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ORIGINAL CONTRIBUTION

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IgE binding to a new cross-reactive structure: a 35 kDa protein in birch pollen, exotic fruit and other plant foods

IgE-Bindung an eine neue kreuzreaktive Struktur: Ein 35 kDa-Protein in Birkenpollen, exotischen Früchten und anderen pflanzlichen Lebensmittteln

Summary Food allergies in birch pollen allergic patients have been shown to be due to cross-reactivities of specific IgE antibodies which are directed against birch pollen allergens with related proteins in fruit, nuts and vegetables. We identified a new cross-reactive structure of 35 kDa in birch pollen and some plant food extracts by Enzyme Allergosorbent Test (EAST) and immunoblot inhibition studies. The 35 kDa birch pollen protein is a minor allergen to which approximately 10-15 % of birch pollen allergic individuals have specific IgE. Our data demonstrate that there is cross-reactivity of this protein with proteins of comparable size from lychee, mango, banana, orange, apple, pear and carrot. While the 35 kDa protein is immunologically independent of the major birch pollen allergen Bet v 1, we also observed IgE binding to a 34 kDa structure which appears to be a Bet v 1 dimer.

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Zusammenfassung Viele Studien belegen, daß das Phänomen der birkenpollenassoziierten Nahrungsmittelallergie auf spezifische IgE-Antikörper zurückzuführen ist, die primär gegen Birkenpollenallergene gerichtet sind und mit verwandten Proteinen in Obst, Nüssen und Gemüse kreuzreagieren. Wir haben ein neues Muster der Kreuzreaktivität identifiziert, das auf einem 35 kDa - Protein aus Birkenpollen beruht. Es handelt sich um ein Minorallergen der Birke, gegen das ca. 10-15 % der Birkenpollenallergiker spezifische IgE-Antikörper aufweisen. Inhibitionsstudien mit dem Enzymallergosorbens-Test (EAST) und mittels Immunoblot zeigen die Kreuzreaktivität dieses Proteins zu Proteinen vergleichbarer Größe aus Litschi, Mango, Banane, Orange, Apfel, Birne und Karotte. Das 35 kDa-Protein ist immunologisch unabhängig von Bet v 1, dem Hauptallergen der Birke. Dagegen haben wir auch ein Protein mit einer Größe von 34 kDa beobachtet, bei dem es sich vermutlich um ein Bet v 1-Dimer handelt.

Schlüsselwörter Pollenallergene – Lebensmittelallergene – exotische Früchte – Kreuzreaktivität – IgE-Immunoblotting

Key words Food allergens – pollen allergens – cross-reactivity – exotic fruit, IgE-immunoblotting.

Introduction

Immediate type allergic reactions (type I allergy) to foodstuffs are becoming increasingly important. These reactions are mediated by IgE antibodies specific for allergens found in various foods. Many combined allergies to respiratory and food allergens have been shown to be due to cross-reactivities of specific IgE. Hypersensitivity to birch pollen, for example, is highly associated with aller-

gy to apple and other fresh fruit, hazelnut, carrot and celery (6, 7, 10, 12, 14). Sensitized patients usually suffer from local symptoms (oral itching, tingling, angioedema of the throat) after ingestion of the food in question (24). Three distinct structures which may be responsible for the phenomenon of birch pollen related food allergy have been identified. The major birch pollen allergen Bet v 1, a 17 kDa protein, has been shown to share common epitopes with proteins of similar size from various fruit and vegetables (8, 9, 18, 27, 35). In addition, the cDNA of Bet v 1 revealed significant sequence homology to some "pathogenesis related proteins" from food plants (5). A second very important cross-reactive structure is the plant pan-allergen profilin (9, 28, 29) which is a minor pollen allergen. Carbohydrate determinants of glycoproteins from pollen and food can also be responsible for IgE cross-reactivities, but the clinical significance of these epitopes is in doubt (1, 2, 6, 32). However, this is also doubtful in the case of the data presented in this study. As a first step to investigate a new phenomenon in the field of food allergy, specific IgE from allergic donors was used to study the immunologic properties of the probably allergenic proteins recognised by these antibodies. Whether these data also reflect the occurrence of symptoms in sensitised patients has to be elucidated by further clinical studies.

We observed that IgE from some patients' sera also bound to a 35 kDa component on birch pollen and apple immunoblots (30). A protein of that size has already been identified as one of the minor birch pollen allergens (15, 16, 20), and our observation suggests that it may be a new cross-reactive structure. Here we show by EAST and immunoblot inhibition experiments that the 35 kDa protein, to which about 10-15 % of birch pollen allergic patients show IgE binding, is immunologically independent of Bet v 1 and is cross-reactive to proteins of the same size from various fruit and vegetables.

