

Isolation and Partial Characterization of a 46-kD Allergen of Bermuda Grass Pollen

Wen-Chia Wu^a Ming F. Tam^b Ho-Jen Peng^c Lai-Chin Tsai^c Chin-Wen Chi^c
Zo-Nan Chang^a

^aInstitute of Biotechnology in Medicine, National Yang-Ming University, ^bInstitute of Molecular Biology, Academia Sinica, and ^cDepartment of Medical Research and Education, Veterans General Hospital-Taipei, Taipei, Taiwan, ROC

Key Words

Bermuda grass pollen · Cyn d Bd46K · Monoclonal antibody · Affinity chromatography · Internal sequencing

Abstract

Cyn d Bd46K, a 46-kD component of Bermuda grass (*Cynodon dactylon*) pollen, had been identified as an allergenic constituent. In the present study two-dimensional (2D) gel electrophoresis illustrated the presence of five acidic isoforms in Cyn d Bd46K, and this molecule was purified by monoclonal antibody (MAb) affinity chromatography for further characterization. Using a digoxigenin-labeled lectin-binding assay, the elucidating protein was disclosed to be a glycoprotein with terminal mannose. The involvement of a carbohydrate moiety in the allergenicity and antigenicity of the elucidated molecule was demonstrated with sodium-periodate-treated Cyn d Bd46K, which reduced binding to its specific MAb and human IgE. We were unable to identify the N-terminal amino acid sequences of Cyn d Bd46K, but some internal amino acid sequences were disclosed by microsequencing some fragments cleaved by *Achromobacter* protease I and fractionated by reversed-phase column chro-

matography. The amino acid sequences of 4 identified Cyn d Bd46K internal peptide fragments were found to be 25–71% identical with that of cytochrome c oxidase III from corn grass pollen. The present study provided important information for future experiments on the molecular cloning of the elucidated allergen.

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Introduction

Bermuda grass pollen (BGP) is an allergen of local and international importance. In Taiwan, 27% of the asthmatic patients show positive immediate skin reactions to its crude extract [1]. It is also an important aeroallergen in Australia, some parts of Europe, and the United States [14, 23].

The characterization of the individual components of an allergen is clinically important. Allergen extracts used in immunotherapy are heterogeneous mixtures containing a variety of proteins. Treatment of allergic patients with these extracts may induce immune responses to some constituents to which the patients were not originally sensitized.

In recent years, we have carried out a series of studies on the analysis of allergenic components of BGP [2–6, 8, 16]. A panel of 16 anti-BGP monoclonal antibodies (MAbs) was generated, and some of their antigens (Cyn d Bd68K, Cyn d Bd58K, Cyn d Bd46K, Cyn d Bd35K, Cyn d Bd25K, Cyn d Bd12K and Cyn d Bd68K, 48K, 38K) were identified by a modified radioallergosorbent test (RAST) to have human IgE-binding capacity [2]. Cyn d 1, the 30 to 34-kD group 1 allergen of BGP, had been isolated and characterized with the use of MAbs [5, 8]. In addition, using these MAbs as probes, 10 full-length cDNA clones of Cyn d 1 have been isolated and sequenced from the BGP cDNA library [6].

A group of 3 anti-Cyn d Bd46K MAbs was also available [2]. The 46-kD molecule identified by these MAbs was recognizable by IgEs in sera of people with BGP allergies with a recognition frequency of 46% [2], suggesting it to be an important allergenic component.

In the present study, this Cyn d Bd46K was isolated in a single purification step using an MAb affinity column. Some of its biochemical and immunological properties were characterized.

Materials and Methods

Monoclonal Antibodies

Anti-Cyn d Bd46K, MAbs 14–16, 17–31 and 18–60 were prepared as described in a previous study [2]. They are all IgG1s, but were obtained from different original wells after fusion of NS-1 myeloma cells with splenic cells from BALB/c mice immunized with BGP. These MAbs reacted with the 46-kD component of BGP and did not cross-react with crude pollen extracts from *Poa annua*, *Zea mays*, *Phalaris minor* and *Sorghum vulgare* var. *saccharatum* [2]. Two additional MAbs, 4–37 and 9–13, were also included in the study; MAb 4–37 shows antigenicity against an epitope on the Cyn d 1 protein backbone [2] while MAb 9–13 reacts against a carbohydrate epitope on the Cyn d Bd68K, 48K, 38K [3].

Gel Electrophoresis and Immunoblotting

2D gel electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were performed as previously described [5]. The isoelectric points were determined according to the method of Hjelmeland et al. [9].

