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## Key Words

Pollen Allergen  
 Bermuda grass  
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# Isolation and Characterization of Group-I Isoallergens from Bermuda Grass Pollen

## Abstract

Two isoallergens of *Cyn d I* were isolated using preparative isoelectric focussing, and were designated *Cyn d Ia* and *b*. These isoallergens differ in their pI, molecular weight (*Cyn d Ia*, 32 kD and *Cyn d Ib*, 31 kD) and their NH<sub>2</sub>-terminal sequence. Four monoclonal antibodies (Mabs) were produced using *Cyn d Ia* as antigen. These Mabs recognized both *Cyn d Ia* and *b*. One of the Mabs recognized four different pI forms of *Cyn d I* on 2D gels. The Mabs also recognized cross-reactive epitopes on proteins from eight other grasses including rye grass, timothy grass, Kentucky bluegrass and Johnson grass.

## Introduction

Bermuda grass (*Cynodon dactylon*; subfamily Chloridoideae) is an important source of seasonal aeroallergens in many areas of the world, especially in tropical and subtropical climates. The allergens of this grass show minimal IgE cross-reactivity with allergens from ryegrass (*Lolium perenne*) and other grasses belonging to the subfamily Pooideae [1-4]. As a consequence, individuals allergic to Bermuda grass require separate diagnosis and therapy [5].

Aqueous extracts of Bermuda grass pollen are highly complex mixtures that contain at least 52 antigenic proteins [6], of which more than 13 are allergens and bind IgE from the sera of individuals allergic to Bermuda grass [6-8]. *Cyn d I* is the major allergen of Bermuda grass pollen, with more than 76% of individuals allergic to Bermuda grass [7, 8] showing sensitivity to this 32-kD protein [9]. In general, at least 50% of the anti-Bermuda grass IgE from any susceptible individual will be directed against the *Cyn d I* allergen.

Although the allergens of ryegrass have been extensively studied, much less information is available regarding the major allergen of Bermuda grass. Only one monoclonal antibody (Mab) raised against *Lol p I* (Mab 3.2; [10]) has been shown to bind to *Cyn d I*. In a recent study by Matthiesen et al. [9], *Cyn d I* was identified as a glycoprotein which bound the lectin concanavalin A (Con A). An isoallergen of *Cyn d I* was subsequently purified using a combination of Con A-sepharose affinity chromatography and carboxymethyl chromatography.

Here we report the isolation, characterization and NH<sub>2</sub>-terminal sequencing of two molecular weight forms of *Cyn d I*. Mabs have been produced using one of these isoforms as an immunogen and these anti-*Cyn d I* Mabs have been used to study of the conservation of epitopes between *Cyn d I* and allergens from other grasses.

## Materials and Methods

### Preparation of Pollen Extracts

Pollens of Bermuda grass (*C. dactylon*), ryegrass (*L. perenne*), timothy grass (*Phleum pratense*), orchard grass (*Dactylis glomerata*), meadow fescue (*Festuca elatior*), salt grass (*Distichlis stricta*), blue grama grass (*Bouteloua gracilis*), Bahia grass (*Paspalum notatum*) and Johnson grass (*Sorghum halepense*) were obtained from Greer Laboratories, Lenoir, N. C., USA.

To prepare the pollen extract which was loaded on the Rotofor, 3 g of Bermuda grass pollen was extracted three times by shaking with 10 ml of 10 mM phosphate-buffered saline (PBS) for 1 h at 4°C. After each extraction, the mixture was centrifuged (700 g, 10 min) and the supernatant collected. After three extractions the supernatants were pooled and filtered through a 3-mm Whatman filter to remove pollen debris. To prepare the protein extracts for separation on sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE), 100 mg of pollen was shaken in 1 ml of PBS overnight at 4°C. The mixture was then centrifuged and the supernatant collected. The protein concentration was determined using the Bio-Rad assay (Bio-Rad, Richmond, Calif., USA).

