



ORIGINAL ARTICLE

## Alpha Amylase Is a Major Allergenic Component in Occupational Asthma Patients Caused by Porcine Pancreatic Extract\*

Hae-Sim Park, M.D., Ph.D.,<sup>†</sup> Hee-Yeon Kim, M.D.,  
You-Jin Suh, M.D., Soo-Jin Lee, M.D.,  
Soo-Keol Lee, M.D., Sun-Sin Kim, M.D., and  
Dong-Ho Nahm, M.D., Ph.D.

*Department of Allergy and Clinical Immunology, Ajou University  
School of Medicine, Suwon, Korea*

### ABSTRACT

*Porcine pancreatic extracts (PPE) are composed of  $\alpha$ -amylase and lipase, which are common components of digestive enzymes. They have been known to cause occupational asthma in exposed workers in pharmaceutical and baking industries, as well as in a laboratory technician, but there has been no report of PPE-induced occupational asthma in medical personnel and their IgE binding components to each component.*

*Four asthmatic subjects showing positive results on PPE-bronchoprovocation testing were enrolled. All of them were nurses working in a university hospital. Their job included grinding and mixing PPE powder for admitted patients. Serum-specific IgE antibodies to PPE,  $\alpha$ -amylase, and lipase were measured by enzyme linked immunosorbent assay (ELISA). To confirm specificity of IgE binding and cross-allergenicity among the three extracts, ELISA inhibition tests were performed. In order to characterize allergenic components within these three extracts, SDS-PAGE and IgE immunoblot analysis were done.*

\*This study was supported by 1999-2-212-003-3 from the interdisciplinary research program of the KOSEF.

<sup>†</sup>Corresponding author. Dr. Hae-Sim Park, M.D., Ph.D., Associate Professor, Department of Allergy and Clinical Immunology, Ajou University School of Medicine, Paldalgu Wonchondong San-5, Suwon, 442-721, Korea. Fax: 031-219-5154; E-mail: hspark@madang.ajou.ac.kr

*Specific IgE antibodies to PPE,  $\alpha$ -amylase, and lipase were detectable by ELISA in all study subjects. An  $\alpha$ -amylase ELISA inhibition test showed significant inhibitions by amylase and PPE, and minimal inhibition by lipase. However, a lipase ELISA inhibition test showed significant inhibitions by  $\alpha$ -amylase and PPE with a lesser degree of inhibition by lipase. Furthermore, IgE immunoblot analysis showed one IgE binding component (55 kDa) within PPE, six components (55 kDa, 43 kDa, 41 kDa, 32 kDa, 31 kDa, 29 kDa) within  $\alpha$ -amylase and two components (31 kDa, 29 kDa) within lipase extracts.*

*These findings suggest that inhalation of PPE powder can induce IgE-mediated bronchoconstriction in exposed nurses. Alpha-amylase is a major allergenic component within PPE.*

**Key Words:** Pancreatic extract;  $\alpha$ -Amylase; Occupational asthma; Specific IgE

## INTRODUCTION

There have been many reports of occupational asthma induced by biological enzymes inhaled in the course of work.<sup>[1-4]</sup> An IgE-mediated mechanism has been implicated in most cases.<sup>[1-4]</sup> Digestive drugs, including porcine pancreatic enzyme, have been widely used in our country. Porcine pancreatic extracts (PPE) are composed of  $\alpha$ -amylase and lipase, which are common components of digestive enzymes. Since occupational asthma due to  $\alpha$ -amylase was first reported by Eloy,<sup>[5]</sup> there have been reports of workers exposed in the baking industry, the pharmaceutical industry, and in the laboratory.<sup>[5-7]</sup> It was also suggested that IgE-mediated response was the major pathogenic mechanism. To the best of our knowledge, this is the first study demonstrating PPE-induced occupational asthma in exposed nurses, and their IgE binding components to each component. Also, allergenic relationships among PPE,  $\alpha$ -amylase, and lipase were identified.

## MATERIALS AND METHODS

### Subjects

Four subjects complaining of asthmatic symptoms when exposed to PPE powder were enrolled. All of them gave their informed consent, which was regulated by the institutional review board of Ajou University Hospital, Suwon, Korea.

Tables 1 and 2 show the clinical characteristics of the four asthmatic subjects. All of them had complained of asthmatic symptoms when handling PPE powder. One had complained of rhinitis

symptoms. Three were atopic. There were no smokers. All of them showed positive results on skin prick test to 1:10 w/v PPE extract. Specific IgE antibodies to the three antigens were detectable by ELISA in the sera of all subjects. All of them showed early asthmatic responses on bronchoprovocation tests to PPE.

