

# IDENTIFICATION OF LATEX UDP GLUCOSE PYROPHOSPHORYLASE (HEV B UDPGP) AS A NOVEL CAUSE OF LATEX FRUIT ALLERGY SYNDROME

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Based on a recently published unusual case of food allergy in a latex-allergic patients, the present study identifies Hev b UDPGP as a novel allergen in natural rubber latex able to cause latex-fruit allergy syndrome and as a novel, potential pan-allergen in vegetable foods.

Food allergy - Latex allergy - Cross-reactivity - Allergens - UDP glucose pyrophosphorylase.

## INTRODUCTION

Latex allergy is frequently associated with severe adverse reactions to plant-derived foods due to the presence of homologous proteins in fruits and vegetables (1, 2). This condition has been called latex-fruit syndrome. Most cases of latex-fruit syndrome are caused by sensitization to hevein (Hev b 6.02); proteins homologous to hevein have been detected in avocado, chestnut, banana, kiwi, tomato, passion fruit, papaya, and mango (3). Other cases seem associated with the sensitization to b-1,3-glucanase (Hev b 2); homologous proteins have been detected in avocado, chestnut, banana, fig, and bell pepper (4). Finally, Hev b 7 seems to cross-react with patatin from potato (5).

We recently reported the case of a latex-allergic man who experienced anaphylaxis following the ingestion of plums and, on a separate occasion, a milkshake containing peach, banana, and grapefruit (6). On skin tests the patient reacted to kiwi, apricot, plum, peach, raw potato, apple, tomato, papaya, mango, and raw chestnut. On immunoblot analysis patient's serum showed a strong IgE reactivity to both latex and plum proteins in the 60-80 kDa range in the absence of reactivity to latex allergens known to be associated with the latex-fruit syndrome. Inhibition experiments demonstrated the cross-reactivity between latex and plum allergens recognized by patient's serum. Patient's reactivity to all those botanically unrelated

fruits suggested sensitization to a highly conserved allergen. Unfortunately, however, immunoblots with other plant-derived foods were negative (6). In the present study we now report the characterization of the allergen responsible of the cross-reaction in this patient.

## METHODS

### Latex

Natural rubber latex (B and C serum fraction [7], supplied by Prof. Esah Yip, Rubber Research Institute of Malaysia) was centrifuged at 20,000 rpm at 4°C for 1 h. Supernatant was gel filtered on a Sephadex G25 column (PD10, Pharmacia, Milan, Italy) and equilibrated with PBS containing 0.05% NaN<sub>3</sub>. Fractions corresponding to proteins showing a MW > 5000 Da (according to instruction's manufacture) were then collected and used as latex extract.

### IgE ELISA

For ELISA experiments the latex extract (10mg/ml; 25mM NaHCO<sub>3</sub>, pH 9.6) was coated in duplicates on polystyrol microtiter wells (Nunc Maxisorp, Nalge Nunc International, Roskilde, Denmark) overnight at 4°C. Non-specific binding sites were blocked with 2% milk in TBS/0.05% Tween 20 (v/v). Coated protein was incubated with patients serum (1:4 dilution) overnight at 4°C. Bound IgE was detected with alkaline phosphatase-conjugated mouse anti-human IgE antibody (1:1000; BD-Biosciences Pharmingen, San Diego,

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Calif., USA) and developed with the p-nitrophenyl phosphate substrate. Color development was measured using an ELISA reader (Spectra Max Plus 384; Molecular Devices GmbH, Munich, Germany) at 405 nm and 510 nm as reference wavelength. Optical density (OD) values from normal human serum (n=5) samples were obtained and standard deviation calculated. All OD values from serum samples (100 µl/well) above mean value plus 3x standard deviations were considered positive. For identifying carbohydrate moieties present on proteins relevant for IgE binding activity inhibition with horseradish peroxidase (HRP; Sigma Aldrich) which contains the relevant plant N-glycans (MuXF, MMXF; [8]) was performed. Therefore, the patient's serum sample was preincubated overnight at 4°C with HRP 10mg/ml. Subsequently, the IgE ELISA was performed as described above.

