g and the supernatants dialyzed against distilled water at 4 °C and lyophilized. In addition, 1 g pollen from birch or timothy grass was shaken in 30 ml distilled water for 4 h at 37 °C and further treated as above or in 30 ml of water or PHEM-Tx buffer and mechanical treatment with an ultraturrax (Ika, FRG). Pollen protein extracts were also obtained by boiling 1 g pollen in SDS sample buffer for 10 min and mechanical treatment with an ultraturrax (Ika, FRG).

Kinetics and Time Course of Protein and Allergen Elution from Pollen

50 mg of birch or timothy grass pollens were placed in Eppendorf tubes, hydrated with 1 ml of distilled water, vortexed briefly and incubated for 0.5, 1, 10 and 30 min, and 1, 2 and 6 h at 4 °C. The protein extracts were then centrifuged for 1 min in a bench centrifuge at 10,000 g. 900 µl of the supernatants were transferred to a new tube and the remaining 100 µl were discharged. The supernatants and pellets were immediately frozen in liquid nitrogen before use. All experiments were done in duplicates. Supernatants were checked for the absence of pollen grains with a light microscope. The protein content of the supernatants was determined using the method of Bradford [19]. The allergen content of the supernatants was estimated by IgE-inhibition studies. For IgE-inhibition studies sera were chosen with specificity to most birch and grass pollen allergens. The reduction in patient's IgE binding to dot-blotted pollen allergens after preabsorption with supernatants was measured by determination of the decrease in bound ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden) in a γ-counter (LKB, Uppsala, Sweden). Measurements of proteins and IgE binding were done as duplicates and the results reflect mean values.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting

Protein extracts were subjected to 14% polyacrylamide gel electrophoresis [20] and blotted onto nitrocellulose [21]. 50 µl of the aqueous supernatants were loaded in each slot. The protein content of the pollen supernatants after different hydration times is shown in the results. In the case of different extraction procedures 1 ml dialysate was lyophilized, resuspended in 100 µl water and 50 µl loaded per slot. In the case of the SDS extract, 1 g pollen was boiled in 10 ml sam-

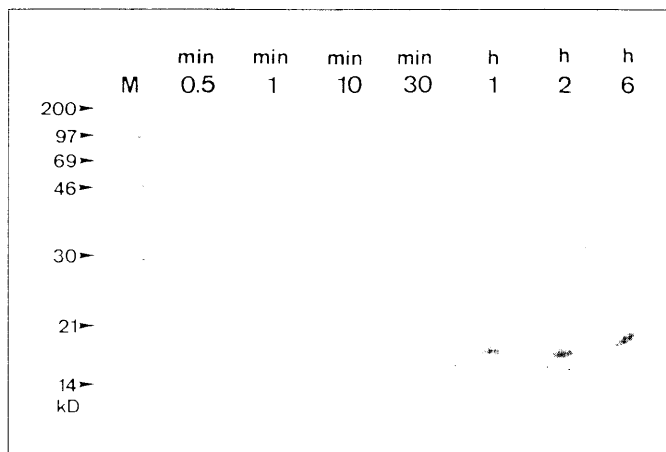


Fig. 1. Coomassie blue-stained gel of birch pollen proteins. Lane M represents the marker. Birch pollen proteins after 0.5, 1, 10 and 30 min and 1, 2 and 6 h of hydration.

ple buffer and 20 μ l thereof were loaded per slot. The nitrocelluloses were either incubated with 1:10 dilutions of sera from pollen allergic patients or with the monoclonal and polyclonal antibodies, diluted 1:500. For incubation of the nitrocelluloses and dilution of the antibodies buffer B (50 mM Na-phosphate, pH 7.5, 0.5% Tween 20, 0.5% bovine serum albumin, 0.05% NaN_3) was used. Bound serum-IgE was detected with 1:10 diluted ^{125}I -labeled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden), bound rabbit antibodies with a 1:2,000-diluted ^{125}I -labeled donkey anti-rabbit antibody (Amersham, Buckinghamshire, UK) and bound mouse monoclonal antibodies were detected with ^{125}I -labeled sheep anti-mouse antibodies (Amersham, Buckinghamshire, UK) diluted 1:1,000. As a control for IgE blots serum from a nonallergic patient was used. A normal rabbit serum, diluted in the same way as the specific antisera, and a mouse monoclonal antibody, raised against a mugwort pollen allergen (unpublished data) without cross-reactivity to birch pollen or timothy grass pollen proteins, were used as negative controls. Blots were washed twice for 5 min and once for 30 min in buffer B, incubated with the primary antibodies overnight at 4°C, washed as described above and then incubated over night with the second antibodies at room temperature. Blots were washed again and dried, exposed to Kodak X-Omat-S films using Kodak intensifying screens (Kodak, Heidelberg, FRG) at -70°C. Time of exposure was between 24 and 72 h.

