

Basidiomycete Allergy: Identification and Characterization of an Important Allergen from *Calvatia cyathiformis*¹

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Abstract. Basidiomycetes were not considered as major aeroallergen sources until spore traps revealed the prevalence of basidiospores, which were in some cases associated with epidemic outbreaks of asthma. More recently, we established that approximately one third of subjects with respiratory allergic disease were skin prick test positive to basidiospore extracts. Bronchial challenge with these extracts induced reversible immediate and late phase bronchospasm in sensitive subjects. Screening individual RAST-positive sera for reactivity to immunoprinted *Calvatia cyathiformis* spore extract indicated that a basic component with pI 9.3 reacted with 63% of test sera. Further analysis by RAST and immunoprint (IP) inhibition showed that this component, *Cal c* Bd9.3, appeared to cross-react with spore extracts from 3 of 5 other species tested. To isolate this component for additional analysis, a protocol was used with two sequential stages of preparative isoelectric focusing (IEF) followed by hydrophobic interaction chromatography. This yielded purified *Cal c* Bd9.3 that retained IgE-binding activity by IP, was a single band by IEF and SDS-PAGE (16 kD) analysis when stained with Coomassie blue. Double diffusion in gel with rabbit antiserum to *Cal c* Bd9.3 demonstrated a single precipitin band in crude extract that was identical to that obtained with purified *Cal c* Bd9.3. By IP this antiserum recognized pure *Cal c* Bd9.3 and the 9.3 band in crude extracts from *C. cyathiformis* and 3 other species, corroborating the cross-reactivity. However, other bands were also recognized. These studies indicate that basidiospores are major fungal aeroallergens, many of which contain a common basic allergen to which most sensitized subjects react.

Introduction

Knowledge of allergens from basidiomycete fungi has progressed rapidly in recent years. Allergenicity has now been demonstrated for some 25 species [1]. Basidiomycete allergens have been analyzed to some degree, from at least three gilled mushrooms, four puffballs and one polypore. However, no basidiomycete allergen has been purified sufficiently to be fully characterized. We determined earlier that two allergens in the puffball *Calvatia cyathiformis* react with a majority of individual sera from skin test positive sub-

jects [2]; one of these has an approximate pI of 9.3. Furthermore, three other species of basidiomycetes have a pI 9.3 allergen, and extracts of all four species inhibit the 9.3 bands on immunoprints [3]. We sought to purify this allergen for full characterization.

Materials and Methods

Due to the relatively basic pI, preparative isoelectric focusing (IEF), adapted from Demeulemester et al. [4], was used to concentrate this allergen from crude spore extract. This was conducted in a gel slurry with a broad ampholyte range, final pH 2-12. The cathodic fraction eluates were collected from several runs, pooled and refocused, again in a gel slurry, final pH 5.4-10.4. The eluants of 9.3 containing fractions were then passed over a phenyl-Sepharose col-

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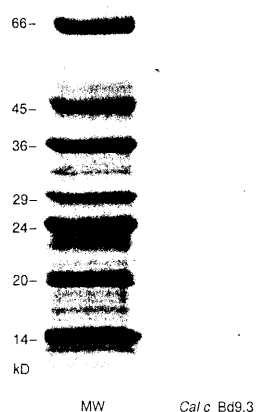


Fig. 1. Gradient (8-18%) SDS-PAGE analysis of purified *Cal c* Bd9.3, indicating a relative molecular mass of approximately 16 kD.

Table 1. Amino acid composition of *Cal c* Bd9.3

Amino acid	Residues, n	Mole percent
asx	13.7	8.379
thr	9.7	5.950
ser	27.0	16.556
glx	18.9	11.564
pro	5.2	3.215
gly	33.6	20.601
ala	12.3	7.511
val	8.0	4.915
met	1.4	0.878
ileu	5.6	3.440
leu	6.3	3.868
tyr	2.6	1.581
phe	6.9	4.242
his	2.3	1.381
lys	7.8	4.787
arg	1.8	1.133
Total residues 163.		

umn (HIC) to remove ampholytes [5]. Column fractions containing protein were dialyzed, aliquoted and held frozen at -70°C . This material was analyzed by gradient (8-18%) SDS-PAGE for purity, amino acid content determined and rabbit antiserum to it produced.

Results

Preparative IEF concentrated the 9.3 allergen in cathodic fractions. HIC retained one protein fraction from eluates of the preparative IEF cathodic fractions. The HIC-retained fraction yielded a single band by analytical IEF that retained the ability to bind IgE from pooled sera of skin test and RAST-positive subjects. This protein also yielded a single band by gradient SDS-PAGE, indicating a relative molecular mass of approximately 16 kD (fig. 1). Amino acid analysis revealed that *Cal c* Bd9.3 was rich in glycine, serine and glutamic acid/glutamate, which together comprised 47 M% of the protein (table 1). The rabbit antiserum to *Cal c* Bd9.3 I formed a single precipitin line with *Cal c* Bd9.3 and also with crude spore extract of *C. cyathiformis*. On immunoprints, this serum recognized purified *Cal c* Bd9.3, and the 9.3 band from crude *C. cyathiformis*, *Coprinus quadrididus*, *Pleurotus ostreatus* and *Psilocybe cubensis*, corroborating cross-reactivity indicated with human sera. The rabbit antiserum also recognized other bands in the crude extracts, although the 9.3 band from each extract was the most intense.

Discussion

Cal c Bd9.3, the pI 9.3 allergen from *C. cyathiformis* spores, is the first allergen purified from basidiomycete fungi. This is a major allergen since it reacts with most (63%) sera from skin test positive subjects. It is also important since it is cross-reactive with allergens from several other basidiomycete species.

We have demonstrated purity by SDS-PAGE, and shown that *Cal c* Bd9.3 has a low molecular weight, about 16 kD. Purification is important since it is a major allergen and cross-reacts with allergens in extracts from other species. This will also aid standardization of extracts and provide a better diagnostic reagent due to the prevalence of reactivity. Further characterization should allow designation of this allergen according to current allergen nomenclature.

References

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