After gel electrophoresis, proteins were either visualized with silver stain or electrophoretically transferred onto a nitrocellulose membrane (Millipore, Bedford, Mass., USA) and treated with 3% (w/v) gelatin as previously described [5]. The membrane was incubated with culture supernatant containing MAbs at room temperature for 1 h, followed by washing with Tris buffer (1 M, pH 7.2) containing 0.05% (w/v) Tween 20. For the 2D gel blots, the MAb-treated membrane was then incubated with iodine-125-labeled goat anti-mouse IgG (BioRad, Richmond, Calif., USA) (2×10^6 cpm/ml) for 1 h. It was then thoroughly washed with phosphate-buffered saline (1 M, pH 7.2) containing 5% (w/v) bovine serum albumin, air-dried

and autoradiographed using Kodak XAR-5 films (Kodak, Rochester, N.Y., USA). For the blots of SDS-PAGE, the MAb-treated membranes were treated with horseradish peroxidase-labeled anti-mouse Ig (BioRad), and then its substrate 4-chloro-1-naphthol (BioRad) as previously described [5].

Affinity Chromatography

Affinity chromatography was performed as previously described [8]. Briefly, an immunosorbent column (1.5 × 15 cm) was made by coupling the MAbs 14–16 and 17–31 to CNBr-activated Sepharose 4B beads (Pharmacia, Uppsala, Sweden) at a concentration of 3 mg/ml [8]. Aliquots of 40 mg BGP extracts, prepared as previously described [5], were loaded onto the column at a flow rate of 5 ml/h with a peristaltic pump. Unabsorbed proteins were removed by washing with phosphate-buffered saline (1 M, pH 7.2), and the desired protein was eluted with two bed volumes of elution buffer (0.1 M citric acid, pH 3.0) at a flow rate of 2 ml/min. The pH of the protein eluted off the column was immediately adjusted to 7.0 with 1 M Tris buffer (pH 10) and then dialyzed against distilled water and lyophilized.

Lectin-Binding Assay

Cyn d Bd46K was transferred to polyvinylidene difluoride membranes (0.5 µg/strip) after electrophoresis. The carbohydrate moieties on Cyn d Bd46K were detected with a glycan differentiation testing kit (Boehringer Biochemica, Mannheim, Germany) according to the manufacturer's instructions. Briefly, the blots were incubated for 1 h at room temperature with a panel of digoxigenin-labeled lectins including *Galanthus nivalis* agglutinin (GNA), *Sambucus nigra* agglutinin (SNA), *Maackia amurensis* agglutinin (MAA), peanut agglutinin (PNA) and *Datura stramonium* agglutinin (DSA). After washing, the lectins bound on the blotted protein were detected with alkaline phosphatase-conjugated sheep anti-digoxinin Fab fragments (diluted 1:1,000) and its specific substrate. Glycoproteins carboxypeptidase Y, transferrin, fetuin and asialofetuin were used as controls for GNA, SNA, MAA, PNA and DSA, respectively.

Periodate Oxidation

Periodate oxidation was performed as described by Woodward et al. [25]. Cyn d Bd46K or BGP was coated onto polyvinylidene difluoride membranes or wells in polyvinyl plates (100 ng/well). After blocking, the plates were rinsed with 50 mM sodium acetate buffer (pH 4.5) and then exposed either to sodium periodate (1 mM) in sodium acetate buffer or to the sodium acetate buffer alone as control for 1 h at room temperature in the dark. At the end of incubation, the wells were washed with sodium acetate buffer and incubated with 1% (w/v) glycine at room temperature for 1 h. The plates were then ready for enzyme immunoassay (EIA) or for RAST as previously described [4].

Amino Acid Sequences of Peptide Fragments of Cyn d Bd46K

The purified Cyn d Bd46K was digested in 100 mM of Na₂CO₃, pH 9.5 with *Achromobacter* protease I (Lys-C) (Wako, Osaka, Japan) at 45 °C for 8 h, with an enzyme-to-substrate ratio of 1:20 (w/w) as previously described [12]. After enzyme digestion, peptide fragments were purified by high-performance liquid chromatography (HPLC) analysis with an ABI model 140B solvent delivery system (Applied Biosystems Inc., Foster, Calif., USA) equipped with a model 1000S diode array detector as previously described [8]. Solvent A was 0.08% (v/v) trifluoroacetic acid in water and solvent B was 80% (v/v)

