

Structure of an IgE-Binding Peptide from Fungal Enolases

Birgit Simon-Nobbe^a Rimantas Kodzius^d Andrey Kajava^e Fátima Ferreira^a
Andreas Kungl^b Gernot Achatz^a Reto Cramer^d Christof Ebner^c
Michael Breitenbach^a

^aInstitute of Genetics and General Biology, University of Salzburg, ^bDepartment of Pharmaceutical Chemistry, University of Graz, and ^cDepartment of Pathophysiology, University of Vienna, Austria; ^dSwiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland; ^eCenter for Molecular Modeling, NIH-CIT, Bethesda, Md., USA

Key Words

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Detailed knowledge of the molecular properties of fungal allergens is a prerequisite for improvements in diagnosis and therapy of allergies caused by molds. *Cladosporium herbarum*, *Alternaria alternata* and *Aspergillus fumigatus* are among the most prevalent causes of mold allergies. Moreover, these species occur worldwide. Compared to allergens from pollens, insects and foodstuffs, little is known presently about the most important allergens of *C. herbarum* and *A. alternata*. One reason for this might be the low quality of commercial mold extracts used for allergy testing [1]. The major allergens of *A. alternata* [1, 2] and *C. herbarum* (unpubl. observations) are secreted proteins that are recognized by about 80 and 60% of the patients sensitized to the given mold species. However, we have previously shown that several other allergens of the molds are cytoplasmic household enzymes and proteins [3]. Prominent among them is enolase, a glycolytic

enzyme that is the second most important allergen in terms of frequency of sensitization to both *C. herbarum* and *A. alternata*. It is generally unclear at present how the intracellular proteins are presented to the immune system. A fraction of the cell's enolase is targeted to the cell wall in *Candida albicans* and *Saccharomyces cerevisiae* [4, 5]. If the same is true for *C. herbarum* and *A. alternata*, this could be one reason for the importance of enolase as an allergen.

We present here the mapping and detailed investigation of an IgE-binding structural epitope of *C. herbarum* enolase. Additionally we are showing that mold enolases are highly cross-reactive and that this cross-reactivity is mostly due to the conserved epitope mentioned above. Furthermore, we are presenting an attempt to explore the allergen repertoire of *C. herbarum* more thoroughly by using the pJuFo cloning system and by robotic hybridization screening of the library.

Ten different but overlapping *C. herbarum* enolase peptides spanning together the whole length of the enzyme's open reading frame were constructed by appropriate PCR reactions and cloning into pGEX-2T (Amersham-Pharmacia-Biotech). The resulting GST fusion polypeptides including the whole length GST-enolase were purified by glutathione affinity chromatography and

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Correspondence to: Dr. Michael Breitenbach
Institute of Genetics and General Biology
University of Salzburg, Hellbrunnerstrasse 34
A-5020 Salzburg (Austria)
Tel. +43 662 8044 5787, Fax +43 662 8044 144, E-Mail michael.breitenbach@sbg.ac.at

tested in IgE immunoblots using eight different patients' sera that had previously been shown to react to *C. herbarum* enolase. Surprisingly, 8/8 patients showed the same pattern of reactivity. They reacted to a 69-amino acid peptide (peptide 9: amino acids 120–189) and additionally only to those peptides which contained the sequence amino acids 120–189, but not to peptides which did not contain this amino acid sequence. Moreover, serum preincubation with peptide 9 completely abolished specific IgE binding to enolase of *C. herbarum* and markedly reduced the binding to enolase of *A. alternata*. We conclude that peptide 9 constitutes or contains (a) major and cross-reactive epitope(s) of fungal enolases.

Cross-reactivity of fungal enolases was also shown by IgE immunoblots of patients' sera recognizing several bands in the protein extract of *C. herbarum*, among them enolase, with protein extracts of *S. cerevisiae*, *C. albicans*, *A. fumigatus* and *A. alternata*. A single band corresponding to enolase (46–48 kD) was recognized in all protein extracts.