### Monoclonal Antibodies

Mab. 3.2 was kindly provided by Dr. D. G. Marsh, John Hopkins Asthma and Allergy Center (Baltimore, Md., USA). It is a Mab raised against *Lol p I* which also recognized cross-reactive epitopes in pollen proteins from eight other grasses including Bermuda grass [10].

Anti-*Cyn d I* Mabs were produced in this study. 50 µg of *Cyn d I* (isolated on the Rotofor; Bio-Rad, Richmond, Calif., USA) in PBS emulsified with RIBI adjuvant (Ribi ImmunoChemical Research, Mont., USA) was used for intraperitoneal immunization of a Balb/c mouse. The mice were boosted with 50 µg of *Cyn d I* interperitoneally at weekly intervals for 3 weeks. Fusion and growth of hybridomas was essentially as described by Harlow and Lane [11]. Single cell cloning was by limiting dilution.

Hybridomas producing anti-*Cyn d I* antibodies were identified using an ELISA assay. ELISA plates were coated overnight with 60 µg/well of soluble Bermuda grass pollen proteins diluted in CAPS buffer (6.67 mM NaCO<sub>3</sub> 35 mM NaHCO<sub>3</sub>, pH 9.6). The wells were then washed 3 times with PBS containing 0.1% Tween 20 and blocked for 30 min with PBS containing 1% bovine serum albumin (PBS/BSA). 100 µl of the primary antibody was added to each well and incubated at room temperature for 60 min, followed by washing (as above) and incubation with β-galactosidase-labelled anti-mouse Ig (Amersham, UK; 1/250 dilution in PBS/BSA, 60 min). After washing, 200 µl of the fluorescent substrate 4-methylumbelliferyl-β-D-galactoside (in 10 mM K<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl, 2 mM MgCl<sub>2</sub>, 0.1% BSA, pH 7.0) was added to each well and incubated at 37°C for 30 min. Fluorescence was detected using fluoroCount 96 fluorometer (Pharmacia LKB, Sweden). To confirm the specificity, antibodies which were positive in the ELISA assay were tested for binding to *Cyn d I* by Western blot analysis of Bermuda grass pollen proteins separated by SDS-PAGE. The isotype of the Mabs was determined using a mouse Mab isotyping kit (Amersham, UK).

### Preparative Isoelectric Focussing (IEF)

Preparative IEF in the Rotofor (Bio-Rad, Richmond, Calif., USA) has been described in detail by Egen et al. [12]. Briefly, 5 ml of ampholyte solution (Bio-Lyte, pH range 3–10; 40%) was added to the pollen extract and the volume adjusted to 50 ml with distilled water.

This mixture was loaded into the Rotofor cell and focussed at 4°C and 12 W constant power. After 4 h, 20 fractions were collected and their pH determined. Fractions containing the proteins of interest were identified with Mab 3.2 on immunoblots after SDS-PAGE. This Mab was raised against purified *Lol p I* but was found to be cross-reactive with group-I homologues from eight other grasses including Bermuda grass [10]. Two groups of fractions containing the two different molecular weight forms of *Cyn d I* (designated *Cyn d Ia* and *b*) were pooled and refractionated in the Rotofor using the same conditions as above, except that samples were focussed for 2.5 h. The pH of each fraction was determined.

### Gel Electrophoresis

**SDS-PAGE.** Protein samples (10 µg of proteins/lane for mini-gels and 80 µg for gradient gels) were denatured under reducing conditions by boiling for 3–5 min in the presence of 25 mM Tris-HCl, 2% SDS, 2% dithiothreitol and 10% glycerol at pH 6.8. Proteins were then separated by SDS-PAGE using 10–15% gradient gels or 12% mini-gels (both with 4% stacking gels) using the buffer system described by Laemmli [13]. Gradient gels were electrophoresed at 20 mA/gel for 1 h, after which the current was increased to 30 mA/gel until the dye-front reached 1 cm from the bottom of the gel. Mini-gels were electrophoresed at 200 V. Gels were then stained with Coomassie brilliant blue R250 or left unstained for electrophoretic transfer to nitrocellulose.