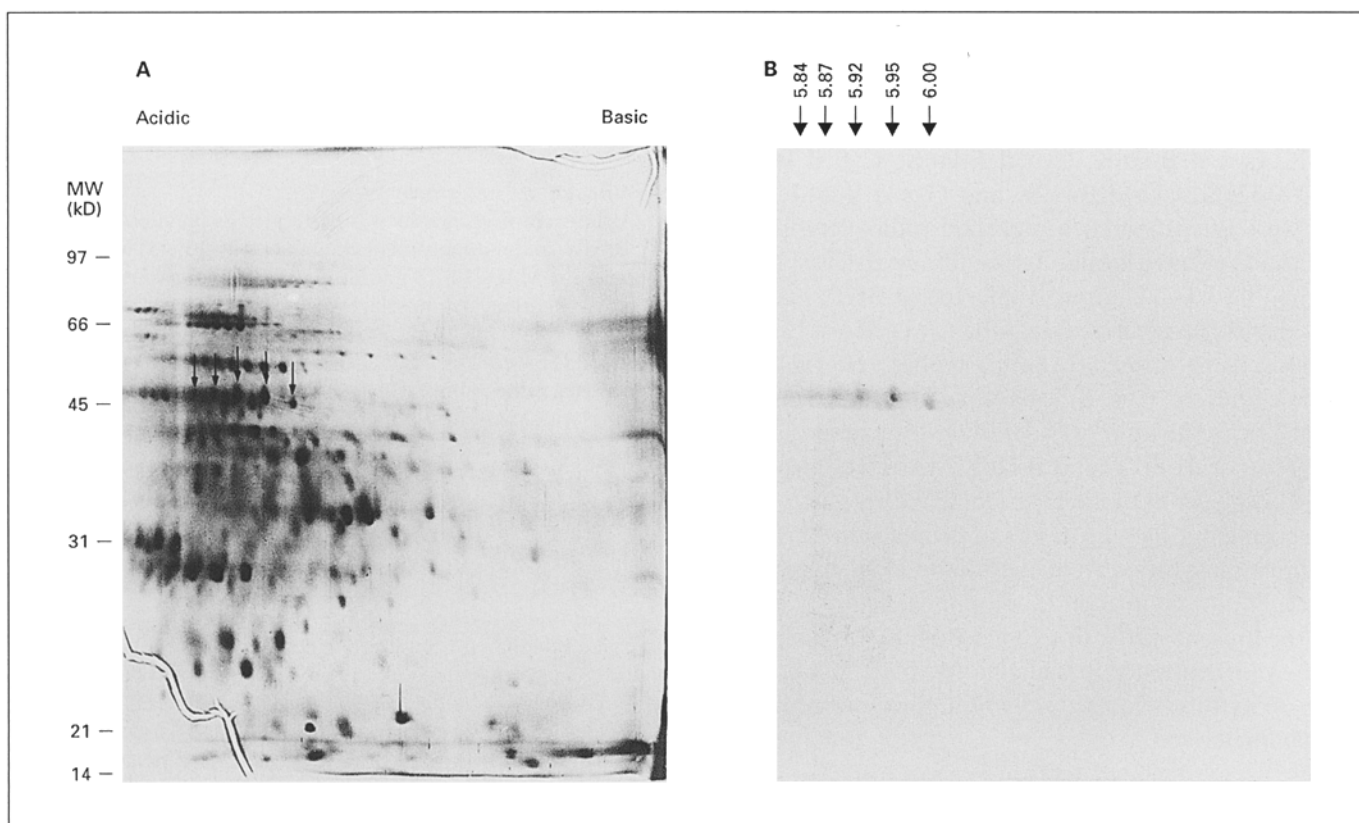


Fig. 1. 2D gel analysis of BGP extract and Cyn d Bd46K. **A** Silver-stained 2D protein profile of crude extract of BGP. **B** 2D gel immunoblot profile of the antigen reacted with MAb 14-16.

acetonitrile in water containing 0.05% (v/v) trifluoroacetic acid. *Achromobacter* protease I digests (100 µg) were loaded onto an Aquapore C8 reversed-phase column (Pierce, Rockford, Ill., USA) (0.46 i.d. × 22.0 cm) that was pre-equilibrated with 12% buffer B. Elution was carried out at a flow rate of 0.6 ml/min. A linear gradient of 1% (v/v) per min of buffer B was started 8 min after sample injection. The elution profile was monitored at 220 nm. Fractions were collected according to the UV absorption profile and were subjected to microsequencing on an ABI gas/liquid-phase model 470A/900A peptide sequencer (Applied Biosystems Inc.) equipped with an online model 120A amino acid phenylthiohydantoin analyzer as previously described [8]. The similarity of the determined amino acid sequences to other proteins was compared with the protein data bank of IntelliGenetics (IntelliGenetics Inc., Mountain View, Calif., USA).