### Preparation of Amylase and Lipase Extracts

The PPE tablets were ground into powder form, which is commonly used in medicine. They were extracted with phosphate-buffered saline [(PBS, pH 7.5), 1:5 wt/vol] at 48°C overnight. The supernatants were used for the bronchoprovocation tests at a concentration of 1:10 wt/vol. For the skin prick test, the 1:5 wt/vol extracts were mixed with an equal amount of sterile glycerine. Some of the supernatant was dialyzed (cut-off molecular weight 6000 D) against 4 L of distilled water at 48°C for 48 hr, and lyophilized at -70°C. Purified powder of  $\alpha$ -amylase and lipase was supplied by Sigma Co. (St. Louis, MO).

### Bronchoprovocation Test with PPE

Airway responsiveness to methacholine was tested according to the method described previously.<sup>[8]</sup> Bronchoprovocation tests were performed according to the procedure used in occupational asthma studies.<sup>[4]</sup> Normal saline was administered from a nebulizer 646 connected to a dosimeter (Devilbiss Co., Doylestown, PA, USA). The subject was asked to breathe the aerosol for 2 min with

**Table 1***Clinical Characteristics of the Study Subjects*

| Case | Age<br>(Year) | Latent Period<br>(Year) | Rhinitis | Skin Reactivity<br>to PPE (mm) <sup>a</sup> | Specific IgE <sup>b</sup> |                   |        |
|------|---------------|-------------------------|----------|---------------------------------------------|---------------------------|-------------------|--------|
|      |               |                         |          |                                             | PPE                       | $\alpha$ -Amylase | Lipase |
| 1    | 25            | 3                       | —        | 4                                           | 520                       | 828               | 65     |
| 2    | 26            | 4                       | —        | 4                                           | 130                       | 379               | 82     |
| 3    | 25            | 3                       | —        | 3                                           | 88                        | 111               | 182    |
| 4    | 23            | 2                       | +        | 3                                           | 74                        | 125               | 100    |

<sup>a</sup>Skin reactivity presented as mean wheal diameter.<sup>b</sup>Specific IgE level presented as absorbance value (positive cut-off: 70 for PPE, 58 for  $\alpha$ -amylase, 54 for lipase).**Table 2***Clinical Features of the Study Subjects*

| Case | Methacholine PC20<br>(mg/mL) | PPE-BPT <sup>a</sup> | Allergen<br>PD20 |
|------|------------------------------|----------------------|------------------|
| 1    | > 25                         | Early                | 0.23             |
| 2    | > 25                         | Early                | 0.35             |
| 3    | > 25                         | Early                | 7.5              |
| 4    | 5.0                          | Early                | 5                |

<sup>a</sup>PPE-BPT: Bronchoprovocation test to porcine pancreas extract.

tidal breathing. This was followed by antigen extracts at concentrations ranging from 1:1000 wt/vol to 1:10 wt/vol. The pulmonary function tests including forced expiratory volume in one second (FEV<sub>1</sub>) and FEF<sub>25–75</sub> were measured with a spirometer (Multi-SPIRO SX/PC, Tempe, AZ, USA) before and 10 min after each inhalation. Then, the FEV<sub>1</sub> and FEF<sub>25–75</sub> were measured every 10 min during the first hour, and pulmonary function tests were performed every hour for 7 hr after the challenge. When the FEV<sub>1</sub> level decreased more than 20% from the baseline value, it was regarded as a positive response.

**ELISA for Specific IgE Antibody to PPE, Amylase, and Lipase, and ELISA Inhibition Tests**

The presence of specific IgE antibodies to the three antigens was determined by ELISA. Microtiter plates (Dynatech, VA, USA) were first coated with 100  $\mu$ L of each antigen (5  $\mu$ L/well),