Electron Microscopy and Immunogold Labeling

Dry pollen grains or pollen grains which had been soaked for 1 min in Beem capsules filled with distilled water were anhydrously fixed on glass slides in *p*-formaldehyde vapor for 24 h at room temperature and then treated with dimethoxypropane to remove water. The grains were then embedded into Lowicryl K4M and sections were obtained as described [22]. The antibodies used for detection of the allergens are described above. As second antibodies goat anti-mouse antibodies coupled to 10-nm gold particles and goat anti-rabbit antibodies coupled to 20-nm gold particles from BioCell (Plano, Marburg, FRG) were used. The following control experiments were carried out: (1) replacement of monoclonal primary antibodies by a

monoclonal antibody of different specificity and of rabbit polyclonal antibody by a normal rabbit serum; (2) double labeling in reverse order using secondary antibodies coupled to 10-nm gold particles before secondary antibodies coupled to 20-nm gold particles; (3) single labeling for *Bet v I* and profilin, and (4) omission of primary antibodies. Details of the labeling procedure have been described [23, 24]. More than 5 sections of each labeling and control were examined to obtain the representative pictures shown in figure 7.

Results

Kinetics of Allergen Release upon Hydration of Pollens

The release kinetics of allergens from pollen of timothy grass (*P. pratense*) and birch (*B. verrucosa*) was monitored by shaking pollens in distilled water for 0.5, 1, 10 and 30 min and 1, 2 and 6 h and detection of the allergens in the supernatant and pellet fractions using Coomassie-stained gels and immunoblots probed with patients' serum IgE or specific antibodies.

The Coomassie-stained gel of the birch pollen supernatants (fig. 1) indicates that most proteins are extracted within 60 min and no remarkable increase in the protein content is observed in the supernatants after longer extraction periods. Similar results were obtained when the gels were silver stained (data not shown). *Bet v I* ranging at 17 kD represents the predominant component of the aqueous supernatants. The major birch pollen allergen *Bet v I* can be detected in the aqueous supernatants after 0.5 min (fig. 2a) and a parallel decrease in the amount of *Bet v I* is observed in the pellet fraction (fig. 2b). Birch profilin appears later (10 min) and in smaller amounts in the supernatants (fig. 3a). Again the pellet fraction shows a decrease in the allergen content (fig. 3b). In the aqueous supernatants from timothy grass pollen, profilin is detected after 0.5 min at 14 kD (fig. 4c) in parallel to *Phl p I* (fig. 4a) and *Phl p V* + group-II allergens (fig. 4b), but *Phl p I* and *Phl p V* + group-II allergens are more prominent components as can be seen in Coomassie-stained gels (fig. 5b). In parallel to SDS-PAGE and immunoblots, the protein content in the supernatants was determined according to Bradford [19] and allergen content was estimated by IgE-inhibition studies. After 10 min of hydration 170 μ g proteins were extracted from 50 μ g birch pollen and 400 μ g proteins were obtained from 50 mg timothy grass pollen. After 2 h 336 μ g were obtained from birch pollen compared to 500 μ g proteins which were extracted from timothy grass pollen. Table 1 shows the increase in total proteins and IgE-binding proteins in the supernatants within 10 min and after 2 h. Most IgE-binding proteins are eluted within the first 10 min.