Results

2D Gel and Immunoblotting

Components of BGP extracts were separated by 2D gel electrophoresis. Over 100 components can be separated and visualized by silver-staining (fig. 1A). Among them, 5

components with pIs ranging from 5.84 to 6.00 were recognized by MAb 14-16 (fig. 1B) as revealed with a 2D immunoblot. The immunoblot profiles of MAbs 17-31 and 18-60 were identical with that of MAb 14-16 (data not shown).

Purification of Cyn d Bd46K by Affinity Chromatography

Approximately 0.4 mg of purified materials were routinely recovered from 40 mg of crude BGP extract with affinity chromatography, representing a 1% yield. The eluted material was analyzed by SDS-PAGE together with the BGP extracts (fig. 2A, lanes 1, 2). These proteins were further transferred onto nitrocellulose membranes to react with either MAb 14-16 (fig. 2B) or anti-Cyn d 1 MAb 4-37 (fig. 2C). As illustrated, a single protein band with an MW of 46 kD was purified from the BGP extract with affinity chromatography (fig. 2A, lane 2). The immunoblot assay demonstrated that the eluted material retained its antigenicity to react with MAb 14-16 (fig. 2B,

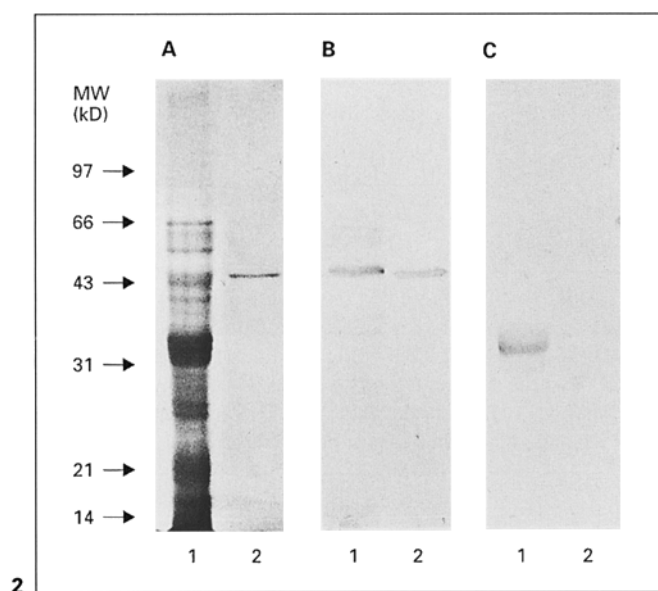


Fig. 2. SDS-PAGE analysis and immunoblotting of proteins isolated from MAb affinity chromatography. After SDS-PAGE, proteins were visualized with Coomassie blue staining (**A**). Proteins were transferred onto nitrocellulose membranes and reacted with MAb 14–16 which reacted with Cyn d Bd46K (**B**) or MAb 4–37 which reacted with Cyn d 1 (**C**). Lanes 1 and 2 are BGP extract (50 µg) and Cyn d Bd46K (2 µg) isolated by affinity chromatography, respectively.

Fig. 3. Lectin binding of Cyn d Bd46K. The binding of lectins GNA (**A**), SNA (**B**), MAA (**C**), PNA (**D**) and DSA (**E**) to the control glycoproteins (lane 1) and Cyn d Bd46K (lane 2) was evaluated by immunoblotting.

lane 2) but did not contain any component recognized by the anti-Cyn d 1 MAb 4–37 (fig. 2C, lane 2). This indicates that there was no Cyn d 1 contamination in the isolated Cyn d Bd46K.

Lectin-Binding Assay

All the control glycoproteins demonstrated strong reactions with lectins GNA, SNA, MAA, PNA and DSA (fig. 3A–E, lane 1). A significant interaction between Cyn d Bd46K and GNA was observed (fig. 3A, lane 2). Mild reactions existed between Cyn d Bd46K and SNA, MAA and PNA (fig. 3B–D, lane 2). In contrast, there was no interaction between DSA and the tested protein (fig. 3E, lane 2).