Materials and methods

Patients' sera

The 76 individual sera used were collected at the Hospital Borkum Riff, Department of Dermatology and Allergology, Borkum, Germany (Dr. H. Aulepp). The criterion for selection was a clinically relevant birch pollen allergy as determined by positive history, skin prick tests and Radio Allergosorbent Tests (RAST). Due to the limited amount of serum, these samples could not be used for the preparation of large pools. Additional sera were purchased from Allergopharma (Reinbek, Germany), including the following pool sera: birch pollen pool Wn 13/32 (n = 7, from birch pollen allergic patients, further clinical data not available), tree pollen pool Zie 39/132 (n = 12, from patients allergic to birch pollen and other kinds of tree pollen) and grass pollen pool 18/100 (n = 11). In addition to the pooled sera, sera from the patients Bo 95, Bo 160 and A 5433 were used for extended immunoblot studies. A serum of a non-allergic individual was taken as a negative control.

Immunochemicals

Rabbit polyclonal anti-human IgE and goat polyclonal affinity-purified biotinylated anti-rabbit IgG were purchased from DAKO (Copenhagen, Denmark), streptavidin-horseradish peroxidase conjugate from Medac (Hamburg, Germany). The rabbit polyclonal antiserum rb-anti-Bet v 1 raised against Bet v 1 was kindly supplied by Allergopharma (Dr. B. Weber, Dr. R. Wahl, Reinbek, Germany). The Bet v 1 specific monoclonal antibody BIP 1 was a gift from Prof. Dr. D. Kraft (Vienna, Austria). Test kits for the determination of specific IgE were obtained from Allergopharma (Reinbek, Germany).

Allergenic extracts

Commercial birch, mugwort and timothy pollen extract were purchased from Allergopharma (Reinbek, Germany). All other extracts used were prepared from fresh material by a low temperature extraction procedure as described elsewhere (33, 35).

Two-dimensional (2-D) electrophoresis

2-D electrophoresis was performed as described (27, 34), the isoelectric focusing step (first dimension) under native conditions in polyacrylamide rod gels, using 9.4 % (v/v) ampholine-carrier (Servalyt T 5-9, Serva, Heidelberg, Germany), the second dimension (SDS-PAGE) under non-reducing conditions with 5 % (w/v) acrylamide stacking gels and 13 % (w/v) resolving gels. Approximately 35 µg of protein were applied for each separation. For immunodetection, the separated proteins were transferred onto nitrocellulose by semi-dry blotting (21) for 45 min at 0.8 mA/cm^2. Immunodetection with patients' sera was performed as described (27), with the sera diluted 1:25 in 0.01 M potassium phosphate buffer containing 0.15 M NaCl, 0.05 % (v/v) Tween 20 and 0.1 % bovine serum albumin (BSA), pH 7.4.

Isolation of allergens by electroelution

The isolation of allergens by electroelution has been described elsewhere (31). Briefly, birch pollen extract was separated by discontinuous SDS-PAGE under reducing conditions according to the method of Lämmli (22), using a 5 % (w/v) acrylamide stacking gel and a 13 % (w/v) resolving gel. Bands were excised after staining with $CuCl_2$ and the protein was eluted using a Centrilutor electroelution device (Amicon, Witten,

Germany). Fractions were tested for purity by SDS-PAGE followed by silver staining. Fractions containing only the desired protein were pooled and the protein content determined according to Bradford (4), with some modifications (33).

EAST and EAST inhibition experiments

Specific IgE was determined by a commercially available Enzyme Allergosorbent Test (EAST). EAST classes correspond to RAST classes of Phadezym RAST (Pharmacia, Freiburg, Germany). For EAST and EAST inhibition experiments, allergen extracts were coupled to cyanogen bromide activated paper disks as described (33). The EAST was performed according to the manufacturers instructions (Allergopharma, Reinbek, Germany). To distinguish more precisely, EAST classes were calculated with one decimal place. For EAST inhibitions the sera were diluted 1:2.5 with incubation buffer (0.05 M Tris-hydroxymethyl-aminomethane, 0.15 M NaCl, 1.0 % Tween 20, 0.3 % BSA, pH 7.4) and for each disk 50 µl serum dilution were incubated with 50 µl inhibitor solution (birch pollen extract, ovalbumin: 250 µg/ml, 35 kDa protein, Bet v 1: 25 µg/ml diluted in incubation buffer). The detection was performed as for EAST but with a colour reaction time of 2 h instead of 1 h.