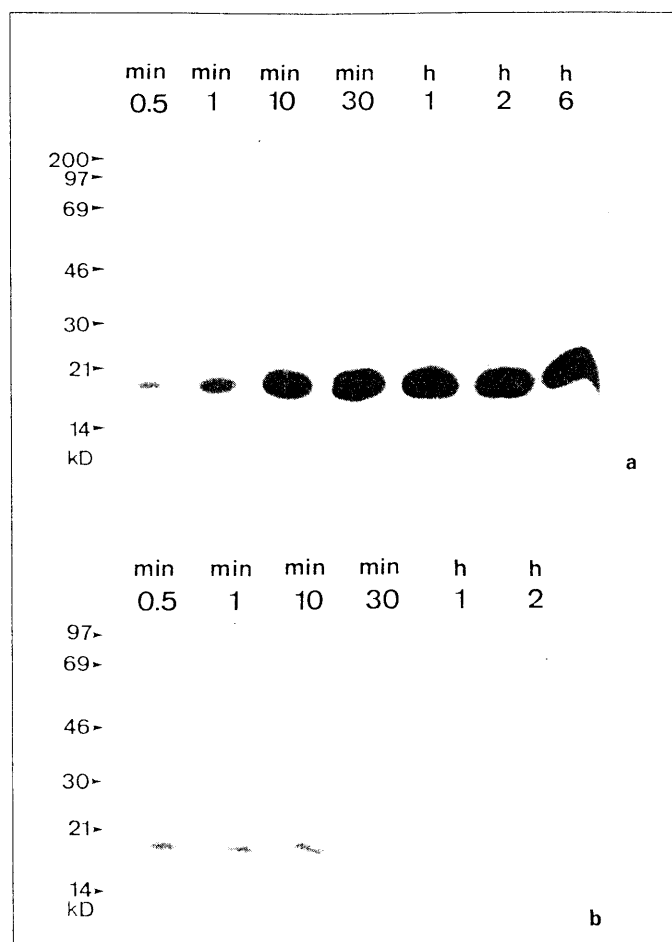


Fig. 2. **a** IgE immunoblots. Birch pollen proteins probed with serum IgE of a patient allergic to *Bet v* I. The supernatants were analyzed after hydration for 0.5, 1, 10 and 30 min and 1, 2 and 6 h. **b** Immunoblot containing proteins extracted from the pellet of hydrated birch pollen which was probed with the monoclonal anti-*Bet v* I antibody, BIP 1. The pellet fractions, which were separated from the supernatants shown above, were analyzed after 0.5, 1, 10 and 30 min and 1 and 2 h of extraction.

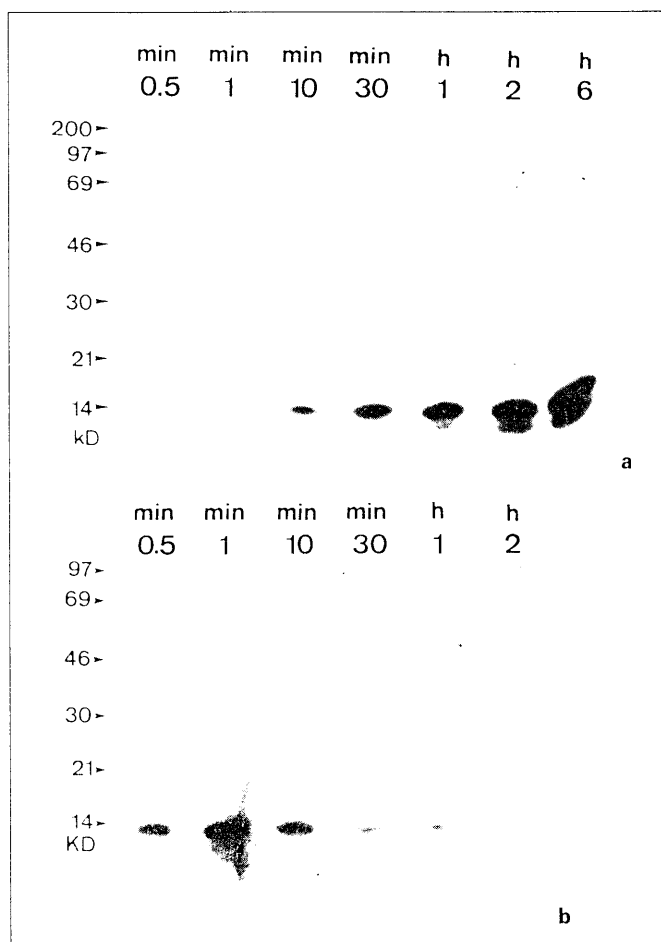


Fig. 3. IgE immunoblots of birch pollen proteins probed with a patient's serum with reactivity to profilin (**a**) or a rabbit antibody raised against recombinant birch profilin (**b**). **a** The supernatants after hydration of birch pollen for 0.5, 1, 10 and 30 min and 1, 2 and 6 h probed with profilin-specific serum IgE. **b** The proteins extracted from the birch pollen pellet fractions after 0.5, 1, 10 and 30 min and 1 and 2 h of hydration which were probed with a rabbit anti-profilin antibody.