Periodate Oxidation

EIA showed that the binding of MAbs 14–16, 17–31 and 18–60 to the sodium periodate-treated BGP was only 17.9%, 18.6% and 18.4% of that of the sodium acetate-treated control (table 1). The interaction between the positive control MAb 9–13 and the sodium periodate-treated BGP diminished by 73% as compared with the sodium acetate-treated control, while the interaction between the

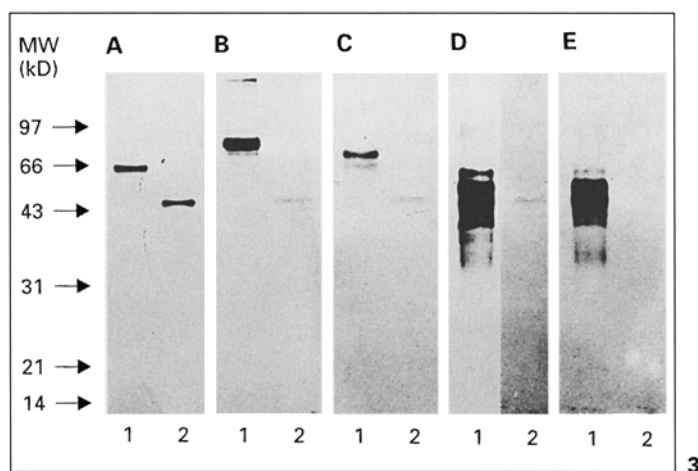


Table 1. Effect of sodium periodate on the epitope recognized by the anti-Cyn d Bd46K MAbs, evaluated using EIA

MAbs	Treatments of BGP extracts	EIA
14–16	sodium acetate	0.662 ± 0.016
	sodium periodate	0.119 ± 0.004
17–31	sodium acetate	0.725 ± 0.013
	sodium periodate	0.135 ± 0.007
18–60	sodium acetate	0.657 ± 0.009
	sodium periodate	0.121 ± 0.005
4–37	sodium acetate	0.435 ± 0.008
	sodium periodate	0.438 ± 0.005
9–13	sodium acetate	0.856 ± 0.021
	sodium periodate	0.225 ± 0.006

Data are the absorbance at OD 405 nm (mean ± SD of triplicates).

negative control MAb 4–37 and sodium periodate-treated BGP remained unchanged.

The effects of periodate oxidation on the IgE-binding capacity of Cyn d Bd46K were evaluated with a RAST (table 2). The binding of IgEs to the untreated Cyn d

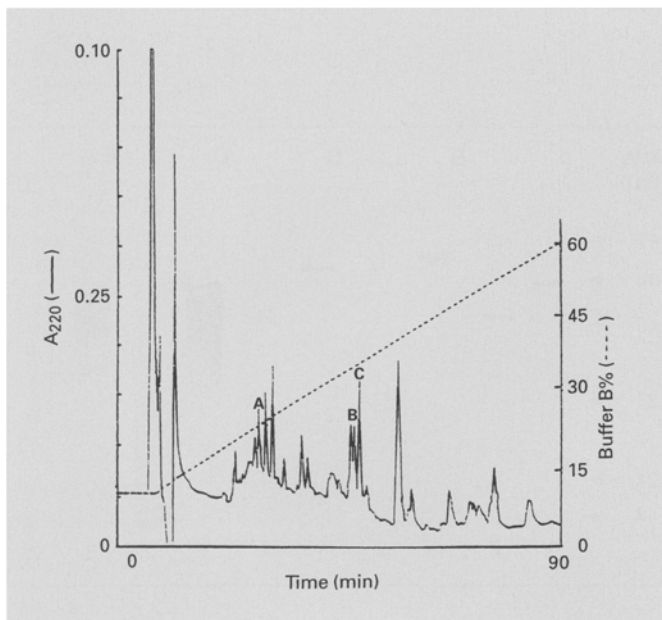


Fig. 4. HPLC profile of Cyn d Bd46K digested by means of *Achromobacter* protease I. Peaks **A**, **B** and **C** were used for amino acid sequencing analysis.

Bd46K was 1,022 cpm, while that of the treated Cyn d Bd46K was 174 cpm. A decrease of 82.9% in IgE-binding capacity was observed after sodium periodate treatment.