Immunoblotting and immunoblot inhibition experiments

Immunoblot studies were performed as described (34). Briefly, extracts were separated by SDS-PAGE under reducing conditions, applying 100 µg of protein per slot (55 mm). The separated proteins were transferred onto nitrocellulose by semi-dry blotting. The membrane was blocked with 0.01 M potassium phosphate, 0.15 M NaCl, 0.3 % Tween 20, pH 7.4. One strip of the blot was stained for total protein using 0.1 % (v/v) India Ink (Pelikan, Hannover, Germany), 1 % (v/v) acetic acid, 0.05 % (v/v) Tween 20 in PBS (13). The remaining part of the blot was cut into 0.3 cm strips and incubated overnight with patients' sera diluted 1:6 in incubation buffer (0.01 M potassium phosphate, 0.15 M NaCl, 0.05 % (v/v) Tween 20, 0.1 % BSA, pH 7.4). Immunodetection of bound specific IgE was performed as described (34). For blot inhibitions, sera were diluted 1:10 in incubation buffer and preincubated with 100 µg protein of birch pollen extract or ovalbumin or 5 µg of 35 kDa protein or Bet v 1 per inhibition for 1 h. Incubation of the blot strips and detection were performed as above.

Microsequencing

For N-terminal sequencing proteins were separated by SDS-PAGE (13 % (w/v) resolving gel) and transferred onto polyvinylidene difluoride (PVDF) membrane (TransBlott, Bio-Rad, Munich, Germany) using a Pegasus blotter (Phase, Lübeck, Germany) for 45 min at 0.8 mA/cm^2. The transfer buffer was 10 mM 3-(cyclohexylamino)-1-propane-sulphonic acid with 10 % (v/v) methanol, pH 11.0 (23). The membrane was stained with Coomassie Brilliant Blue (Merck, Darmstadt, Germany) as described (25). Protein bands were excised and used for microsequencing by Edman degradation with a 473 A pulsed liquid protein sequencer with an on line phenylthiohydantoin amino acid analyzer (Applied Biosystems, Weiterstadt, Germany). Prior to sequencing, three on-membrane deblocking methods were applied for the 35 kDa protein according to Hirano et al. (17) to remove N-terminal acetylserine or acetylthreonine, formyl groups, and pyroglutamic acid.

Results

In a first screening, 76 sera of birch pollen allergic individuals were tested for IgE binding to the 35 kDa protein on one-dimensional birch pollen immunoblots. Nine sera (12 %) gave positive results. The two pool sera Wn 13/32 and Zie 39/132 also showed strong IgE binding to the 35 kDa band. Two individual sera (A 5433 and Bo 160) and the birch pollen pool serum Wn 13/32 were selected for further studies on the cross-reactivity pattern of the 35 kDa allergen (see below). All three sera also reacted to some degree with the major birch pollen allergen Bet v 1, but none showed IgE reactivity to profilin. Almost all sera of birch pollen allergic patients tested exhibited IgE binding to a diffuse band at 34 kDa on birch pollen immunoblots. This band was not detectable by india ink staining or in silver stained gels.

Two-dimensional electrophoresis

To characterise the 35 kDa allergen and the 34 kDa component, birch pollen extract was separated by 2-D electrophoresis and blotted for immunodetection with suitable sera (Zie 39/132 and Bo 95). Two blots showing the respective protein spots are presented in Figure 1. The isoelectric point of the main spot of the 35 kDa allergen was determined as 7.4, with isoallergens being found between pI 6.2 and pI 7.5 (Figure 1A). The diffuse spot of the 34 kDa protein, however, was detected at an isoelectric point of about 5.7, similar as for the major birch pollen allergen Bet v 1 at 17 kDa (Figure 1B).

Isolation of allergens by electroelution and testing for purity

For specific inhibition studies, the 35 kDa protein as well as Bet v 1 were isolated. We chose the technique of electroelution from excised protein bands after separation by SDS-PAGE. This method allows the isolation of proteins in a single step when antibodies for affinity purification are not available. Because the 35 kDa allergen and the 34 kDa component (only present in small