Allergens Are Obtained by Aqueous Protein Extraction Methods

Different protein extraction methods were applied for the isolation of allergens from birch and timothy grass pollen. In particular we used aqueous hydration of pollen grains, detergents which allow the isolation of membrane proteins and cytoskeletal proteins such as profilin and harsh extraction procedures, such as boiling the tissue in SDS sample buffer combined with grinding of the tissue. The Coomassie brilliant blue-stained protein gels of birch pollen protein extracts (fig. 5a) and timothy grass pollen protein extracts (fig. 5b) illustrate the outcome of the dif-

ferent methods. Among the aqueous extraction procedures and the method using Triton X-100 (fig. 5, lanes 2–7) little differences are observed regarding the protein pattern. Boiling of pollen in SDS-sample buffer and grinding with an ultraturrax (fig. 5, lane 8) shows that in addition to allergens a number of nonallergenic proteins can be obtained. All aqueous extraction procedures yield high amounts of the major allergens, *Bet v* I from birch pollen and group-I, group-V and group-II/III allergens from timothy grass pollen. Profilins are extracted in smaller amounts by aqueous extraction. In contrast to the pollen allergens, nonallergenic proteins such as heat shock pro-

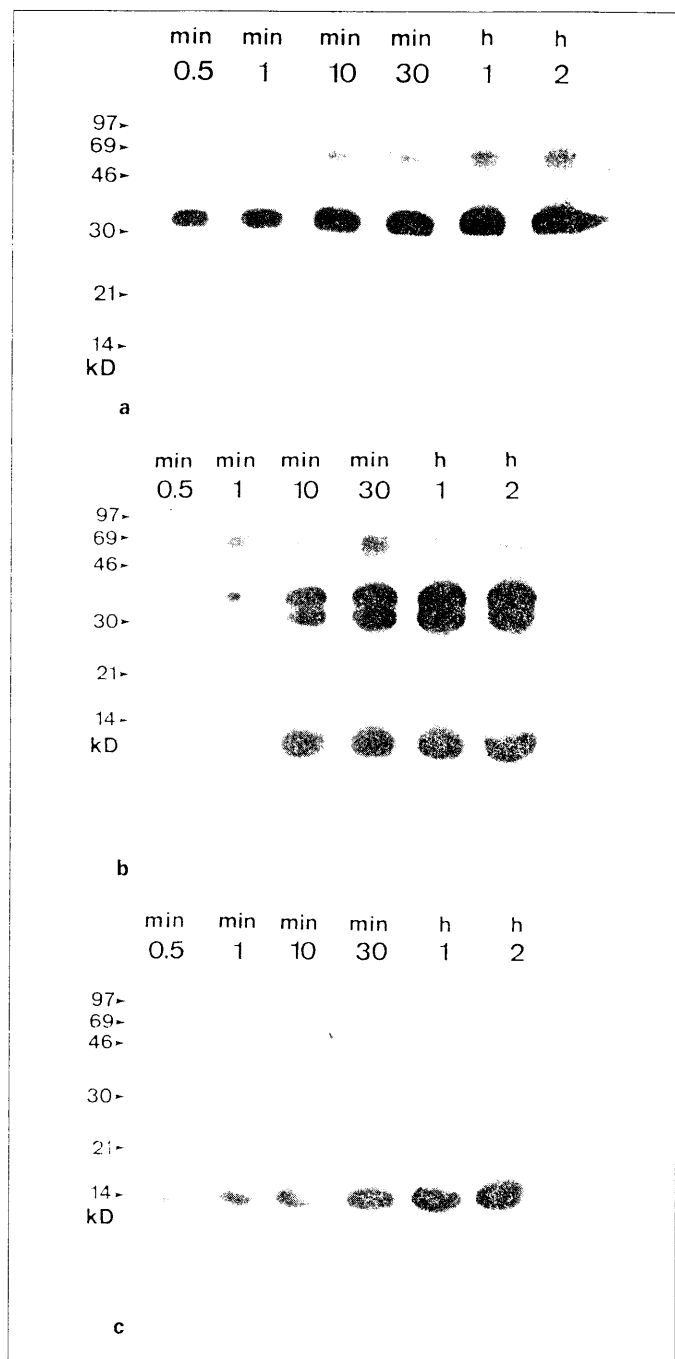


Fig. 4. Immunoblots of timothy grass pollen proteins probed with a rabbit anti-*Lol p I* antibody (**a**), or a rabbit anti-group-V/II antibody (**b**) and a rabbit anti-recombinant birch profilin antibody (**c**). Supernatants after 0.5, 1, 10 and 30 min, and 1 and 2 h of hydration of timothy grass pollen.

