

Yin-Mei Chiung¹
 Bai-Ling Lin²
 Chin-Hui Yeh³
 Chu-Yung Lin³

¹Institute of Occupational
 Safety and Health

²Institute of Molecular
 Biology, Academia Sinica,
 Taipei, Taiwan, ROC

³Department of Botany,
 National Taiwan University

Heat shock protein (hsp 70)-related epitopes are common allergenic determinants for barley and corn antigens

IgE reactive components of barley and corn were compared using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with sera from workers exposed to complex bioaerosols. The antibody made against *Arabidopsis* heat shock protein (hsp 70) was used to identify those components equivalent to hsp 70 in molecular size. Components with a molecular mass of 69 kDa and 33 kDa were positively reacted, and immunoblots of two-dimensional polyacrylamide gel electrophoresis were compared.

Keywords: Allergen / Corn / Barley / Hsp70

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1 Introduction

Long-term inhalation of grain dust was reported to be a cause of occupational asthma [1]. Skin tests and broncho-provocation tests proved that corn dust is a cause of respiratory syndromes [2] related to the workplace. Several proteins isolated from grain powders of *Gramineae* were reported to be similar in amino acid sequence and immunological properties [3, 4]. A comparison of the epitopes results in a clear correlation of cross reactivity between suspected antigens [5]. Prevalent studies of serological examination of allergens may exhibit the effect on health caused by bioaerosol exposure, as well as the common allergenic proteins from different sources [6]. Over 50% of the feeds used on pig farms is made of corn flour; therefore, it is reasonable to consider this a possible work-related allergic bioaerosol on pig farms. In this study, we examined sera from the workers with ELISA and immunoblotting using extracts of barley and corn flour, and discovered similarities in these two allergens. Many heat shock proteins (hsp) were recognized by immunochemical studies [7]; these might also be possible allergens in fungi [8]. Antigenic cross-activity of heat shock protein (hsp 70) with a 70 kDa component was proved by amino acid sequence alignment in *Penicillium citrinum* [9]. An IgE-binding protein was reported homologous to hsp90 in the *Aspergillus fumigatus* cDNA study [10]. Allergens DerfII, screened from the *Dermatophagoides farinae* cDNA library, were reported to be significantly homologous in the carboxyl-terminal region of the hsp 70 family [11]. It is meaningful to find causes to

explain the roles hsp-related molecules play in immunological pathology, especially the cross-reactive epitopes.

In the serum screen of allergic workers at pig-farm houses, we found a high rate of positive reactions with IgE both to barley and corn extracts, which were significantly associated, when analyzed by χ^2 on ELISA and immunoblots of SDS-PAGE protein gels. Among the components with high occurrence, 69 kDa molecules were shown to be a possible common antigen in similarity. An antibody against C-terminal amino acids of hsp 70 from *Arabidopsis* was used to identify these potential allergens in extracts of corn and barley.

2 Materials and methods

2.1 Serum samples tested

The sera were collected from 133 pig-farm workers in southern Taiwan, from March to May. Twenty-seven workers (20.4%) had allergy problems due to biological causes, and six workers were sensitive to feed dust, according to questionnaires.

2.2 Allergen extracts, enzyme-linked immunoassay

Barley and corn flour used in the preparation of extracts were reground and extracted with 0.5 M phosphate-buffered saline (PBS), pH 7.0, for 18 h at 4°C, and the soluble proteins were concentrated with ammonium sulfate, resolved in bicarbonate buffer, and lyophilized, after dialysis. The dried powder were prepared to 2–2.5 mg/mL solutions, and used in ELISA and electrophoresis, following the methods used in our clinical routine [12]. For ELISA tests, the aliquots in pH 9.6 were coupled to polystyrene microtiter plates for 18 h at 4°C. Sera (50 μ L) at a 1:10 dilution in PBS containing 0.05% Tween (PBST), pH 7.0, were for specific IgG, and in a 1:4 dilution for specific IgE.