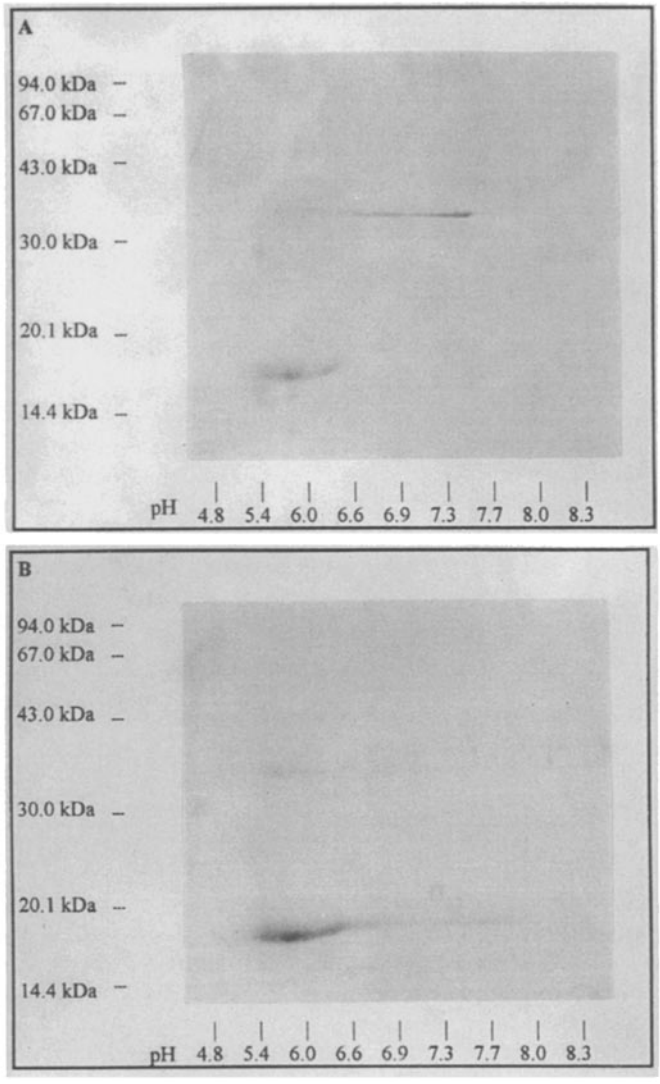

Fig. 1 IgE reactivity of sera Zie 39/132 tree pollen pool (A) and Bo 95 (B) (serum dilution 1:25) to birch pollen extract after 2-D PAGE and semi-dry electroblotting onto nitrocellulose membranes showing the 35 kDa protein (A) as well as Bet v 1 at 17 kDa (A and B). The diffuse spot at approximately pI 5.7 repesents the 34 kDa structure (B).

amounts) are situated closely together on SDS-PAGE gels, complete separation was problematic. To test for purity, the isolated proteins were run on a gel and silverstained (Figure 2 A) as well as blotted again to be detected with a serum recognising the 34 kDa component. While immunodetection revealed a slight impurity of the 34 kDa protein in the 35 kDa protein solution, we were surprised to find the 34 kDa band in the blot of isolated Bet v 1, too. When detected with the polyclonal serum rb-anti-Bet v 1 or the monoclonal Bet v 1 specific antibody BIP1, the 34 kDa band was recognised in addition to Bet v 1 (Figure 2 B), suggesting that it may represent a Bet v 1 dimer.

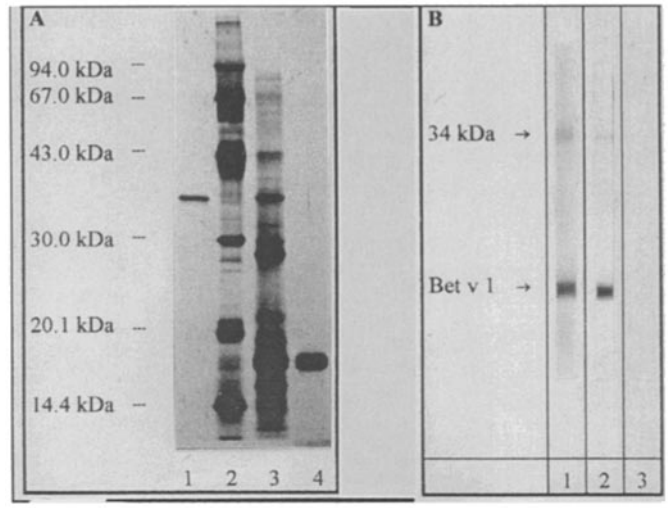

Fig. 2 A: silver stained SDS-PAGE gel of the isolated proteins: 1: 35 kDa protein; 2: molecular weight marker; 3: birch pollen extract; 4: Bet v 1.
B: binding of the polyclonal serum rb-anti-Bet v 1 (1) and the monoclonal antibody BIP 1 (2) to the isolated Bet v 1 protein electroblotted onto nitrocellulose after SDS-PAGE. 3: buffer control.