and left at 4°C overnight. Each well was washed three times with 0.05% Tween-phosphate-buffered saline (PBST), and the remaining binding sites were blocked by incubation with 250  $\mu$ L of 3% bovine serum albumin (BSA)-PBST for 1 hr at room temperature. The wells were then incubated for 2 hr at room temperature with 100  $\mu$ L of either the patient's serum (undiluted) or unexposed control sera from 20 patients showing negative responses to common inhaled allergens as well as to PPE. After washing three times with PBST, 100  $\mu$ L of 1:1000 vol/vol biotin-labeled goat anti-human IgE antibodies (Sigma Co., St. Louis, MO) were added to the wells and incubated for 2 hr at room temperature. The wells were then washed three times with PBST and incubated with 1:1000 vol/vol streptavidin-peroxidase (Sigma Co., St. Louis, MO) for 30 min before another washing step followed by incubation with 100  $\mu$ L ABTS (2,2'-azinobis-3-ethyl-benzthiazoline sulfonic acid in a citrate phosphate buffer) for 10 min at room temperature. The reaction was stopped by the addition of 50  $\mu$ L 2 M sodium azide, and the absorbance was read at 410 nm by an automated microplate reader. All assays were performed in triplicate. Positive cut-off values of specific IgE bindings were derived from mean+2 standard deviations of 20 unexposed controls, as described previously.

**SDS-PAGE and Immunoblotting**

Porcine pancreatic amylase, and lipase extracts (10 mg/mL) were dissolved in a simple buffer (0.5 M *tris*-HCl, pH 6.8, 2.5 mL, glycerol 2.0 mL, 10% wt/vol sodium dodecyl sulfate 4.0 mL, 0.1% bromophe-

nol blue 0.5 mL, and distilled water 1.0 mL) and heated at 100°C for 3 min. Ten microliters of molecular weight markers (6–250 kDa) (Novex, San Diego, CA) and antigen solutions were added to a Novex precast 12% *tris*-glycine gel (acrylamide) for the separation of antigens. Electrophoresis was performed with a Novex Mini-cell for 90 min at 130 constant voltage. The gel was fixed and stained with Coomassie brilliant blue. For immunoblotting, the gel was soaked in a transfer buffer (12 mM *tris*, 96 mM glycine in 20% methanol) for 10 min. Electroblotting was carried out for 90 min at 200 mA using a Novex western transfer apparatus. The blotted polyvinylidene difluoride membrane (Novex, San Diego, CA) was then treated with PBST containing 5% bovine serum albumin for 1 hr to block non-specific protein binding. The sera pool of four patients and five unexposed controls was diluted to 1:2 vol/vol with PBST. The membrane was then incubated with the patient's serum and control sera for 18 hr at room temperature. After being washed with PBST, the membrane was incubated with biotinylated anti-IgE antibodies (1:1000 wt/vol, Vector Laboratories, Inc., Burlingame, CA) for 3 hr at room temperature. The membrane was washed again with PBST and incubated for 1 hr with streptavidin-alkaline phosphatase (1:1000 wt/vol) (Sigma Chemical Co., St. Louis, MO). As a substrate solution, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma Co., St. Louis, MO) was applied to the membrane until positive bands appeared. After the reaction, the membrane was washed with distilled water and observed.

## RESULTS

### Allergenic Relationships Among PPE, Amylase, and Lipase

Figure 1 shows PPE (a) and  $\alpha$ -amylase (b) ELISA inhibition test results with serial additions of PPE, amylase, lipase, and *D. pteronyssinus* antigens. Significant inhibitions were noted with additions of  $\alpha$ -amylase and PPE in a dose-dependent manner, while minimal inhibitions were noted by lipase and *D. pteronyssinus* antigens. A lipase ELISA inhibition test showed significant inhibitions by amylase and PPE, with a lesser degree of inhibitions by lipase extracts, and minimal inhibitions by *D. pteronyssinus* antigen, as shown in Fig. 2.

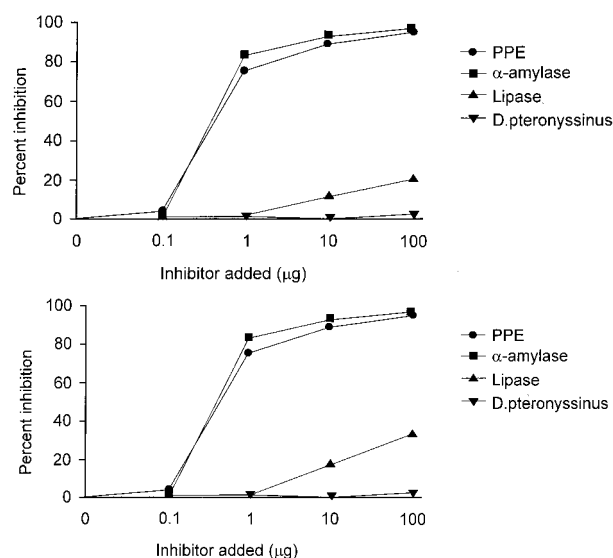


**Figure 1.** Inhibition results of PPE (a) and  $\alpha$ -amylase (b) ELISA with serial additions of PPE,  $\alpha$ -amylase, lipase, and *D. pteronyssinus* antigens.