Correspondence: Dr. Y. M. Chiung, Institute of Occupational Safety and Health, 4th Fl., 132, Min Sheng E. Rd., sec 3, Taipei, Taiwan, ROC

E-mail: chiun@mail.cla.gov.tw

Fax: +886-2-27197261

Abbreviations: AP, alkaline phosphatase; hsp, heat shock protein

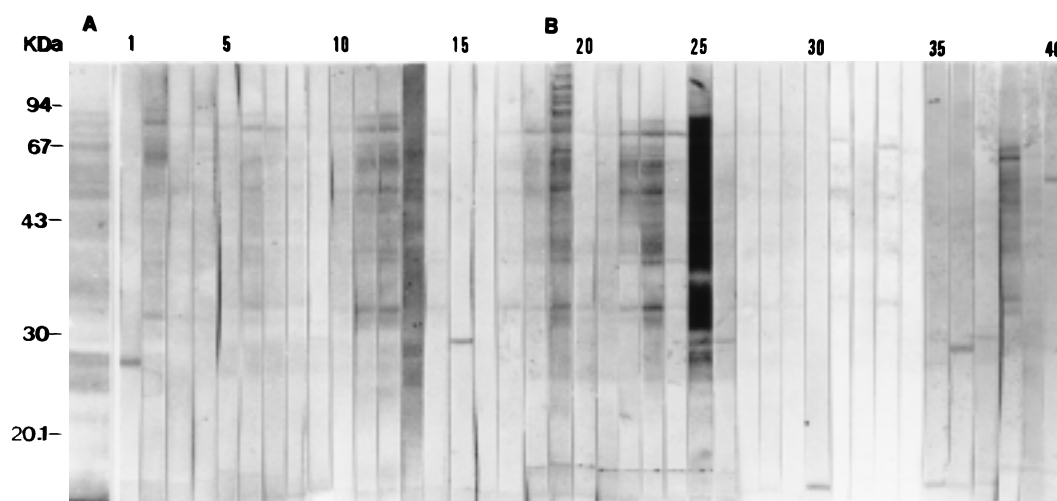


Figure 1. Identification of IgE-binding components by alkaline phosphatase conjugated anti-human IgE antibodies after SDS-PAGE of the crude extract of corn with sera from pig-farm workers. (A) Corn extract; (B) profiles of IgE-binding components.

Cord blood sera were used as negative control; those samples showing an over fourfold increase in color reactions were considered to be positive.

2.3 Electrophoresis and immunoblot analysis

The protein samples, reduced with 2% SDS, at 100°C for 5 min, were separated by 12.5% SDS-PAGE after transfer onto PVDF membrane; the blotted membranes were incubated overnight with the sera separately. The alkaline phosphatase (AP)-conjugated anti-human IgG ϵ antibody was reacted at room temperature. Anti-Hsp 70 polyclonal antibodies against *Arabidopsis* were used to identify related molecules in barley and corn extracts. The reactions were visualized with enhanced luminal detection with the AP or ECL Western blotting system. Two-dimensional (2-D) gel electrophoresis was performed with carrier ampholytes pH 3–pH 10 in the first dimension and SDS-PAGE in the second dimension, following the methods of O'Farrell.

2.4 Statistical analysis

The correlation of allergic components and the exposure groups were analyzed by SPSS.

3 Results and discussion

Respiratory symptoms of workers and its correlation with specific IgE against feed flour were described previously [13]. The results of ELISA showed that nine workers have IgE-positive reactions to the corn extract, 11 by CAP methods in routine clinical examination. In ELISA five workers exhibited significant reactions to barley extract.

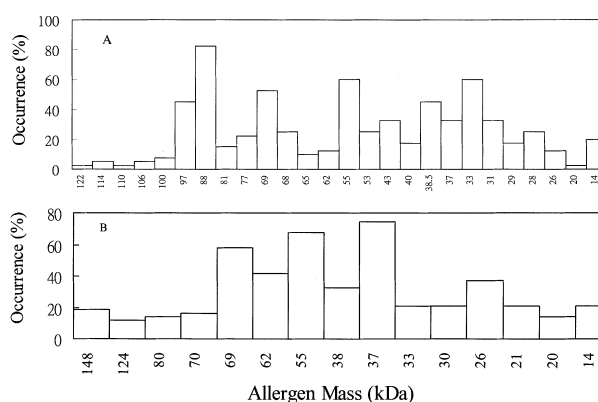


Figure 2. Allergrams of the crude extracts of corn and barley flour recognized by sera of pig-farm workers. (A) Corn; (B) barley; % of occurrence was calculated as number of samples with positive reactivity to specific components divided by total positive samples.