EAST and EAST inhibition studies

For characterisation of the 35 kDa birch pollen allergen's cross-reactivity pattern, three sera strongly reacting with the protein band (Wn 13/32 birch pollen pool serum and the individual sera A 5433 and Bo 160) were investigated for specific IgE in EAST and EAST inhibition experiments with extracts of various fruit and vegetables coupled to self-prepared allergen disks. Extracts of two other pollen species (mugwort and timothy) were included in the study. After determination of the EAST class, EAST inhibitions were performed with whole birch pollen extract, the isolated 35 kDa and Bet v 1 proteins and ovalbumin as a negative control. The results are summarised in Table 1. The 35 kDa protein significantly inhibited IgE binding of the tested sera to extracts from various fruit and vegetables, e.g. apple, lychee and carrot, but not to mugwort pollen extract. Binding to timothy pollen extract was not inhibited by birch pollen extract at all. Bet v 1 also inhibited IgE binding to some but not all of the extracts which showed inhibition by the 35 kDa protein.

Immunoblot studies

To identify the proteins cross-reacting with the 35 kDa birch pollen allergen in various extracts, the three selected sera were used for immunoblot studies. Blot inhibitions were performed with the 35 kDa protein as well as with Bet v 1 to test whether the two structures

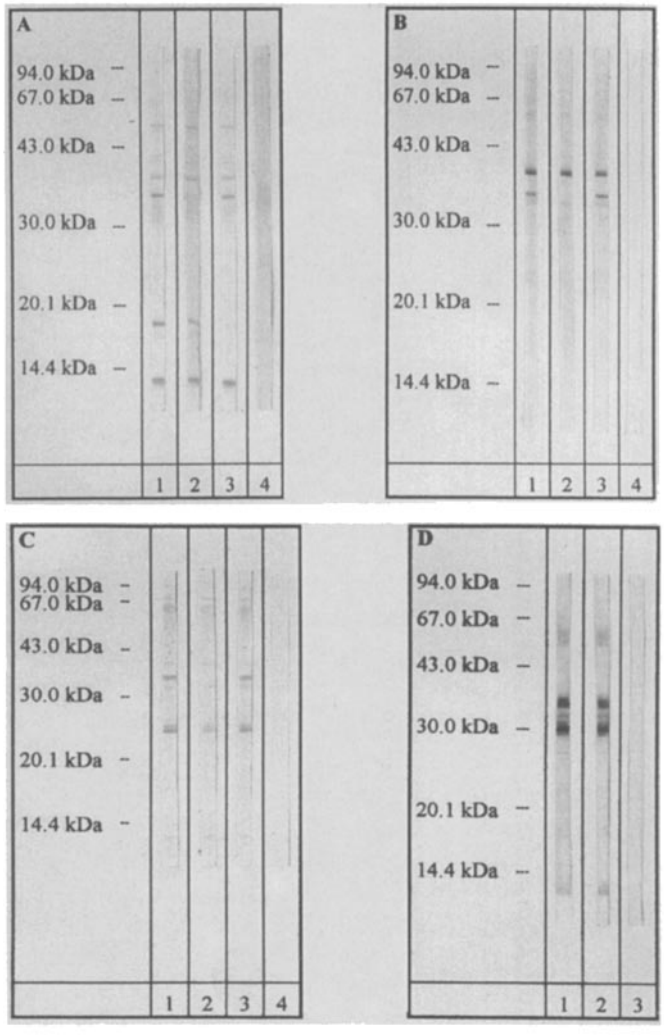

Fig. 3 A-C: Inhibition of IgE binding of serum Wn 13/32 to apple (A), lychee (B) and orange (C) extract separated by SDS-PAGE and transferred to nitrocellulose with the isolated 35 kDa and Bet v 1 proteins (5 µg/inhibition). 1: uninhibited; 2: inhibited with the 35 kDa protein; 3: inhibited with Bet v 1; 4: buffer control. D: Inhibition of IgE binding of serum grass pollen pool 18/100 to timothy grass pollen extract separated by SDS-PAGE and transferred to nitrocellulose with the isolated 35 kDa protein (5 µg). 1: uninhibited; 2: inhibited with the 35 kDa protein; 3: buffer control.