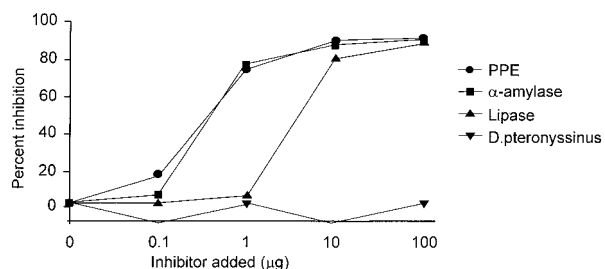


**Figure 2.** Inhibition results of lipase ELISA with serial additions of PPE,  $\alpha$ -amylase, lipase, and *D. pteronyssinus* antigens.

### Gel Electrophoresis and IgE Immunoblotting

In order to determine the protein components of PPE,  $\alpha$ -amylase, and lipase, the extracts were analyzed by a 12% SDS-PAGE. Figure 3 shows that PPE,  $\alpha$ -amylase, and lipase had various protein bands ranging from 6 to 100 kDa. Figure 4 shows the bindings of specific IgE in patients (P) and control sera (C) on a blotted nitrocellulose membrane. With PPE extracts, there was one strong band bound to IgE antibody with molecular weight of 55 kDa. Six components (55 kDa, 43 kDa, 41 kDa, 32 kDa, 31 kDa, 29 kDa) in  $\alpha$ -amylase and two components (31 kDa, 29 kDa) in lipase were also noted.

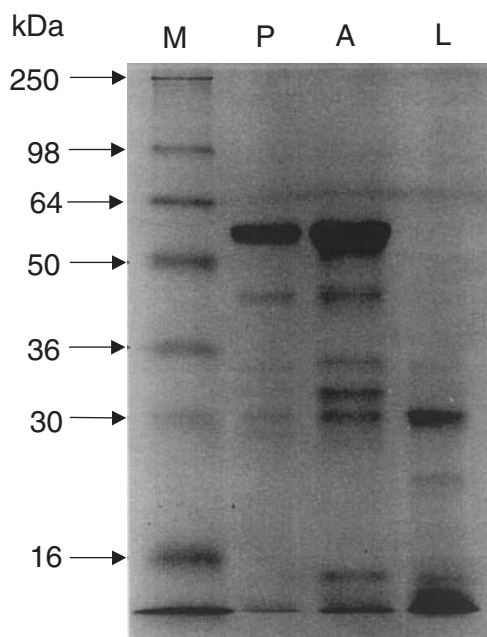


**Figure 3.** Protein staining of SDS-PAGE (12%) of porcine pancreatic extract (P),  $\alpha$ -amylase (A), and lipase (L) extracts. M: Marker protein.

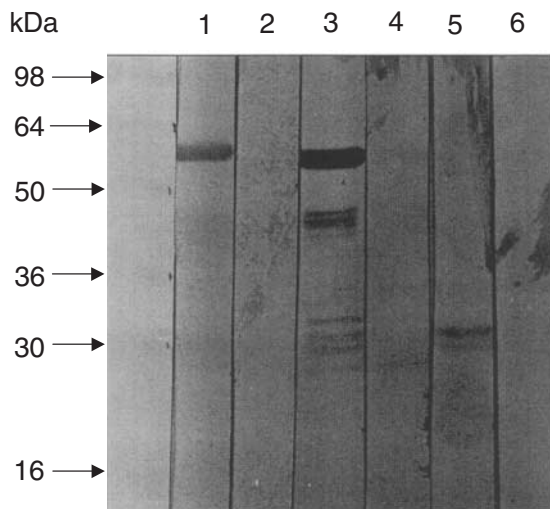


**Figure 4.** IgE immunoblot analysis of porcine pancreatic extract,<sup>[1,2]</sup>  $\alpha$ -amylase<sup>[3,4]</sup> and lipase<sup>[5,9]</sup> extracts in patients<sup>[1,3,5]</sup> and control sera.<sup>[2,4,9]</sup>

## DISCUSSION

This study demonstrates that PPE powder inhalation can induce occupational asthma in exposed

nurses working in a hospital. They had work-related asthmatic symptoms when exposed to PPE powder. All of them showed positive responses on skin prick test to PPE extracts prepared by PPE powder to which they were exposed. Also they showed early asthmatic responses to PPE bronchoprovocation test. When the bronchial challenge test with PPE was performed on two house dust mite-sensitive asthmatic patients with similar levels of airway hyperresponsiveness to methacholine, they showed no response. This indicates that the bronchoconstrictive response in this study's patients was a specific one. Ten healthy volunteers and 10 asthmatic patients with no history of exposure to PPE showed negative responses to skin prick tests with PPE.