Homology-based structural modeling of *C. herbarum* enolase on the very highly homologous (74% identical amino acids with minimal gaps introduced) known three-dimensional structure of *S. cerevisiae* enolase-1 [6] resulted in a model for the structure of *C. herbarum* enolase. Peptide 9 in the context of this modeled structure showed a rather extended conformation spanning the diameter of the globular protein molecule twice and reaching the surface three times. Several plausible B cell epitopes are predicted where basic amino acids of peptide 9 are exposed on the surface. In vitro site-directed mutagenesis will show which one of the predicted epitopes is indeed involved in IgE binding.

Next, peptide 9 was expressed in a 6 × His-tagged form without a carrier protein and purified by nickel-chelate affinity chromatography. The resulting pure polypeptide was soluble and CD spectroscopy showed a stable structure quite different from the structure in the context of enolase with a relatively large amount of the β -pleated sheet. This polypeptide did not bind IgE. It is interesting to speculate how bacterial GST could display a 'helper' function for folding and/or stability of the natural conformation of peptide 9.

Our previously published [3] and unpublished *C. herbarum* allergens (7 in total) were all cloned by antibody screening of a cDNA expression library in λ -ZAP. As this method has clear limitations, we attempted to explore the complete repertoire of *C. herbarum* allergens by means of the phage display method [7]. First, the pJuFo plasmid was modified to enable directed cloning and the use of methylation protection of the cDNA inserts. Recombinant phages were immunoselected with a mixture of serum IgE from three *C. herbarum*-positive patients. 5,300 recombinant phages from the remaining library were then plated, picked and ordered by repeated hybridization screening. At least 13 new allergens were discovered, including some sequences showing high sequence homology to previously described allergens of *A. fumigatus*. Two new allergens display a high sequence homology to human proteins and are new candidates for so-called autoallergens [8].

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References

- 1 Unger A, Stöger P, Simon-Nobbe B, Susani M, Crameri R, Ebner C, Hintner H, Breitenbach M: Clinical testing of recombinant allergens of the mold *Alternaria alternata*. *Int Arch Allergy Immunol* 199;118:220–221.
- 2 De Vouge MW, Thaker AJ, Curran IHA, Zhang L, Muradia G, Rode H, Vijay H: Isolation and expression of a cDNA clone encoding an *Alternaria alternata* Alt a 1 subunit. *Int Arch Allergy Immunol* 1996;111:385–395.
- 3 Achatz G, Oberkofler H, Lechenauer E, Simon B, Unger A, Kandler D, Ebner C, Prillinger H, Kraft D, Breitenbach M: Molecular cloning of major and minor allergens of *Alternaria alternata* and *Cladosporium herbarum*. *Mol Immunol* 1995;32:213–227.
- 4 Chaffin WL, Lopez-Ribot JL, Casanova M, Gozalbo D, Martinez JP: Cell wall and secreted proteins of *Candida albicans*: Identification, function, and expression. *Microbiol Mol Biol Rev* 1998;62:130–180.
- 5 Pardo M, Monteoliva L, Pla J, Sanchez M, Gil C, Nombela C: Two-dimensional analysis of proteins secreted by *Saccharomyces cerevisiae* regenerating protoplasts: A novel approach to study the cell wall. *Yeast* 1999;15:459–472.
- 6 Stec B, Lebioda L: Refined structure of yeast apo-enolase at 2.25 Å resolution. *J Mol Biol* 1990;211:235–248.
- 7 Crameri R, Suter M: Display of biologically active proteins on the surface of filamentous phages: A cDNA cloning system for selection of functional gene products linked to the genetic information responsible for their production. *Gene* 1993;137:69–75.
- 8 Appenzeller U, Meyer C, Menz G, Blaser K, Crameri R: IgE-mediated reactions to autoantigens in allergic diseases. *Int Arch Allergy Immunol* 1999;118:193–196.

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