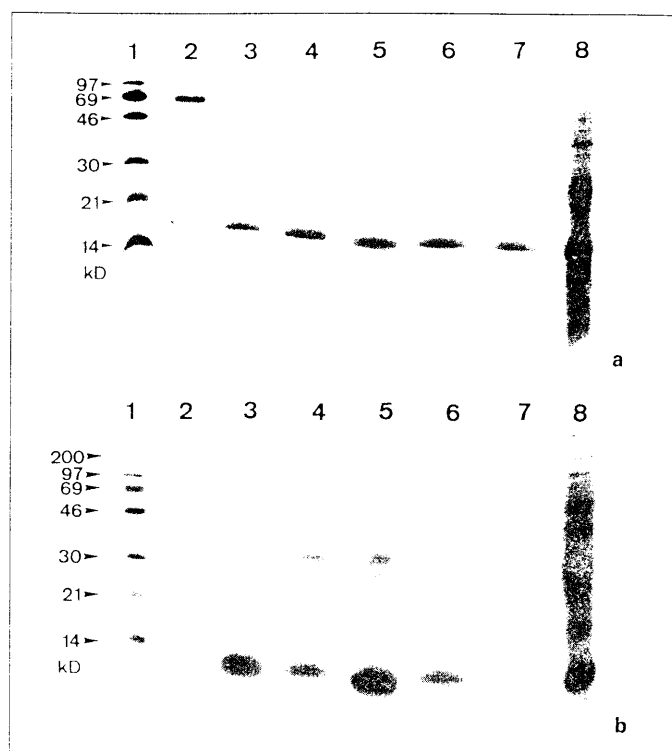


Fig. 5. Coomassie blue-stained SDS-polyacrylamide gels containing birch pollen proteins (**a**) or timothy grass pollen proteins (**b**) which were extracted by different procedures. Lane 1 represents the marker; lane 2, proteins extracted in distilled water at 37°C; lane 3, proteins extracted in distilled water at 4°C. Proteins in lane 4 were extracted in buffer containing Triton X-100; in lane 5 a potassium phosphate buffer was used; proteins in lane 6 and 7 were extracted as in lane 3 and 4 using an ultraturrax, and in lane 8 pollen was boiled in SDS-sample buffer.

Table 1. Elution kinetics of total proteins and IgE-binding proteins from birch (*B. verrucosa*) and timothy grass (*P. pratense*) pollen after hydration

	Birch		Timothy grass	
	0.5 to 10 min	10 min to 2 h	0.5 to 10 min	10 min to 2 h
Increase in IgE-binding components in the aqueous supernatants, %	63	23	37	7
Increase in proteins in the supernatants, %	59	49	25	20

The values displayed were obtained by comparing protein amounts and percentages of IgE inhibition in the 0.5- and 10-min and 2-hour fractions.

tein 70 are obtained preferentially by harsh extraction procedures, such as boiling the tissue in SDS sample buffer and mechanical grinding (fig. 6).

Immune Electron Microscopic Localization of Allergens in Dry and Hydrated Birch Pollens

Birch pollen grains were hydrated for 1 min and the distribution of two well-characterized birch pollen allergens, *Bet v* I and birch profilin (*Bet v* II) was compared with dry