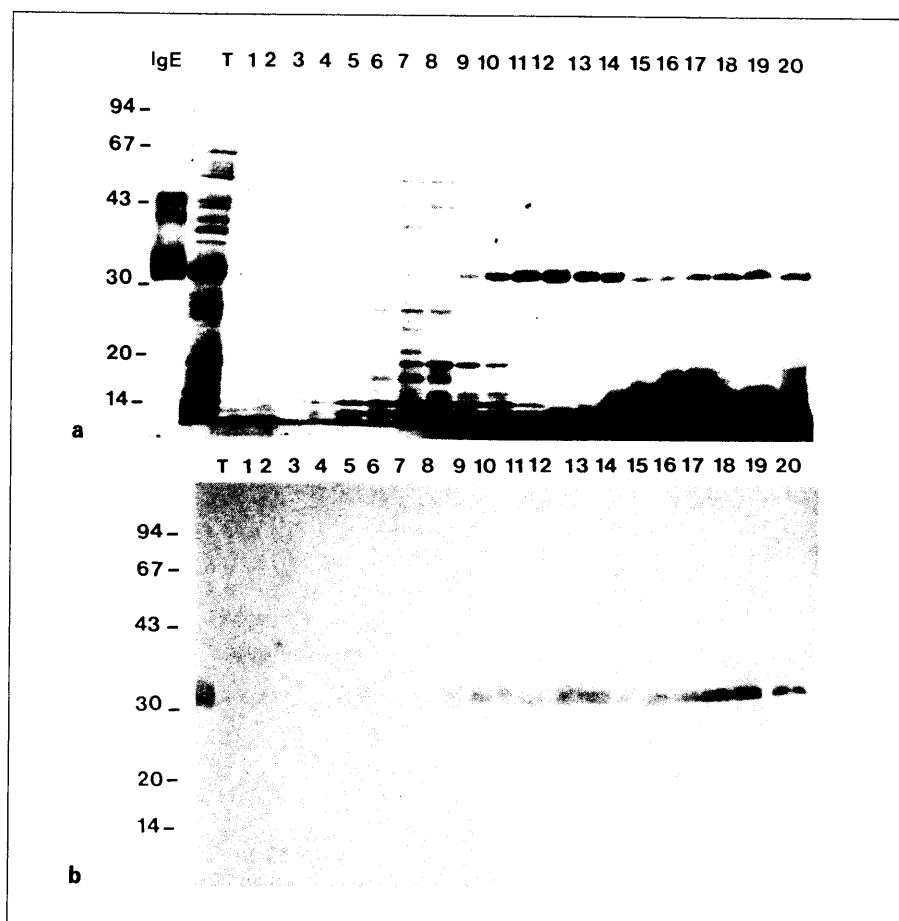
**2D-Gel Electrophoresis.** Soluble rye grass pollen proteins (10 µg/gel) were separated in a mini-Protean II 2-D cell (Bio-Rad, Richmond, Calif., USA) according to the manufacturers instructions. Proteins were diluted 1:1 in first-dimension sample buffer (9.5 M urea, 2% CHAPS, 5% mercaptoethanol, 1.6% Bio-Lyte 5/7 ampholyte, 0.4% Bio-Lyte 3/10 ampholyte). 13 µg of protein was applied per gel and the sample overlayed with first-dimension sample overlay buffer (9.5 M urea; 0.8% Bio-Lyte 5/7 ampholyte, 0.2% Bio-Lyte 3/10 ampholyte; bromophenol blue). The first-dimension gel was run for 3.5 h with upper chamber of 20 mM NaOH and lower chamber buffer of 10 mM H<sub>3</sub>PO<sub>4</sub>. The second-dimension gel was overlayed with SDS sample equilibration buffer (62.5 mM Tris-HCl, pH 6.8; 2.3% [w/v] SDS; 5% [v/v] β-mercaptoethanol; 10% [w/v] glycerol; bromophenol blue). The second-dimension gel was run for 45 min on 12% SDS-polyacrylamide mini-gels as described above. Gels were silver stained to reveal the protein profile.