Much higher positive rates were observed by SDS-PAGE and immunoblot examination. Among the sera tested, 40 (30.1%) showed IgE reactivity to protein extracts of corn. Three kinds of barley antigens found to have significant correlations to the exposure level [14] are the components of 55, 37, and 30 kDa in molecular mass, calculated from the results of a 32.6% positive rate immunoblot examination. Heterogeneity in the IgE-binding pattern was found both in corn (Fig. 1) and barley; in individual cases significant similarity in component binding and reaction intensity was exhibited ($P < 0.01$). The most prevalent antigens were the 88 kDa component in corn and the 37 kDa component in barley; suspected common antigens with similar molecular mass were also noticed (Fig. 2). Components with molecular masses between

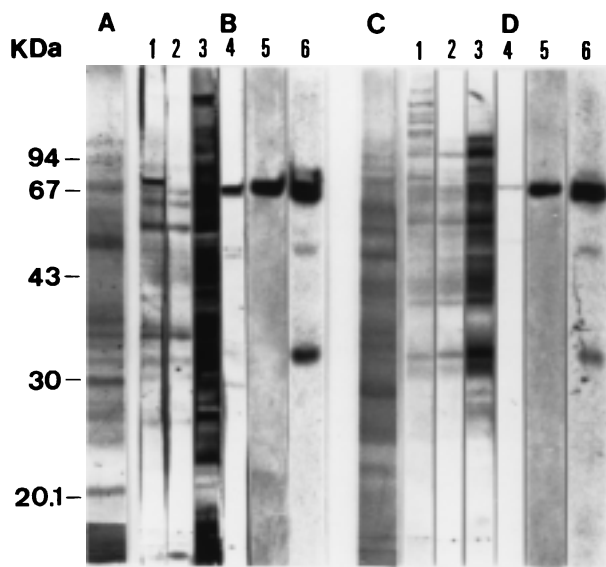


Figure 3. Antibody-binding components of barley and corn in one-dimensional immunoblots. (A) Barley extracts; (B) components of barley extract recognized by antibodies; (C) corn extracts; (D) components of corn extract recognized by antibodies. The profiles of proteins recognized by IgE of workers with respiratory syndrome, B1~3 and D1~3; asthma patient, B4 and D4; anti-hsp70, B5, 6 and D5, 6.

68–70 kDa appeared in both extracts, and reacted with positive samples. A component of 69 kDa was reactive with polyclonal antibodies against *Arabidopsis* hsp 70. Another component of 33 kDa was also recognized, possibly due to greater degradation of the corn extract preparation (Fig. 3). The allergenic components of three workers with respiratory syndromes were identified to have different patterns, but all showed significant reactions to the 69 kDa molecules suspected in hsp 70 proteins. A sample from an asthma patient allergic to *Penicillium* also showed positive reactions to the 69 kDa components contained in corn and barley. The positive reaction rates among 43 allergic workers were 20.9% and 11.6% with barley and corn respectively, in ELISA tests, but no positive reactions were detected among nonallergic workers. This result reveals that barley and corn are causative agents related to the workplace. Immunoblot tests provided additional information in comparison of these two occupational allergen studies. Samples showing positive reactions with IgE to these two extracts were significantly similar when analyzed by χ^2 , both on ELISA and immunoblots of SDS-PAGE protein gels.

Extracts of barley and corn were also analyzed by 2-D gel electrophoresis (Fig. 4), followed by immunoblotting with pooled sera. It showed a large portion of the allergenic