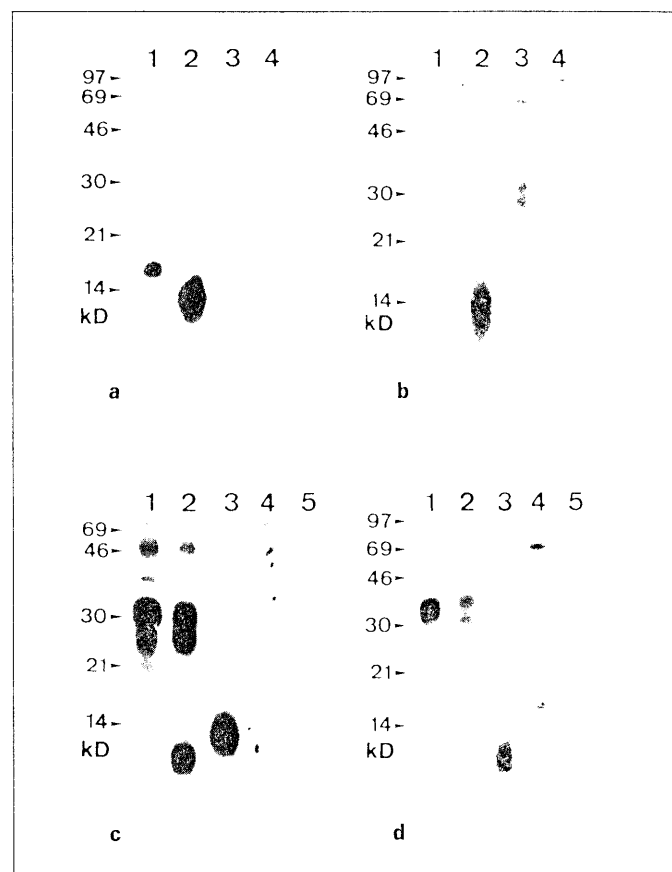


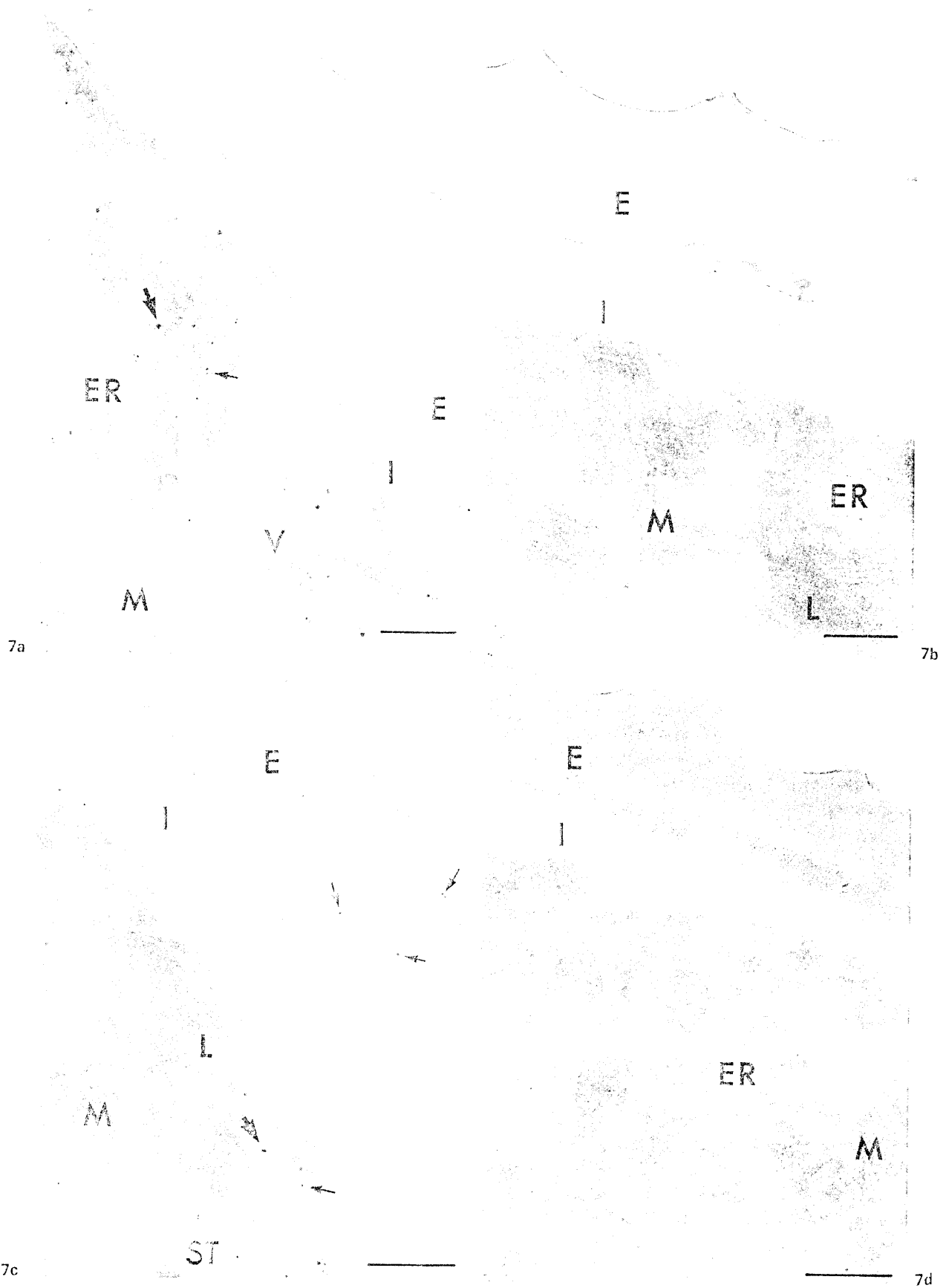
Fig. 6. Immunoblot comparison of protein extraction procedures. Birch pollen proteins (**a, b**) and timothy grass pollen proteins (**c, d**) were extracted in an aqueous buffer containing Triton X-100 (**a, c**) or by boiling the pollen in SDS-sample buffer and applying mechanical grinding (**b, d**). In the case of birch pollen the nitrocellulose strips were incubated with the anti-*Bet v* I monoclonal antibody BIP 1 (lane 1); in lane 2 with a rabbit anti-profilin antibody; in lane 3 with a rabbit anti-tomato heat shock protein 70 antibody, and lane 4 reflects the buffer control without addition of the primary antibody. The nitrocellulose strips containing timothy grass pollen extracts were incubated with a rabbit anti-group I antibody (lane 1), a rabbit anti-group V/II antibody (lane 2), a rabbit anti-birch profilin antibody (lane 3), the rabbit anti-tomato heat shock protein 70 (lane 4), and in lane 5 without primary antibody (buffer control).