are immunologically independent. Bands inhibitable by the 35 kDa protein could be identified in apple, pear, lychee, mango, carrot, orange and banana, but not in cherry or coriander. Inhibition of the IgE binding of birch pollen pool serum Wn 13/32 by the 35 kDa protein and Bet v 1 is shown in Figure 3 for apple, lychee and orange blots. Figure 4 shows the detection of the 35 kDa cross-reactive bands in apple, lychee, orange, pear, mango, carrot, and banana as well as the cherry blots with the three sera tested. The cross-reactive proteins are all of uniform size (34-35 kDa) but not very abundant in most extracts, i.e. hardly or not at all detectable by india ink staining, while the birch pollen protein is one of the major bands on india ink stained blots. IgE binding to the 35 kDa birch pollen protein (not shown) or to the 35 kDa food proteins was not inhibitable by Bet v 1, showing that they are immunologically independent of Bet v 1, or by ovalbumin, which was used as an inhibition control. A nonallergic control serum did not bind to any of the proteins in the range of 34 - 35 kDa (Figure 4, lanes number 4). However, weak nonspecific staining of some bands occurred with the control serum. The serum recognised a high molecular weight band in lychee (Figure 4 B) and mango (Figure 4 E). Some other bands of the control serum were also present in the buffer control (Figure 4, lanes 5), for example a very abundant protein in cherry (Figure 4 H). These bands were therefore regarded as irrelevant. The isolated 35 kDa protein did not inhibit binding of the grass pollen pool serum 18/100 to a timothy grass pollen blot (Figure 3 D).

Microsequencing

SDS-PAGE separated birch pollen extract was blotted onto a PVDF membrane, the 35 kDa band and the 34 kDa region excised (although, in case of the latter, there was not enough protein to be visible in Coomassie staining) and submitted to N-terminal microsequencing. For the 34 kDa component only the first six amino acids could be determined due to the low amount of protein, but these (G V F N Y E) were completely identical to the published sequence of Bet v 1 (5, 19) and to previous own microsequencing results. Direct sequencing of the 35 kDa protein failed, probably due to the protein being blocked at the N-terminus. Attempts to deblock the protein by (i) treatment with trifluoracetic acid vapor to remove N-terminal acetylserine or acetylthreonine, (ii) treatment with 0.6 M HCl to remove formyl groups, and (iii) treatment with pyroglutamyl peptidase to remove pyroglutamic acid were equally unsuccessful.

Discussion

The frequency of IgE binding to the 35 kDa protein we observed in our serum screening (12 %) is in agreement with previous studies of other groups who detected a minor birch pollen allergen of the same size (15, 16, 20). In EAST inhibition studies with the isolated 35 kDa birch pollen protein we showed that it is cross-reactive to proteins in various plant foods, but not to mugwort or timothy pollen extract, binding to which was not inhibitable by either the 35 kDa protein or Bet v 1, or by birch pollen extract, respectively. This result shows that the 35 kDa protein is not a plant pan-allergen similar to profilin (28) which is present in almost all kind of pollen and plant food. Immunoblot inhibitions clearly

Table 1 EAST and EAST inhibition with birch pollen extract and isolated allergens for three different sera and various fruit, vegetables and two pollen species

	Wn 13/32				A 5433				Bo 160			
Allergen Disk	EAST class	% inhibition by birch	35 kDa	Bet v I	EAST class	% inhibition by birch	35 kDa	Bet v I	EAST class	% inhibition by birch	35 kDa	Bet v I
apple	3.3	93	56	64	3.1	73	34	50	2.4	98	90	43
pear	3.4	93	79	23	3.0	77	55	16	3.0	97	94	16
lychee	3.5	96	90	–	2.5	81	71	–	3.2	95	88	–
mango	3.3	96	91	–	1.9	79	69	–	2.9	97	93	–
peach	3.0	86	68	22	2.4	67	58	20	1.3	n.t.	n.t.	n.t.
orange	3.0	97	82	–	2.0	86	59	–	2.1	100	97	–
cherry	3.8	93	67	64	2.5	70	46	48	2.4	97	76	56
carrot	3.7	92	85	–	2.1	79	64	–	2.5	100	95	–
coriander	3.0	94	64	44	2.3	89	55	58	1.7	95	84	–
parsley	2.4	88	56	32	1.9	n.t.	n.t.	n.t.	0.3	n.t.	n.t.	n.t.
basil	2.2	88	60	–	1.3	n.t.	n.t.	n.t.	0.7	n.t.	n.t.	n.t.
hazelnut	2.9	89	56	44	2.4	53	–	–	3.1	93	72	76
celery	2.7	76	46	47	2.0	n.t.	n.t.	n.t.	0.4	n.t.	n.t.	n.t.
potato	2.0	80	37	28	0.2	n.t.	n.t.	n.t.	0.4	n.t.	n.t.	n.t.
banana	2.8	95	68	–	2.4	n.t.	n.t.	n.t.	2.0	100	87	–
pea	2.5	80	50	24	1.8	n.t.	n.t.	n.t.	0.5	n.t.	n.t.	n.t.
sunflower	2.7	97	40	28	1.8	n.t.	n.t.	n.t.	0.6	n.t.	n.t.	n.t.
onion	3.0	43	17	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
zucchini	3.0	59	–	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
kiwi	2.2	45	19	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
strawberry	2.8	45	21	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
grape	2.8	42	–	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
tomato	2.8	53	25	–	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Brussels sprouts	2.5	76	41	26	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
mugwort	2.5	40	–	–	3.1	20	n.t.	n.t.	0.1	n.t.	n.t.	n.t.
timothy	4.3	–	n.t.	n.t.	4.1	–	n.t.	n.t.	0.2	n.t.	n.t.	n.t.