### Immunoblot Analysis

Proteins separated by 2D-PAGE and gradient SDS-PAGE were transferred to nitrocellulose (BA 0.45 µm, Schleicher and Schuell, Dassel, FRG) according to Towbin et al. [14]. Proteins from mini-gels were transferred at 200 mA for 3 h and gradient gels at 120 mA overnight. After protein transfer, unreacted binding sites were blocked by incubation of the nitrocellulose filters in powdered milk (10% in PBS).

Western blots were incubated with Mab 3.2 or in pooled sera from 10 individuals allergic to Bermuda grass. Mab 3.2 was diluted 1:1,000 in PBS/BSA. Mab binding was visualized by incubation in a solution of peroxidase-labelled, anti-mouse Ig antibody (Dakopatts) followed by addition of the enzyme substrate as described by Singh and Knox [15]. Human serum was diluted 1:4 in 150 mM PBS/BSA. IgE binding was visualized by incubation of the blot in <sup>125</sup>I-labelled anti-human IgE (Kallestad; diluted 1:6 in PBS/BSA) followed by autoradiography.

**Fig. 1.** Separation by SDS-PAGE of fractions obtained by preparative IEF and identification of *Cyn d I*. Fractions obtained from preparative IEF of Bermuda pollen proteins were separated by SDS-PAGE. **a** The gel was stained with Coomassie brilliant blue R250. The lane labelled, IgE, shows binding of IgE from pooled sera binding to a Western blot of Bermuda grass pollen proteins. **b** Proteins from the same gel (**a**) were transferred to nitrocellulose and the Western blot screened with Mab 3.2. In both panels, T refers to total soluble Bermuda grass pollen proteins, and 1–20 to fractions harvested from the first separation on the Rotofor. Samples are loaded from most acidic to most basic fraction. Black shadows in the low molecular weight region of lanes 10–20 (**a**) are due to the presence of ampholites in these fractions.



The binding of Con A to *Cyn d I* proteins was investigated by incubation of nitrocellulose blots of Bermuda grass pollen proteins in the lectin, Con A (50 µg/ml in H<sub>2</sub>O) for 2 h. The binding of Con A was visualized by incubation with horseradish peroxidase (50 µg/ml in H<sub>2</sub>O) for 1 h followed by addition of the enzyme substrate as described by Singh and Knox [15].

#### *NH*<sub>2</sub>-Terminal Amino Acid Sequencing

*Cyn d I* proteins (purified by IEF in the Rotofor) were recovered from SDS-PAGE gels by electrotransfer onto polyvinylidene difluoride membrane (Millipore) using 10 mM CAPS-10% methanol (pH 11.0) as the transfer buffer. Proteins were then visualized by staining with Coomassie brilliant blue R250, destained in methanol-acetic acid-water (50:10:40, v/v/v) and washed extensively with deionized water. The *NH*<sub>2</sub>-terminal amino acid sequence of both *Cyn d I* proteins was determined as described by Ward et al. [16].

*Cyn d I* proteins, isolated by Rotofor, were also purified using reverse-phase HPLC [18] and the *NH*<sub>2</sub>-terminal amino acid sequence of *Cyn d Ib* protein determined.

## Results

Electrophoretic separation of Bermuda grass pollen extracts followed by Coomassie blue staining showed a prominent band with a molecular weight of 31–32 kD (fig. 1). This was identified as *Cyn d I* by its ability to bind Mab 3.2 and IgE from the sera of individuals allergic to Bermuda grass pollen (fig. 1).