pollen grains. Figure 7a shows an ultrathin section of a dry birch pollen grain. Using double immunogold labeling of *Bet v* I and birch profilin both allergens are found to be uniformly distributed in the cytoplasm, whereas after 1 min of hydration in distilled water, *Bet v* I can be stained in the exine and on the surface of the pollen grain (fig. 7c). Profilin, against which only around 10–20% of the patients are sensitized, stains less dense and, after 1 min of hydration, no profilin was found on the surface of the pollen grain. The distribution of gold particles in a number of sections obtained from dry and hydrated pollen grains was evaluated. The sections shown were chosen as representative pictures and reflect the trend observed in different labelings. Control sections of dry and hydrated birch pollens stained with nonimmune rabbit or mouse sera, which had been protein G affinity purified and contained the same protein concentration as the specific antibodies (fig. 7b, d), gave almost no background staining, indicating the specificity of the labeling procedure.

Prediction of the Hydrophilicity of Pollen Allergens and Amino Acid Analysis

To compare the primary structure of allergens with a nonallergenic protein, we calculated the hydrophilicity [25] of the birch pollen allergens, *Bet v* I [1] and birch profilin [2], and the timothy grass pollen allergens, *Phl p* I (unpublished data), *Phl p* V (unpublished data), and timothy grass profilin, and compared the computer prediction with a nonallergenic cytoplasmic protein, the heat shock protein 70. In the case of the grass pollen allergens *Phl p* I and *Phl p* V, the hydrophobic leader peptides were included in the analyses. No remarkable difference is observed in the patterns derived from the allergens in comparison to tomato heat shock protein 70. All calculated proteins show

Fig. 7. Transmission electron micrographs of ultrathin sections of birch pollen-immunogoldstaining with *Bet v* I- and profilin (*Bet v* II)-specific antibodies. **a, b** Sections of dry pollen grains fixed anhydrously showing wall and cytoplasm. **c, d** Pollen grains which were hydrated for 1 min in water. **a, c** The sections were incubated with anti-*Bet v* I (small arrows, small gold particles) and anti-profilin antibodies (big arrows, large gold particles). **b, d** Sections which were incubated with normal rabbit sera and mouse antibodies without specificity for pollen proteins which had been passed over a protein G affinity column. The bars represent 0.25 μ m. E = Exine; ER = endoplasmatic reticulum; I = intine; L = lipid; M = mitochondrion; ST = starch; V = vesicle. Allergens are stained in the cytoplasm and after 1 min of hydration *Bet v* I is also observed in the pollen wall, exine and on the outer membrane. The sections, which were incubated with the control antibodies, showed almost no background staining.



long hydrophilic and hydrophobic stretches. No marked differences regarding the hydrophilicity patterns and amino acid composition of allergens and the nonallergenic heat shock protein 70 were observed.

Discussion

It is well established that proteins of high aqueous solubility and low molecular weight, which are derived from airborne particles such as pollen, mite feces, mould spores and cat or dog dander, are likely to behave as allergens [26–36]. In case of pollen, hydration may be considered as the critical event when allergens are liberated upon contact with the respiratory mucosa of allergic patients.

