The cross-reactive calcium-binding pollen allergen, Phl p 7, reveals a novel dimer assembly

Supplementary material

Results:

Reduction of IgE recognition of Phl p 7 by mutation of calcium-binding domains or fragmentation

A recombinant Phl p 7 mutant (Mut-4) containing mutations in the calcium-binding domains and two synthetic fragments comprising the complete N-terminal (N-term) or the C-terminal (C-term) calcium-binding domain were compared with the wild-type Phl p 7 allergen regarding IgE binding capacity (Fig. 6). Nine out of ten sera from Phl p 7-allergic patients displayed strong (lanes 1–5, 7–10) and one serum weaker (lane 6) IgE reactivity to Phl p 7. The IgE binding capacity of the Phl p 7 mutant (Mut-4) was strongly reduced for four sera (lanes 4–6, 8) and completely abolished for six sera (lanes 1–3, 7, 9, 10). None of the ten sera showed IgE reactivity to the Phl p 7 fragments (lanes 1–10). No reactivity was observed when serum from a non-allergic patient or buffer without addition of serum was used (lanes N, b). Bound IgE antibodies were detected with ^{125}I-labeled anti-human IgE antibodies and visualized by autoradiography.

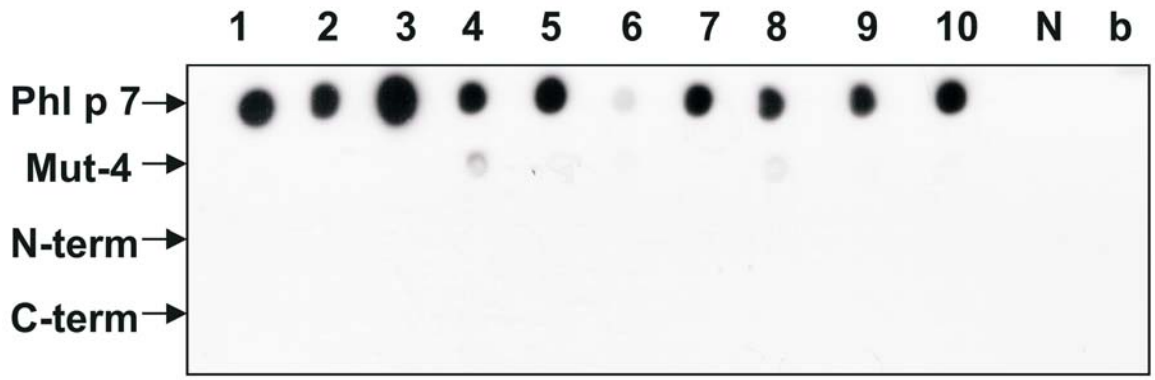

Figure 6. Reduced IgE binding capacity of a Phl p 7 mutant and Phl p 7 fragments.
Recombinant Phl p 7, a rPhl p 7 mutant with two amino acid exchanges in each of the calcium-binding domains (Mut-4) as well as two fragments containing the N-terminal (N-term) or the C-terminal (C-term) EF-hand were exposed to sera from ten Phl p 7-allergic patients (lanes 1–10), to serum from a non-allergic individual (lane N) or buffer alone (lane b).

Material and Methods:

Generation and purification of the Phl p 7 mutant and two Phl p 7 fragments

A Phl p 7 mutant containing two mutations in each calcium-binding domain was engineered by site-directed mutagenesis using the Chameleon double-stranded site-directed mutagenesis kit (Stratagene, East Kew, Australia) (Deng and Nickoloff, 1992).
The following mutations were made: First EF-hand: 17D $\rightarrow$ 17A, 24E $\rightarrow$ 24A; second EF-hand: 52D $\rightarrow$ 52A, 59E $\rightarrow$ 59A). The mutant was expressed in *Escherichia coli* and purified as described for Phl p 7 (Niederberger *et al.*, 1999).
Peptides representing the N-terminus without methionine (amino acid 2–37) or the C-terminus (amino acid 37–78) of Phl p 7 were synthesized by using a Fmoc (9-fluorenyl-methoxy-carbonyl)-strategy with HBTU [2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium-hexafluorophosphat]-activation (0.1 mmol small-scale cycles) on the Applied Biosystems (Foster City, CA) peptide synthesizer Model 433A, purified by HPLC and checked by mass-spectrometry (Focke *et al.*, 2001).

Comparison of the IgE binding capacity of Phl p 7, the Phl p 7 mutant and the two Phl p 7 fragments by dot blot experiments

Recombinant Phl p 7, the Phl p 7 mutant as well as the two Phl p 7 fragments were dotted onto nitrocellulose strips (1μg/dot) (Schleicher & Schüll, Dassel, Germany) and exposed to sera from Phl p 7-allergic patients, as described (Niederberger *et al.*, 1999). Bound IgE antibodies were detected with ^{125}I-labeled anti-human IgE antibodies (Pharmacia, Uppsala, Sweden) and visualized by autoradiography using KODAK X-OMAT films and intensifying screens (Kodak, Heidelberg, Germany) (Valenta *et al.*, 1992).

References:

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