Amino Acid Sequences of Peptide Fragments of Cyn d Bd46K

We could not identify the N-terminal amino acid sequence of Cyn d Bd46K although Cyn d Bd46K was stable under Edman degradation. In order to obtain more information about the internal amino acid sequence of Cyn d Bd46K, the protein purified in the affinity column was digested with *Achromobacter* protease I and the digested peptides were separated by HPLC (fig. 4). At least 20 fragments were obtained. Fractions A, B and C, as indicated in figure 4, were selected for sequencing analysis according to their peak shape. Peak A contained two peptides and yielded amino acid phenylthiohydantoin signals of unequal intensity. The sequences were deduced to be VATLAATEATPN and DGHVFREK. The amino acid sequences of peptides B and C were determined to be PFVYIYS and LGIDDXVIF, respectively. The sequencer did not yield any signal for the sixth residue of peptide C, which might be indicative of a cysteine residue, a glycosylation or a phosphorylation site.

Table 2. RAST shows the binding between IgE antibodies of pooled sera to Cyn d Bd46K which had been treated with sodium periodate

Serum	Cyn d Bd46K treated with	
	acetate buffer	sodium periodate
Allergic sera	1,022 ± 52	174 ± 37
Normal sera	85 ± 38	ND

Cyn d Bd46K which bound to the polyvinyl plates was reacted either with acetate buffer or sodium periodate. Afterwards, these Cyn d Bd 46Ks were incubated with pooled sera from patients allergic to BGP, and the specific IgE binding was detected by ¹²⁵I-labeled anti-IgE MAbs [4]. Data are the mean ± SD of triplicates of pooled sera (n = 12) and are shown as count per minute.

These amino acid sequences were compared with the protein data bank of IntelliGenetics (table 3). Peptide B (PFVYIYS) shares 71% identity with the 254th–261th residues (PFVSIYW) of cytochrome c oxidase polypeptide III from corn grass (*Zea mays*) pollen [13]. In addition, VATLAATEATPN, DGHVFREK and LGIDD/VIF share 25–33% identity with the 23–34th, 115th–122th, and 88th–96th residues, respectively, of the same protein.

Discussion

Cyn d Bd46K was identified to contain 5 components with pIs ranging from 5.84 to 6.00 (fig. 1). This shows clearly that it is not a group IV allergen which is a 50- to 56-kD basic allergen [10]. The narrow pI ranges of Cyn d Bd46K are different from the broad pI ranges of Cyn d 1. The Cyn d 1 isoforms exhibit pIs ranging from 5 to 9 [6] and a maximum variation in amino acid sequence of 16% [unpubl. results]. The 5 isoforms of Cyn d Bd46K with close pI values may represent proteins with highly identical amino acid sequences or components with identical amino acid sequences but different degrees of glycosylation, amidation or oxidation.

In this study Cyn d Bd46K was successfully isolated by affinity chromatography; the material eluted by MAb immunosorbence was a single 46-kD protein (fig. 2A, lane 2) which reacted with anti-Cyn d Bd46K MAb (fig. 2B, lane 2) and contained no peptide that reacted with anti-Cyn d 1 MAb (fig. 2C, lane 2). In addition, a

Table 3. Comparison between amino acid sequences of some fragments of Cyn d Bd46K and the cytochrome c oxidase peptide III of corn grass pollen

Fragments of Cyn d Bd46K	corn cytochrome c oxidase polypeptide III	Amino acid sequence	
		identity %	homology %
VAT <u>L</u> AATEATPN	²³ <u>L</u> GALATTVGGVM ³⁴	25	50
DGHVFRE <u>K</u>	¹¹⁵ I <u>G</u> GIWPPK ¹²²	25	50
<u>P</u> FVYIYS	²⁵⁵ <u>P</u> FVSIYW ²⁶¹	71	71
<u>L</u> GIDDXVIF	⁸⁸ <u>L</u> FIVSEVMS ⁹⁶	33	44

X = Unidentified; underlined = identity.

RAST (table 1) disclosed that the purified material retained its IgE-binding capacity. This elucidated allergen was then subjected to further characterization. Although the yield was too low to accumulate a sufficient quantity with which to study the nature of the carbohydrates in detail, the presence of carbohydrates on Cyn d Bd46K was detected by specific binding of lectins (fig. 3). The presence of a substantial amount of mannose in the purified molecule was demonstrated by its strong interaction with GNA, which recognizes high mannose N-glycan chains [18]. SNA and MAA recognize sialic acids linked to galactose in the $\alpha(2-6)$ and $\alpha(2-3)$ positions, respectively [17, 24]. The positive reaction between these lectins and the purified molecule disclosed the presence of sialic acids on Cyn d Bd46K. These results indicate that Cyn d Bd46K is a glycoprotein with terminal mannose.