Separation by SDS-PAGE of the fractions obtained by preparative IEF of Bermuda grass pollen extracts revealed that the majority of proteins focussed in the pH range 4–6 (fractions 5–10, fig. 1a). *Cyn d I*, however, focussed in fractions 10–20 with a pH range of 6–10. The proteins in fractions 10–13 which bound Mab 3.2, had a slightly higher molecular weight (32 kD) than those in fractions 15–20 (31 kD; fig. 1b). The intermediate fraction 14 contained both proteins that bound Mab 3.2. These proteins have been designated *Cyn d Ia* (32 kD) and *Cyn d Ib* (31 kD).

**Table 1.** NH<sub>2</sub>-terminal sequences of *Cyn d I* isoallergens and other group-I allergens

| Allergen                      | NH <sub>2</sub> -terminal amino acid sequence                       |
|-------------------------------|---------------------------------------------------------------------|
| <i>Cyn d I a</i> <sup>1</sup> | AMGD <u>K</u> PGPXITATYGD <u>K</u> XLDA <u>K</u> XA <u>F</u> D<br>T |
| <i>Cyn d I b</i> <sup>1</sup> | AIGD <u>K</u> PGPXITAXYGX <u>K</u> XLXA                             |
| <i>Cyn d I b</i> <sup>2</sup> | AIGDKPGPKITATYXXXKWLEAKATFYGSNPRGAA                                 |
| <i>Cyn d I</i> [9]            | AMGDKPGPXITATYGDKWLDKATFYG                                          |
| <i>Lol p I</i> <sup>3</sup>   | IAKVpPGpKITA EYGDKWLDKXT                                            |
| <i>Lol p I</i> <sup>4</sup>   | IAKVPPGPNITAEYGDKWLDKSTWYGKPT                                       |
| <i>Poa p I</i> [24]           | IAKVpPGpXITATYGDKWLDKST                                             |
| <i>Pha a I</i> [23]           | IAKVPPGXITAEYGDKWLD                                                 |

Bold face amino acids differ between different *Cyn d I* isoallergens. Underlined amino acids indicate that the designation is tentative. X = Unknown amino acid; p = hydroxyproline residues. Where two amino acids have been identified at the same position, the second amino acid is shown on the line below.

<sup>1</sup> Determined after transfer to PVDF membrane.

<sup>2</sup> Determined after HPLC purification.

<sup>3</sup> From amino acid analysis [20].

<sup>4</sup> From sequence of cDNA clone [20].

Fractions 10–13 containing *Cyn d Ia* were pooled and refractionated. *Cyn d Ia* was found in all fractions but dominated the protein complement of fractions 13–20 (results not shown). These fractions had a pH of 6.5, which gives an approximate estimate of the pI *Cyn d Ia*.

In order to purify *Cyn d Ib*, fractions 15–20 of the original IEF preparation were pooled and refractionated. *Cyn d Ib* was found in all fractions but dominated the protein profile of fractions 1–12 (results not shown). These fractions had a pH of 7.4, an indication of the pI of *Cyn d Ib*.

Purified *Cyn d Ia* and *Ib* were assessed for their ability to bind to IgE from the serum of allergic individuals. Both fractions bound IgE from the sera of 9 of 10 Bermuda grass-allergic patients (results not shown).

#### NH<sub>2</sub>-Terminal Amino Acid Sequence

The two *Cyn d I* components show minor amino acid sequence variations in their NH<sub>2</sub>-terminal regions (table 1). *Cyn d Ia* has methionine at residue 2, aspartic acid at residue 20 and alanine at residue 24, while *Cyn d Ib* has isoleucine at residue 2, glutamic acid at residue 20 and threonine at residue 24. In *Cyn d Ia*, the most likely amino acid at position 24 was alanine but threonine was also tentatively assigned to this position. The designation of aspartic acid at position 26 is tentative.