Serum dilution: 1:2.5. Inhibitor concentrations: birch pollen, ovalbumin: 250 µg of protein/ml, purified allergen fractions: 25 µg/ml. Non-specific binding and inhibition with ovalbumin were taken as negative controls. Inhibition values of less than 15 % were considered as negative (-). n.t. = not tested.

revealed that the 35 kDa protein is immunologically independent of Bet v 1. The 35 kDa cross-reactive bands in various fruit and vegetable extracts were inhibitable by the birch pollen 35 kDa protein but not by Bet v 1.

The fact that, in the EAST inhibition studies, the 35 kDa protein and Bet v 1 inhibitable portions taken together sometimes yield values higher than 100 % is probably due to an impurity of the Bet v 1 dimer in the isolated 35 kDa protein which may be relevant for inhibition of extracts containing proteins highly cross-reactive to Bet v 1. While the IgE binding of the tested sera to some fruit and vegetable extracts was found to be inhibitable to a significant degree by both Bet v 1 and the 35 kDa protein in EAST inhibition experiments (e.g. apple, pear, peach, cherry), only the 35 kDa protein seemed to be of relevance for the cross-reactivity with lychee, mango, orange, carrot and banana. In the case of carrot, this result is surprising because carrot has been shown to contain a Bet v 1 related protein (9, 14, 27). However, the carrot protein is not always recognised by sera from patients sensitised to Bet v 1 and is not recognised by the Bet v 1-specific monoclonal antibody BIP 1 (9, 27 and own observations), suggesting that it may be immunologically less related to Bet v 1 due to sequence variations among the allergens. IgE binding to some other plant food extracts (e.g. coriander, parsley, pea) was inhibitable by the 35 kDa protein to a lesser degree. These extracts may contain small amounts of proteins cross-reactive to the 35 kDa protein. Cherry revealed no 35 kDa b and on immunoblots (Figure 4 H), but showed a high degree of cross-reactivity in the EAST. This effect may be due to intensive IgE binding to the Bet v 1 dinner or to the limited detection treshold of immunoblotting.

Interestingly, the 35 kDa protein cross-reacts with proteins from some plant foods which are not enclosed in the common birch pollen related food allergy cluster. Allergy to exotic fruit like lychee, mango and banana co-occuring with allergy to birch pollen may now in some cases be explainable by sensitisation to the 35 kDa cross-reactive structure. Allergy to lychee, however, has in one case been shown to be caused by a sensitisation to profilin (11).