The binding of three MABs to the sodium-periodate-treated Cyn d Bd46K was reduced by more than 80% compared to the control (table 1). This suggests that the carbohydrate moiety is involved in the epitopes of those MABs. Mild periodate oxidation at acidic pH cleaves carbohydrate vicinal hydroxyl groups without altering the structure of polypeptide chains [25]. A RAST identified that more than 80% of the IgE-binding capacity of Cyn d Bd46K was lost after treatment. These data indicate that carbohydrates appear to play an important role in the allergenicity and antigenicity of Cyn d Bd46K, as reported in the literature [7, 11, 22]. For BGP, Smith et al. [19] found that recombinant Cyn d 1 obtained from a bacterial expression system does not bind human IgE whereas that obtained from the yeast expression system does. In addition, the carbohydrate moiety had also been reported to play an important role in the immune response of BG60, another important 60-kD allergen of BGP [21].

Recombinant cDNA technology has been successfully applied in allergology either for sequencing or obtaining

recombinant allergens [15]. Either human allergic sera or MABs can be used as probes to screen the cDNA clones from the cDNA library; this technology has been successfully applied to the cloning of Cyn d 1 [6, 19] and Cyn d 7 [20]. However, the findings that both the allergic sera and MABs specific to Cyn d Bd46K react against the carbohydrate moiety (tables 1, 2) indicate that these antibodies are not suitable for future cloning studies. In addition, the N-terminal amino acid sequence of Cyn d Bd46K could not be determined, ruling out the possibility of obtaining a full-length cDNA clone by reverse transcriptase-polymerase chain reaction. Alternatively, degenerate nucleotide primers can be designed according to the internal amino acid sequences of the studied protein and be used as probes [15]. The present study disclosed some internal amino acid sequences of Cyn d Bd46K by microsequencing some enzyme-cleaved, HPLC-fractionated fragments (fig. 4, table 3). In the future, some degenerate primers can be designed and constructed for the molecular cloning of the gene of the Cyn d Bd46K.

Interestingly, the data bank revealed 25–71% identity and 44–71% homology between the determined internal amino acid sequences of Cyn d Bd46K and some fragments of cytochrome c oxidase polypeptide III of corn grass pollen [13] (table 3). The cytochrome c oxidase polypeptide III from corn grass was shown to be a representative of that from common sunflower (*Helianthus annuus*) and broad bean (*Vicia faba*); the data bank revealed that these proteins which contain 265 amino acids share a highly conserved 95.4% identity. The relationship between Cyn d Bd46K and cytochrome c oxidase III remains unclear and can be clarified only after further sequencing analysis of Cyn d Bd46K.

In conclusion, the present study partially characterized Cyn d Bd46K. The elucidating allergen was disclosed to be an acidic glycoprotein with terminal mannose, and car-

bohydrates played an important role in its allergenicity as well as antigenicity. Some internal amino acid sequences were identified to provide information for the design of some degenerate primers for further cloning of Cyn d Bd46K cDNAs.