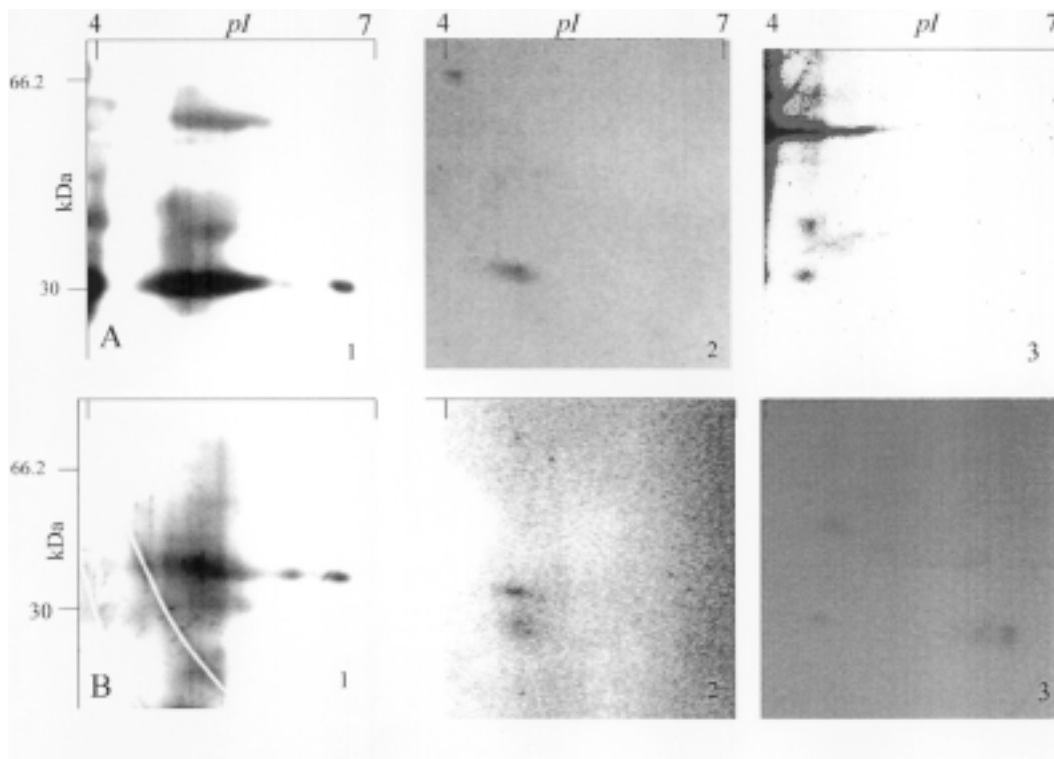


Figure 4. A comparison of components of barley and corn flours in 2-D gel electrophoresis and immunoblots recognized by human IgE antibodies and anti-hsp70 antibodies. (A) Barley; (B) corn; (1) silver stain; (2) anti-hsp70 antibody with HRP; (3) anti-human IgEε chain with AP.

components over pI 4.5. Both of the susceptible hsp 70-related components around 69 and 33 kDa showed positive reactions with anti-hsp 70 antibody, but only the degraded ones exhibited weak reactions when examined by pooled sera. Consequently, the components around 33 kDa in molecular mass and pI 5.0 were both reacted with human IgE and antibody to hsp70. Considering the positive reaction results of 69 kDa and 33 kDa in SDS-PAGE immunoblots in an hsp-related epitope examination, the 33 kDa protein might be a degraded fragment of 69 kDa, a possible protein in the hsp 70 family.

Among the allergens found in the *Poaceae* species plant, pollen-specific maize proteins showed significant sequence homology with other specific proteins from monocot and dicot plants [15, 16]. Some of these allergens were only expressed in pollen, not in vegetable and floral tissue [17]. Cross-reactivity of cereal grains in species of the *Poaceae* family was confirmed by absorption test, and a trend towards positive correlation was indicated for RP16ID [18]. In our study, although hsp 70-related epitopes are suggested as being the result of a possible participation of IgE-binding structure on 69 and 33 kDa molecules in cross-reactivity between barley and corn, more evidence is required. The similarity of the positive reactions to the components barley and corn extracts in pig-farm workers may result from the specificity of *MHC* genes of workers and a common exposed environment. The simultaneous exposure to barley and corn dust may have an enhancing effect on common antigens or common epitopes of different antigens. Reports on hsp 70 allergens are quite diverse, including mold, insects and plants, but few discuss the different allergenic factors. Heat shock proteins are reported to have multiple roles of clinical significance; several have been reported to induce delayed-type hypersensitivity reactions, some are associated with the development of protective cellular immunity or adverse auto-antibodies [19], and only few showed IgE reactivity towards the sera of asthma patients. This significant pathological complexity was reported to be due to the different Hsp families and their sequences. The antigenic diversity, whether there is any similarity in the structure of related epitopes recognized by human IgE in heat shock proteins, and its pathological significance, requires further study.

4 Concluding remarks

The results in this study showed a partial similarity between protein components in extracts of flours made from grains of barley and corn. A common epitope in the components of 69 kDa and its degraded molecules was recognized by an antibody preparation against a plant hsp

70. Molecules with molecular mass and pI values similar to hsp 70 degraded components reacted positively with serum IgE of allergic workers. A common allergenic determinant site related to hsp 70 was suggested.

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