#### Specificity of Mabs Raised Against *Cyn d I*

After cell fusion and screening by ELISA, twelve hybridoma lines were identified that secreted antibodies capable of binding to proteins from a Bermuda grass pollen extract in an ELISA assay.

From the original twelve cultures, four hybridoma lines were isolated which produced Mabs against *Cyn d I*. The isotypes of the four Mabs were determined. 3A2, 3C2 and 4D2 all have a G1 heavy chain and 1D1 has a G2b heavy chain. 3A2 and 4D2 have kappa light chains and 1D1 and 3C2 have lambda light chains.

The specificity of Mabs 1D1, 3A2, 3C2 and 4D2 for Bermuda grass pollen proteins was assessed. On Western blots of Bermuda grass pollen extract all the Mabs recognise proteins with molecular weights of 31–32 kD (fig. 2), the same proteins recognized by Mab 3.2. These proteins appear to correspond to *Cyn d Ia* and *Cyn d Ib*. Mabs 3A2 and 4D2 also recognize a 29-kD protein. This protein bound IgE from the sera of individuals allergic to Bermuda grass pollen but with a much lower frequency than *Cyn d I* (31 and 32 kD; results not shown).

The lectin Con A bound to all isoforms of *Cyn d I* that could be identified on a one-dimensional gel (fig. 2). This indicates that *Cyn d I* is a glycoprotein with  $\alpha$ -D-glucoside or  $\alpha$ -D-mannoside carbohydrate moieties. Both *Cyn d Ia* and *b* bound Con A (results not shown).

The pI of *Cyn d I* has been recorded as between 6.0 and 6.8 [9]. This suggests that as well as having isoforms with different molecular weights, *Cyn d I* also has isoforms with different pI. Mab 4D2 was used to screen a Western blot of Bermuda grass pollen proteins separated by 2D-electrophoresis to identify these isoforms. An internal pI marker (Carbamylated CPK, Pharmacia) was used to estimate the pIs of the different isoforms. Four isoforms of *Cyn d I* with pIs of 7, 6.95, 6.89 and 6.7 were identified using this Mab (fig. 3).

#### Identification of Cross-Reactive Epitopes on Proteins from Other Grass Genera

The distribution of proteins in other grasses that share common antigenic epitopes with *Cyn d I* was studied using the Mabs raised against *Cyn d I*.

All grass pollens showed strongly staining components with molecular weights between 30 and 35 kD. Irrespective of their different epitope specificity an *Cyn d I*, the Mabs raised against *Cyn d I* (1D1, 3A2, 3C2 and 4D2) had almost identical binding patterns to cross-reactive proteins in the pollen of eight other grasses (fig. 4).

All four Mabs bound to two proteins with similar molecular weights (which are hard to distinguish on minigels) in Bermuda grass pollen extracts, and Mabs 3A2 and 4D2 also bound to a 29-kD protein. In timothy pollen extracts, the Mabs bound to two components with molecular weights of 33 and 36 kD. In ryegrass and orchard extracts the Mabs bound strongly to one major component (molecular weight 35 kD) and less strongly to a component with a molecular weight of 30 kD. The Mabs bound to four proteins, with molecular weights of 26, 28, 30 and 35 kD, in kentucky bluegrass extract. All Mabs bound to at least one protein in grama grass, salt grass, bahia grass and Johnson grass and Mab 3A2 also bound to a 25-kD allergen in grama grass (fig. 4).