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References

- 1 Chang HN, Lin FM, Chang YF. Correlation of skin test to RAST, and PRIST in asthmatic patients. *Chin Med J* 34:376-383;1984.
- 2 Chang ZN, Tsai LC, Chi CW, Wang MC, Shen HD, Lee DT, Han SH. Analysis of allergenic components of Bermuda grass pollen by monoclonal antibodies. *Allergy* 46:520-581;1991.
- 3 Chang ZN, Chi CW, Sun HF, Tsai LC, Lee DT, Han SH. A unique human IgE-binding epitope on the Bermuda grass pollen recognized by mouse lambda-type monoclonal antibodies. *Clin Exp Allergy* 21:503-509;1991.
- 4 Chang ZN, Liu CC, Peng HC, Tsai LC, Han SH. A common allergenic epitope of Bermuda grass pollen shared by other grass pollens. *J Biomed Sci* 1:93-99;1994.
- 5 Chang ZN, Liu CC, Tam MF, Peng HJ, Tsai JJ, Han SH. Characterization of the isoforms of the group I allergen of *Cynodon dactylon*. *J Allergy Clin Immunol* 95:1206-1214;1995.
- 6 Chang ZN, Peng HJ, Lee WC, Chen TS, Chua KY, Tsai LC, Chi CW, Han SH. Sequence polymorphism of the group I allergen of Bermuda grass pollen. *Clin Exp Allergy* 29:488-496;1999.
- 7 Haavik S, Smestad Paulsen B, Wold JK. Glycoprotein allergens in pollen of timothy. V. Significance of the carbohydrate moiety for the immunological activity of a basic glycoprotein allergen. *Int Arch Allergy Appl Immunol* 87:231-237;1987.
- 8 Han SH, Chang ZN, Chi CW, Peng HC, Liu CC, Tsai JJ, Tam M. Use of monoclonal antibodies to isolate and characterize Cyn d 1, the allergen of Bermuda grass pollen. *J Allergy Clin Immunol* 92:549-558;1993.
- 9 Hjelmeland LM, Nebert DW, Chrambach A. Electrofocusing of integral membrane proteins in mixtures of zwitterionic and nonionic detergents. *Anal Biochem* 95:201-208;1979.
- 10 Jaggi KS, Ekramoddoullah AKM, Kisil FT, Dzuba-Fischer JMM, Rector ES, Sehon AH. Identification of two distinct allergenic sites on ryegrass-pollen allergen, Lol p IV. *J Allergy Clin Immunol* 83:845-852;1989.
- 11 Koshte VI, Kagen SL, Aalberse RC. Cross-reactivity of IgE antibodies to caddis fly with arthropoda and mollusca. *J Allergy Clin Immunol* 84:174-183;1989.
- 12 Lin RY, Shen HD, Han SH. Identification and characterization of a 30 kD major allergen from *Parapenaeus fissurus*. *J Allergy Clin Immunol* 92:837-845;1993.
- 13 McCarty DM, Hehman GL, Hauswirth WW. Nucleotide sequence of the *Zea mays* mitochondrial cytochrome oxidase subunit III gene. *Nucleic Acids Res* 16:9873;1988.
- 14 Meyers RL, Berns-Mason AW, Trayer KH, Feldman BH, Rosengren HN. Isolation and characterization of the dialysate from Bermuda grass pollen. *J Allergy* 47:100-101;1971.
- 15 Mohapatra SS, Nicodemus CF, Schou C, Valenta R. Recombinant allergens and epitopes. *Allergy Clin Immunol News* 2:45-48;1994.
- 16 Shen HD, Wang SR, Tang RB, Chang ZN, Su SN, Han SH. Identification of allergens and antigens of Bermuda grass (*Cynodon dactylon*) pollen by immunoblot analysis. *Clin Allergy* 18:401-409;1988.
- 17 Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ. The elderberry (*Sambucus nigra* L.) bark lectin recognizes the Neu5Ac(α 2-6)Gal/GalNAc sequence. *J Biol Chem* 262:1596-1601;1987.
- 18 Shibuya N, Goldstein IJ, Van Damme EJM, Peumans WJ. Binding properties of a mannose-specific lectin from the snowdrop (*Galanthus nivalis*) bulb. *J Biol Chem* 263:728-734;1988.
- 19 Smith PM, Suphioglu C, Griffith J, Theriault K, Knox B, Singh MB. Cloning and expression in yeast *Pichia pastoris* of a biologically active form of Cyn d 1, the major allergen of Bermuda grass pollen. *J Allergy Clin Immunol* 98:331-343;1996.
- 20 Smith PM, Xu H, Swoboda I, Singh MB. Identification of a Ca^{2+} binding protein as a new Bermuda grass pollen allergen Cyn d 7: IgE cross-reactivity with oilseed rape pollen allergen Bra r 1. *Int Arch Allergy Immunol* 114:265-271;1997.
- 21 Su SN, Shu P, Lau GX, Yang SY, Huang SW, Lee YC. Immunologic and physicochemical studies of Bermuda grass pollen antigen BG60. *J Allergy Clin Immunol* 98:486-494;1996.
- 22 Tretter V, Altmann F, Kubelka V, Marz L, Becker WM. Fucose α 1-3 linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. *Int Arch Allergy Immunol* 102:259-266;1993.
- 23 Trinca JC. Pollens used in the treatment of hay fever and asthma in eastern Australia. *Med J Aust* 49:969-972;1962.
- 24 Wang WC, Cummings RD. The immobilized leucoagglutinin from the seeds of *Maackia amurensis* binds with high affinity to complex-type Asn-linked oligosaccharides containing terminal sialic acid-linked α -2,3 to penultimate galactose residues. *J Biol Chem* 263:4576-4585;1988.
- 25 Woodward MP, Young WW, Bloodgood RA. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. *J Immunol Methods* 78:143-153;1985.