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Mutational epitope analysis and cross-reactivity of two isoforms of Api g 1, the major celery allergen

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Abstract

For better understanding the cross-reactivity between the major birch pollen and celery allergens, Bet v 1 and Api g 1, respectively, putative epitope areas and structurally important positions for IgE-binding of the isoforms Api g 1.01 and Api g 1.02 were point mutated. The IgE binding capacities were measured in ELISA, the IgE cross-reactivity between the isoforms, mutants and Bet v 1 investigated by ELISA-inhibition experiments with serum pools from patients with confirmed celery allergy (DBPCFC). Api g 1.01 displayed a clearly higher frequency and capacity of IgE binding than Api g 1.02. In Api g 1.01, substitution of lysine against glutamic acid at amino acid position 44, a key residue of the Bet v 1 "P-loop", increased the IgE-binding properties. Structural instability due to proline insertion at position 111/112 resulted in loss of IgE binding of Api g 1.01, but not of Api g 1.02. Between Api g 1.01 and Api g 1.02 only partial cross-reactivity was seen. The data suggest that the IgE epitopes of the two isoforms are distinct and that in contrast to Api g 1.01, the "P-loop" region plays an important role for IgE binding of celery allergic subjects to Api g 1.02. Understanding and investigation of the molecular mechanisms in celery allergy is an important step to generate hypoallergenic proteins for safe and efficacious immunotherapy of food allergy.

Keywords: Celery; Epitopes; Food allergy; Isoform; Mutation

1. Introduction

Allergy to celery tuber (celeriac) is a common plant food allergy in Europe, in particular in Switzerland, France and Germany. In Switzerland, it ranks among the most prevalent food allergies (Etesamifar and Wüthrich, 1998) and has been reported as the most frequent cause of food anaphylaxis (Rohrer et al., 1998). Approximately 50% of celery allergic subjects in Switzerland present a case history of systemic allergic reactions (Ballmer-Weber et al., 2000; Luttkopf et al., 2000). Clinically, celery allergy is associated to both birch pollen and mugwort pollen allergy, which is due to the existence of cross-reacting allergens in the respective pollen, which are also thought to represent the sensitizing allergens. The known allergens of celery tuber are the major allergen Api g 1.01 (Breiteneder et al., 1995) and its isoform Api g 1.02 (Hoffmann-Sommergruber et al., 2000), the minor allergen profilin Api g 4 (Scheurer et al., 2000) and Api g 5 (Bublin et al., 2003) a protein showing homology to FAD (flavin adenine dinucleotide) containing oxidases. The allergenicity of Api g 5 depends on *N*-glycans containing xylose and fucose residues and does not depend on the protein sequence. Several studies have shown that Api g 1 is the most important allergen in celery tuber (Breiteneder et al., 1995; Vieths et al., 1995; Hoffmann-Sommergruber et al., 1999; Luttkopf et al., 2000). The clinical presentation of celery allergy differs from other pollen related food allergies and appears to include more severe symptoms than oral allergy syndrome (Mari et al., 2005). Moreover, clinical reactivity to heat-processed celery has been confirmed by double-blind, placebo-controlled food challenges (Ballmer-Weber et al., 2002). Therefore, studying the IgE epitope structure of Api g 1 and IgE binding to its isoforms is of

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particular interest, because a specific set of epitopes or different stability characteristics could be responsible for a different clinical reactivity to this Bet v 1 related allergen. Due to the polyclonal nature of the IgE response, it is difficult to analyse directly the structure of immune complexes between IgE and allergens. Therefore, most studies on IgE epitopes of Bet v 1 related allergens have focused on site-directed mutagenesis (Ferreira et al., 1998; Scheurer et al., 1999; Neudecker et al., 2003; Wiche et al., 2005; Ma et al., 2006) or, more recently on direct affinity selection and enrichment of IgE binding surface structures utilising libraries of peptide mimics (Mittag et al., 2006). In the present study we focused on the "P-loop" which has been suggested as a major epitope area on Bet v 1 (Mirza et al., 2000) and homologous food allergens (Holm et al., 2001; Neudecker et al., 2003; Mittag et al., 2006). In addition, we found that introduction of a proline residue in position 112 of Bet v 1, or equivalent position of homologous food allergens led to an almost complete reduction of IgE binding capacity of Bet v 1 and the apple allergen Mal d 1 (Son et al., 1999) as well as the cherry allergen Pru av 1 (Scheurer et al., 1999; Neudecker et al., 2003) which could in the case of Pru av 1 be attributed to a complete loss of secondary structure. Therefore, the relevance of this position for the allergenicity of Api g 1 was also studied. In an initial screening the relevance of Api g 1.01 and Api g 1.02 was analysed for the first time in a study population in which food allergy to celery had been confirmed by double-blind, placebo-controlled food challenges. Our data revealed that the epitope structure of Api g 1 and its cross-reactivity of Bet v 1 with Api g 1 isoforms is complex and that mutation of positions equivalent to amino acid 112 of Bet v 1 has a significant impact on the structural stability of Api g 1 isoforms.