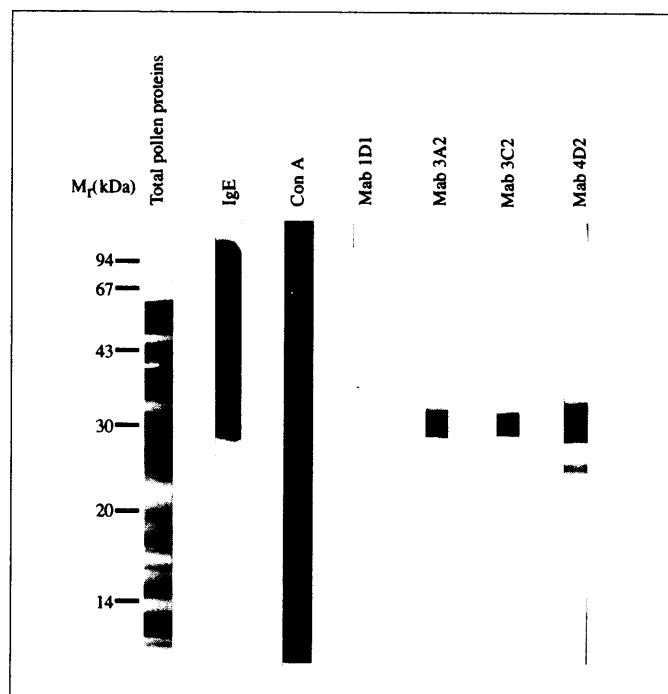
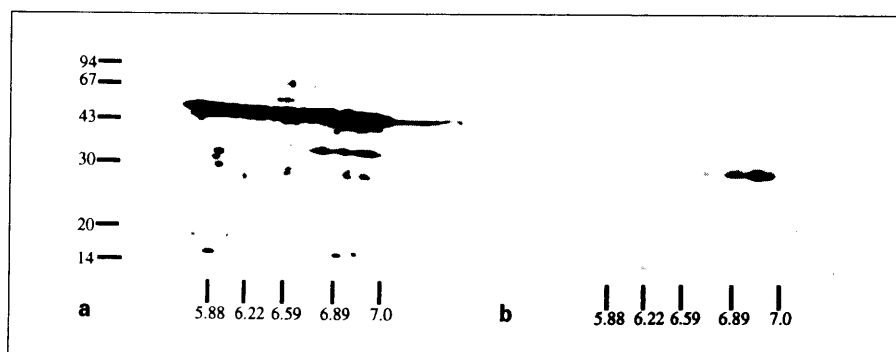


**Fig. 2.** Specificity of IgE from human sera, Con A and anti-*Cyn d I* Mabs for Bermuda grass pollen proteins. Soluble Bermuda grass pollen extract was separated by SDS-PAGE and either stained with Coomassie brilliant blue R250 (total pollen proteins) or transferred to nitrocellulose and the Western blots screened with pooled IgE from serum of Bermuda grass-allergic individuals.

#### Discussion

The major allergen of Bermuda grass, *Cyn d I*, has been described as a protein with a molecular weight of 32 kD [9], and we now show that *Cyn d I* exists as two molecular weight forms – designated *Cyn d Ia* (32 kD) and *Cyn d Ib* (31 kD). Both are glycoproteins and bind IgE

**Fig. 3.** Specificity of anti-*Cyn d I* Mab for Bermuda grass pollen proteins separated by 2D-PAGE. Soluble Bermuda grass pollen proteins were separated by 2D-PAGE and either stained with Coomassie brilliant blue R250 (total pollen proteins; **a**) or transferred to nitrocellulose (**b**) and the Western blots screened with Mab 4D2. Molecular mass ( $M_r$ ) in kilodaltons is denoted at the left and pI below. The internal pI marker (Carbamylated CPK; Pharmacia) appears as a band at 43–45 kD (**a**).





both are glycosylated. The differences in molecular weight may be due to the extent of glycosylation of the proteins or to differences in the size of the polypeptide chain. Further characterization of *Cyn d I* at the molecular level should answer this question.

Isoallergens have been defined as 'a group of allergens which possess experimentally indistinguishable immunological properties and closely related physicochemical structures' [1]. The proteins isolated here can therefore be defined as isoallergens as they do not appear to differ in any immunological characteristics.