Previous immunochemical studies could not provide sufficient information about the structure and biological functions of allergens and whether the allergenic potency of a protein could be linked to its intrinsic properties. The characterization of allergens by molecular cloning techniques enabled us to obtain sequence data and thus information regarding the possible biological functions of allergens [37]. The proteolytic activity of allergens, as was suggested for mite allergens [38], and the presentation of *Lol p* IX (*Lol p* Ib, *Lol p* V) [39] via starch granules are discussed as examples of a linkage between allergenicity and properties of allergens. However, the possibility of eliciting an IgE response in animals by injection of low doses of the antigen in combination with aluminium hydroxide as adjuvant [9] contradicts the idea that the allergenicity of a protein may be linked directly to its intrinsic properties. An important role of presentation of proteins via the respiratory mucosa in the course of sensitization is suggested by a number of data showing that important food allergens represent homologs of pollen proteins. Indeed a considerable number of food-allergic patients cross-react with the homologous pollen proteins [40, 41], whereas IgE reactivity against a pollen protein does not necessarily imply reactivity to the homologous food allergen. By including data on the genetic restriction of the IgE response towards certain allergens [42], one would favor the concept that, in genetically predisposed individuals, type-I allergy is likely to occur upon sensitization with accessible proteins via the respiratory tract.

We then investigated parameters such as aqueous solubility, kinetics of release, and distribution of well-defined tree and grass pollen allergens. For this purpose the solubility of pollen allergens was compared with a nonallergenic protein, the heat shock protein 70. In addition we studied the kinetics of allergen enrichment in aqueous su-

pernatants obtained by pollen hydration, and monitored the distribution of the major birch pollen allergen, *Bet v* I and birch profilin, in dry and hydrated pollen by immune electron microscopy. The comparison of different extraction procedures showed that aqueous extraction methods, in particular shaking pollens for minutes in water lead to the release of large amounts of birch pollen allergens (*Bet v* I and birch profilin) and timothy grass pollen allergens (*Phl p* I, *Phl p* V, and timothy grass profilin). Protein extracts which were prepared by short-term hydration of pollen preferentially contained large amounts of major allergens. Harsh extraction procedures, such as boiling pollen grains in sample buffer, delivered a mass of additional nonallergenic proteins such as heat shock protein 70 [13], indicating that mostly allergenic proteins are available rapidly upon hydration. Compared to other nonallergenic proteins less *Bet v* I is extracted by boiling pollen in SDS sample buffer which may explain the weak *Bet v* I reactivity in this extract (fig. 6b, d). At 37 °C an additional unidentified protein of 69 kD could be extracted from birch pollen with distilled water (fig. 5a, lane 1). As yet nothing is known regarding this protein which probably represents a minor cross-reactive allergen. We noted that a number of pollen-allergic patients display IgE reactivity to a protein of a similar molecular weight present in tree, grass and weed pollen. Profilin, which represents a cytoskeletal protein, was less efficiently extracted by aqueous hydration than major allergens such as *Bet v* I, *Phl p* I, *Phl p* V, and group-II/III allergens. The time course of pollen hydration shows that those allergens which are major allergens for up to 90% of the patients such as *Bet v* I, *Phl p* I, and *Phl p* V can be detected earlier and in larger amounts in the supernatants of hydrated pollen. Using serum IgE, birch profilin is detected later in the aqueous supernatants compared to timothy grass profilin. In fact timothy grass profilin accounts for IgE binding in 20–25% of grass pollen-allergic patients [3, 17], whereas birch profilin is a target for IgE antibodies in only 10% of the birch pollen-allergic patients [16]. The observation that the frequency of sensitization against an allergen may be linked to its release kinetics is also supported by the immune electron microscopic studies with hydrated birch pollen, which show in a double-labeling procedure that *Bet v* I can be stained after 1 min of hydration in the exine and on the surface of the pollen grain, whereas profilin is still localized in the cytoplasm.

In accordance with previous findings, it is suggested that an important prerequisite for a protein to behave as a major allergen is a high degree of accessibility upon hydration. Low molecular weight proteins which can easily

be dissolved out of airborne particles such as pollens are likely to sensitize individuals via the mucosa of the respiratory tract. However, a direct linkage between the biological function of allergens or their distribution in certain compartments of the pollen, such as the starch granules [43], and their ability to elicit an IgE response seem to be less likely because the allergens which we investigated, *Bet v I* a pathogenesis-related plant protein [1], profilin a cytoskeletal protein [2–5] and the grass pollen allergens with still uncharacterized functions, *Phl p I* and *Phl p V*, are located in different compartments of the pollen grain, represent proteins with different or unknown functions and are derived from far distantly related plant species such as monocots (timothy grass, *Phleum pratense*) or dicots (birch, *Betula verrucosa*).

When comparing the amino acid composition and the hydrophilicity pattern of the investigated allergens with a

nonallergenic cytoplasmic protein such as tomato heat shock protein 70 [13], no significant differences were found. Our study therefore suggests that the allergenic properties of allergens are rather correlated with their solubility in large amounts upon hydration and low molecular weight than with common intrinsic properties.

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