2. Materials and methods

2.1. Patients sera

In total 66 sera were included in this study. Twenty-three were from patients with a confirmed food allergy to celery tuber (celeriac) as demonstrated by a positive reaction in a double-blind, placebo-controlled food challenge (DBPCFC) with celery. Detailed clinical data such as case history of adverse reactions to celery and symptoms in response to DBPCFC as well as skin tests and serological data have been published elsewhere (Ballmer-Weber et al., 2000, 2002; Luttkopf et al., 2000). As controls, 22 sera were taken from birch pollen allergic patients without celery allergy, confirmed by a negative open food challenge test with fresh celery tuber, and 21 sera from healthy donors without allergy were included. Written informed consent was obtained from all study participants.

2.2. Cloning and mutagenesis of the recombinant celery allergens

Recombinant (r) Api g 1.0101 was generated as previously described (Neudecker et al., 2003). Api g 1.0201 cDNA (GenBank accession no. Z75662) was amplified from mRNA isolated from celery tuber by RT-PCR using the gene specific primers Api g 1.02(+)Nde 5′-GTA CAT ATG GGT GTC CAA AAG ACC GTG GTT GAG GCT and Api g 1.02(−)Bam 5′-CTA GGA TCC TCA AGC AAG AAA CTG CAA GTT TGC TAG (restriction sites underlined) and cloned in pCRII-Topo vector for sequence verification (Invitrogen, Karlsruhe, Germany). For expression the cDNA was directionally ligated in the pET-11a vector (Novagen, Schwalbach, Germany) between *Nde*I and *Bam*HI restriction sites. Mutagenesis reactions were performed with the QuikChange® Site-Directed Mutagenesis Kit (Stratagene, Amsterdam, The Netherlands) using either the Api g 1.0101-pET11a or the Api g 1.0201-pET11a construct as template. Two Api g 1.0101 variants were generated. One single point mutation was inserted at position 44 ($K^{44}E$) using the mutagenic primers Apig1.01K^{44}E(+) 5′-GCT TAC AAG AGT GTA GAA ATC ***GAG*** GGA GAT GGT GGA CC-3′, Apig1.01K^{44}E(−) 5′-CCA GGT CCA CCA TCT CC***C TC***G ATT TCG ACA CTC TTG-3′ and one at amino acid position 111 ($S^{111}P$) primed by Apig1.01S^{111}P(+) 5′-CT GCT GAT GGA GGA ***CCC*** ATT TGC AAG ACC ACT GCC ATC-3′ and Apig101S^{111}P(−) 5′-GGC AGT GGT CTT CCA AAT ***GGG*** TCC TCC ATC AGC AGT TGG C-3′. One mutation of Api g 1.0201 at position 11($C^{111}P$) was introduced using the primers Apig102C^{111}P(+) 5′-GTG CCA ACC GAC GGA GGT ***CCC*** ATA GTG AAG AAC ACC ACC-3′ and Apig102C^{111}P(−) 5′-GGT GTT CTT CAC TAT ***GGG*** ACC TCC GTC GGT TGG CAC AAC AAC G3′ (mutated regions in bold italic). All mutation reactions were performed according to the manufacturers instructions.

2.3. Expression and purification of recombinant celery allergens

For expression as non-fusion proteins, the mutated plasmids were transformed into *Escherichia coli* BL21(DE3) competent cells (Novagen, Schwalbach, Germany). Protein synthesis was induced overnight at 23 °C by adding isopropyl-β-D-thiogalactoside (IPTG) (Carl Roth, Karlsruhe, Germany) to a final concentration of 1 mM, after cell growth was photometrically monitored to reach an optical density of 0.8 at 600 nm wavelength. Subsequently, cells were harvested by centrifugation, resuspended in lysis-buffer (50 mM NaH_2PO_4, 500 mM NaCl pH 7.0, 5 U/ml Benzonase (Merck, Darmstadt, Germany)) and lysed by repeated freezing and thawing. After 1 h rotation the extract was clarified by centrifugation for 30 min at 15,000 × *g*. The allergens were purified by preparative SDS-PAGE (Prep Cell Model 491, Bio-Rad, München, Germany) according to manufacturers instructions. The fractions that contained the pure allergen were pooled, dialyzed against 1/10 MOPS-buffer (2 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS), 0.5 mM sodium acetate, 0.1 mM EDTA, pH 7.0), precipitated with acetone and redissolved in 1/10 MOPS-buffer. Recombinant non-fusion Api g 1.0101 was purified as described (Neudecker et al., 2003). Due to low expression levels obtained with the pET11a construct generated in the present study, rApi g 1.0201, was applied as C-terminally hexahistidine tagged pro-