#### *Homology between Group-I Allergens of Different Grasses*

The NH<sub>2</sub>-terminal sequences of a number of group-I allergens have been determined and they show strong homology [21–24] (table 1). There are few amino acid substitutions between these allergens; in the first 15 amino acids there is only difference between the NH<sub>2</sub>-terminal sequence of *Lol p I* and those of *Poa p I* and *Phl p I*. *Cyn d I* has more amino acid substitutions in the NH<sub>2</sub>-terminal amino acid sequence compared to other grasses (eight differences in the first 24 amino acids) and only two of these substitutions are considered as conservative. *Cyn d I*, unlike *Lol p I* and *Poa p I* does not contain hydroxyproline in its NH<sub>2</sub>-terminal sequence.

Use of anti-*Cyn d I* Mabs has identified cross-reactive epitopes on proteins of 8 grasses from three subfamilies, the Pooideae (ryegrass, Kentucky bluegrass, timothy grass and orchard grass), Chloridoideae (salt grass and grama grass) and Panicoideae (Bahia grass and Johnson grass). Other studies have also identified proteins cross-reactive with group-I allergens from grasses of other subfamilies. A group-I component was identified in *Phragmites communis* (common reed) [25] a member of the subfamily Arundinoideae, although other reports suggest that only a low degree of allergenic cross-reactivity exists between this allergen and ryegrass [26]. The anti-*Cyn d I* Mab 3A2 used in this study also binds to 31- and 32-kD cross-reactive proteins in rice [P. Theerakulpisut, personal commun.], which is a member of the subfamily Bambusoideae. Proteins from rice pollen have shown cross-allergenicity with those of timothy and orchard grass [27]. These results suggest that the group-I allergens are indeed widespread in the grass family. This conservation of group-I allergens in grasses from many subfamilies suggests that they may have an important role in pollen function or development. The proteins are released from pollen soon after coming in contact with moisture, suggesting a role in pollen stigma interactions during pollination.

There is abundant evidence of the lack of cross-reactivity between the allergens of Bermuda grass and other grass pollen allergens, particularly those from grasses in the subfamily Pooideae [2–4, 28]. *Cyn d I* is not precipitated in radioimmuno-electrophoresis by polyclonal antisera raised against *Lol p I* (other group-I allergens are precipitated) [9] and rabbit anti *Lol p I* polyclonal antibodies do not bind *Cyn d I* on Western blots [29]. In contrast, polyclonal antisera raised against *Cyn d I* precipitated (although weakly) *Lol p I*, *Dac g I*, *Phl p I* and *Poa p I* in radioimmuno-electrophoresis, and recognized 28- to 35-kD proteins in ten other grasses [9].

An explanation for these results is that antibodies are raised against certain dominant epitopes on group-I allergens. *Cyn d I* appears to have different dominant epitopes to those on group-I allergens of grasses from the subfamily Pooideae (possibly due to differences in the tertiary structure of the protein when compared to other grasses). These epitopes are still present on the other allergens and so many Mabs raised against *Cyn d I* recognized group-I allergens from grasses in other subfamilies, as shown by Matthiesen et al. [9].

By using tryptic digestion of *Lol p I*, Esch and Klapper [30, 31] identified an IgE-binding peptide corresponding to the major IgE epitope of *Lol p I*. Similar digests have been made on group-I proteins from other grasses and the sequence of the major IgE-binding peptides was found to be highly conserved in all the grasses tested. The existence of this highly conserved IgE epitope explains the allergenic cross-reactivity between closely related grasses [31]. We have recently reported the cloning and sequencing of cDNAs encoding *Lol p I* [20]. The complete amino acid sequence of *Lol p I* has been deduced [19, 20] and we have found that the IgE-binding peptide is actually the COOH terminus of the protein [20]. Cloning and sequencing of *Cyn d I* will allow its amino acid sequence to be determined. Comparison of the COOH terminal of *Cyn d I* with that of *Lol p I* may identify the molecular basis for the observed lack of allergenic cross-reactivity between these two proteins.

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