## 2D Electrophoresis

5µl of the latex extract solution (at a concentration of 10mg/ml) were diluted with rehydration buffer (urea 7M, thiourea 2M, CHAPS 4%, BioLyte Ampholyte, Bio-Rad, 0.2%, DTT 65mM, BBF traces) up to a total volume of 125 µl and applied to 7cm ReadyIPG strips (Bio-Rad), pH range 3-10NL. The strips were focused on Protean IEF Cell (Bio-Rad) after a 14h passive rehydration followed by a rapid linear voltage slope until 30000 Vhrs were reached.

Prior to SDS-PAGE, the strips were equilibrated in NuPAGE sample buffer (Invitrogen) containing DTT 50mM in the first step and iodoacetamide 65mM in the second one. Proteins were then separated according to their molecular weights on NuPAGE ZOOM gels 12% (Invitrogen), using MOPS-SDS as running buffer, 200 V constant. Gels were either stained with Coomassie Blue (Fig.1A) or electroblotted onto 0.2µm nitrocellulose membrane (Sigma) in a semi-wet blot module (Invitrogen) and reversibly stained with Red Ponceau.

## Immunoblotting

Membranes were blocked 2x15' with TBS pH 7.4/Tween 0.3% and incubated overnight with patient's or control serum diluted 1:2 in TBS/Tween 0.05%/gelatin (Bio-Rad) 0.05%. 2D-immunoblots were rinsed with TBS/Tween 0.03% and incubated with alkaline phosphatase conjugated goat anti-human-IgE/PALK, Pharmingen, diluted 1:750 in TBS/Tween 0.05%/gelatin (Bio-Rad) 0.05% for one hour. After the washing steps, immunoblots were developed with phosphatase substrate BCIP/NBT (Bio-Rad) (Fig.1B and 1C).

## Protein identification

The spots in the gel corresponding to the positive spots in the immunoblotting experiment were cut from the gel and destained overnight with a solution of 25 mM ammonium bicarbonate and 50% acetonitrile. The proteins were digested "in gel" with trypsin (Promega, Madison, WI, USA) as described by Hellmann and co-workers (9).

The tryptic peptide mixtures were analysed by LC-MS/MS using an Agilent (Palo Alto, CA, USA) 1100 series nano HPLC coupled to an Agilent XCT Plus ion trap fitted with a nano electrospray nebulizer. The chromatographic separations were run on a C18 nanocolumn Zorbax 300SB 150 x 0.075 mm (Agilent, Palo Alto, CA, USA). Injection volume was 1 µL and flow rate 300 nL/min. Gradient mobile phase composition was adopted: 95/5 for the first ten minutes to 45/55 in 35 min and then to 70/30 in 5 min. Eluent A: water 0.1% formic acid 5% acetonitrile, eluent B: acetonitrile 0.1% formic acid. Capillary voltage was 1600 V and fragmentation voltage was 1.3 V.

For elaboration of the LC and MS/MS data the Data Analysis software (Agilent, Palo Alto, CA, USA) was used. Data interpretation was achieved by Mascott software (Matrix Science Ltd, London UK).

## RESULTS

IgE binding to components of the latex extract could be demonstrated by ELISA. Preincubation of the serum sample with HRP reduced IgE binding by only 27% as determined by OD values compared to the positive control suggesting that in this case the antibody response is primarily directed against protein determinants and not carbohydrates of a HRP type. Patient's serum recognized two main immune-reactive spots in latex extract. The two main immunoreactive spots, labelled as 1 and 2 in Figure 1B, showed an approximate MW of 52 kDa and a pI of 4.9 and 5.1,

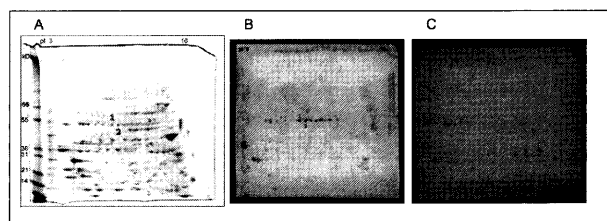


**Figure 1:** A: 2-D electrophoresis of latex protein extract. Solubilized latex proteins were separated in the first dimension using 7 cm pH3-10 NL IPG strips and in the second dimension using 12% SDS-PAGE. The numbers indicate the two spots excised and identified. B: 2-D immunolabeling of latex protein extract with specific IgE from patient's serum. The numbers indicate the two immunoreactive spots corresponding to the identified proteins. C: negative control with a non allergic patient serum.