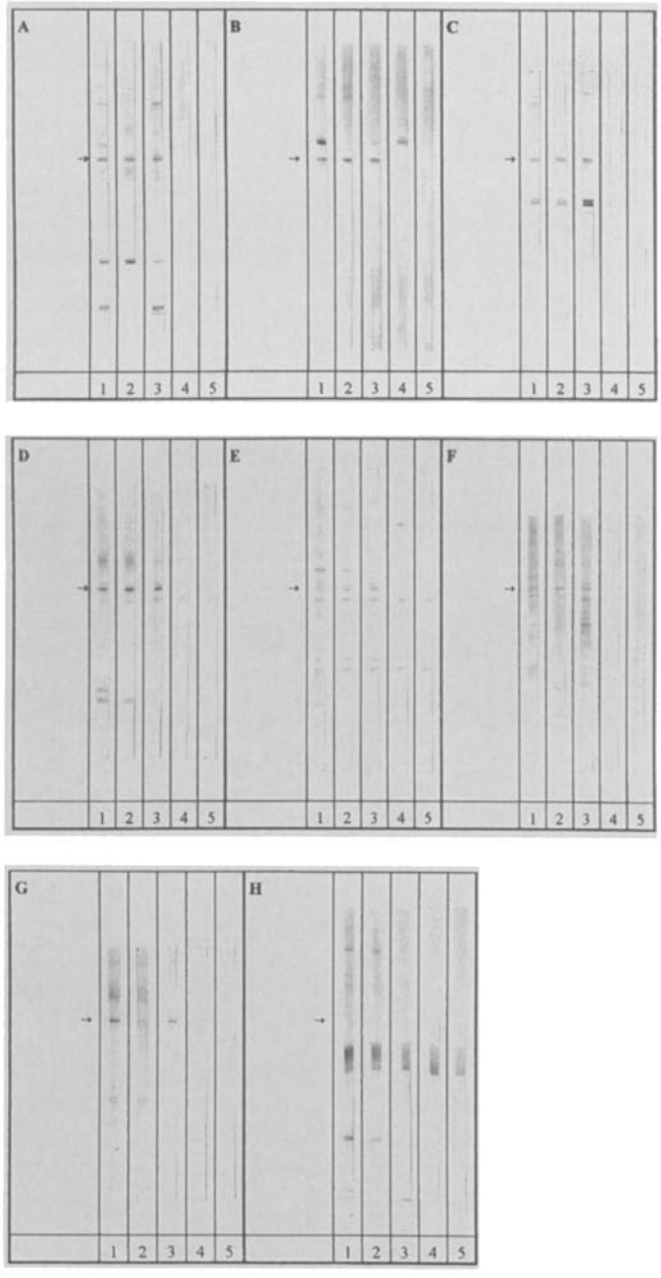

Fig. 4 Immunoblot analyses for detection of the 35 kDa cross-reactive proteins in apple (A), lychee (B), orange (C), pear (D), mango (E), carrot (F), banana (G) and cherry (H). Sera: 1: Wn 13/32; 2: A 5433; 3: Bo 160; 4: control serum from a non-allergic individual; 5: buffer control. The position of the bands inhibitable with the isolated 35 kDa protein is marked.

Since the detection of specific IgE to food proteins does not always correspond to allergic symptoms (3), further studies to highlight the clinical importance of sensitisation to the 35 kDa allergen are necessary. Clinical data from patient Bo 160, whose serum was investigated in this study, suggest that there may be a clinical relevance for the 35 kDa protein: This patient suffers from symptoms to birch pollen, apple, banana, carrot, hazelnut and walnut. Our immunoblot studies indicate that at least in the case of banana the 35 kDa protein may be responsible for an allergic reaction (compare figure 4, lane 3). In skin prick tests, the patient Bo 160 also shows a positive reaction to lychee, but nothing is known about possible symptoms to this fruit. Further work on the clinical relevance of the 35 kDa cross-reactive structure is in progress.

Because direct N-terminal sequencing of the 35 kDa protein was not possible, we believe that it is N-terminally blocked. The blocking group appears to be neither acetylserine or -threonine nor a formyl group or pyroglutamic acid, because deblocking reactions to remove these groups did not yield products which could be sequenced successfully. Further work to elucidate the molecular structure of the 35 kDa birch pollen allergen and the corresponding food proteins is in progress.

During our studies on the 35 kDa cross-reactive protein we identified a 34 kDa component in birch pollen extract, which was present in very low amounts and not detectable by silver or india ink staining but only by immunodetection. We conclude that this component is a Bet v 1 dimer, because (i) it is recognised by all patient sera strongly reacting with Bet v 1 but not by sera which do not contain Bet v 1 specific IgE, (ii) the isoelectric point is the same as for Bet v 1, (iii) it was found in isolated Bet v 1 purified from the 17 kDa region of SDS-PAGE gels, (iv) the 34 kDa component is recognised in addition to Bet v 1 by the rabbit polyclonal serum rb-anti-Bet v 1 and the Bet v 1 specific monoclonal antibody BIP 1, (v) the six N-terminal amino acids are identical to the published sequence of Bet v 1 (5, 19). We propose that a small proportion of Bet v 1 is present as a dimer and the dimer may also be formed during storage and sample preparation of the purified allergen. Recently, a dimer of the timothy grass pollen allergen Phl p 1 has been observed (26). In this case the dimer was only present when electrophoresis was performed under non-reducing conditions, indicating that dimerisation occured via disulphide bond formation. Because we detected the same amount of the Bet v 1 dimer under reducing (one-dimensional electrophoresis) and non-reducing (two-dimensional electrophoresis) conditions, the dimerisation of Bet v 1 is unlikely to be due to disulphide bond formation. The mechanism of dimerisation remains unclear.

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