respectively. These were finally identified as UDP glucose pyrophosphorylase (Hev b UDPGP) by searching in NCBI database updated till 18 Oct 2005 by Mascot software. Because the amino acid sequence of UDPGP from latex is unknown, its identification was achieved by homology with *Arabidopsis thaliana* (gi | 14532836) (Table 1). Thanks to the high homology of this enzyme in plants (more than 80% of identical amino acid sequences) it was possible to sequence by MS/MS 6 internal peptides from spot 1 and 9 internal peptides from spot 2, with amino acid sequences identical to those from *Arabidopsis thaliana* UGPase. A sequence coverage of the UDPGP sequence of 27% and 18% has been obtained, respectively.

|     |                                          |
|-----|------------------------------------------|
| 1   | MAATATEKLP QLKSAVDGLT EMSENEKSGF         |
|     | INLVSRYLSG EAQHIEWSKI                    |
| 51  | QTPIDEIVVP YDKMANVSED ASETKYLLDK         |
|     | LVVLK <b>LNGGL GTTMGCTGPK</b>            |
| 101 | SVIEVRDGLT FLDLIVIQIE NLNNKYNCKV         |
|     | PLVLMNSFNT HDDTQKIVEK                    |
| 151 | YTKSNVDIHT FNQSKYPRVV ADEFVPWPSK         |
|     | GKTDKDGWYP PGHGDVFPPL                    |
| 201 | MNSGKLDLAF SQGK <b>EYVFIA NSDNLGAIVD</b> |
|     | <b>LK ILKHLIQN KNEYCMEVTP</b>            |
| 251 | <b>KTLADVKGKT LISYEGK VOLLEIAQVPDEH</b>  |
|     | <b>VNEFKSIEKF</b> KIFNTNNLWV             |
| 301 | NLKAIKKLVE ADALKMEIIP NPKEVDGVKV         |
|     | <b>LQLETAAGAA</b> IRFFDNAIGV             |
| 351 | NVPRSRFLPV KATSDLLLVQ SDLYTLVDGF         |
|     | <b>VTRNKARTNP</b> TNPAIELGPE             |
| 401 | FKKVASFSLR FKSIPSIVEL <b>DSLKVS</b> GDVW |
|     | FGSGVVLK GK VTKANAGTK                    |
| 451 | LEIPDNAVLE NKDINGPEDL                    |

**Table 1:** Sequence of the putative UDP glucose pyrophosphorylase from *Arabidopsis thaliana* (gi | 14532836). Identification of spot 1 and 2 of latex extract (Fig 1A) has been obtained after MS/MS sequencing of their tryptic peptides. Peptides in bold character show identical sequences in putative UDP glucose pyrophosphorylase from *Arabidopsis thaliana* (gi | 14532836) and in protein contained in spots 1 and 2, which have been therefore identified as latex UDP glucose pyrophosphorylase. Peptides in italics are unique for spot 2.

## DISCUSSION

UDP-glucose pyrophosphorylase (UGPase) is found in all prokaryotic and eukaryotic organisms; it catalyses the formation of UDP-glucose (UDP-Glc), a key precursor for polysaccharide synthesis. In addition to its key function in primary carbohydrate metabolism

in plants, UDP-Glc in all eukaryotes is essential in the synthesis of carbohydrate moiety of glycolipids, glycoproteins and a variety of secondary metabolites, among other functions. When analyzed at the derived protein level, there was more than 80% identity for plant UGPases (10). For this reason UGPase has been easily identified by homology in *Arabidopsis thaliana* database, being the complete amino acid sequence of this protein from latex still unknown. UDP glucose pyrophosphorylase (Hev b UDPGP) has been considered as a potential allergenic protein in natural rubber latex (11). However, it was not included into the most updated list of latex allergens that was recently published (12). We were lucky to find and study a patient that was virtually monosensitized to latex UDP glucose pyrophosphorylase. This allowed us to show that Hev b UDPGP is clinically relevant and cross-reacts with homologous proteins in vegetable foods.

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