The pathogenetic mechanism of occupational asthma caused by PPE may be an IgE-mediated reaction, since skin prick tests with this extract showed positive responses. Serum IgE antibodies specific to PPE,  $\alpha$ -amylase, and lipase were detected by ELISA. The ELISA inhibition results suggested specificity of IgE binding to  $\alpha$ -amylase, PPE, and lipase, as specific inhibitions were obtained with various dilutions of homologous antigens. However, both  $\alpha$ -amylase and PPE induced significant inhibitions to the same degree for both PPE and  $\alpha$ -amylase ELISA inhibition tests, while minimal inhibitions by lipase and *D. pteronyssinus* were seen. Lipase ELISA inhibition test results showed significant inhibitions by PPE and  $\alpha$ -amylase, with a lesser degree of inhibitions by lipase. Also, IgE immunoblot analysis showed IgE binding components on the PPE,  $\alpha$ -amylase, and lipase antigens. These results suggest that PPE inhalation can induce IgE-mediated bronchoconstriction in exposed nurses.  $\alpha$ -Amylase within PPE is a major allergenic component in PPE-induced bronchoconstriction.

Regarding allergenic relationships among PPE,  $\alpha$ -amylase, and lipase, both PPE and  $\alpha$ -amylase ELISA inhibition tests demonstrated that PPE and  $\alpha$ -amylase may have extensive cross-allergenicity. Furthermore, IgE immunoblot study also showed that PPE and  $\alpha$ -amylase extracts shared one strong IgE binding component (55 kDa), and that cross-allergenicity existed between amylase, PPE, and lipase on lipase ELISA inhibition test. Compared to PPE and  $\alpha$ -amylase, there was a lesser degree of inhibition by lipase on lipase ELISA inhibition tests. The IgE immunoblot analysis showed that lipase had two IgE binding components. All the subjects had positive response to PPE,  $\alpha$ -amylase,

and lipase together. No one showed an isolated positive response to lipase on IgE-ELISA. These results may suggest that PPE and  $\alpha$ -amylase have extensive cross-allergenicity, and that  $\alpha$ -amylase is a major allergenic component in PPE. Although it is impossible to completely exclude the possibility of lipase as an allergen, as the decreased inhibitory capacity of lipase in this study could simply be due to the fact that the antibodies produced in these nurses were lower affinity antibodies for lipase compared to PPE and  $\alpha$ -amylase, positive binding to lipase antigen in this study may be caused by cross-allergenicity with  $\alpha$ -amylase antigen. Further studies will be needed with a larger group of patients to clarify the allergenicity of lipase.

Several authors have suggested the important role of atopy in the development of IgE-sensitization to occupational allergens with high molecular weight.<sup>[10–12]</sup> In this study, three out of four asthmatic patients had atopy, although our previous study demonstrated no significant association between IgE-sensitization to PPE or  $\alpha$ -amylase, and atopic status in exposed nurses working in the same environment.<sup>[13]</sup> This is comparable with Wiessmann and Baur's study.<sup>[14]</sup>

In conclusion, this study demonstrates that commonly used drug powders, such as PPE, can induce occupational asthma in exposed nurses working in a hospital. Evidence is presented to indicate that  $\alpha$ -amylase included in PPE is a major allergenic component that can induce IgE-mediated bronchoconstriction. Cross-allergenicity between  $\alpha$ -amylase and lipase was noted.

## REFERENCES

1. Cartier, A.; Malo, J.-L.; Pineau, L.; Dolovich, J. Occupational Asthma Due to Pepsin. *J. Allergy Clin. Immunol.* **1984**, *73*, 574–577.
2. Pauwels, R.; Devos, M.; Callens, L.; van der Straeten, M. Respiratory Hazards from Proteolytic Enzymes. *Lancet*, 1978; *i*, 669.
3. Bernstein, J.A.; Bernstein, D.I.; Warrington, R.; Bolin, T.; Warren, C.P.; Bernstein, I.L. Occupational Asthma Induced by Inhaled Egg Lysozyme. *Chest* **1993**, *103*, 532–535.
4. Park, H.S.; Nahm, D.H. New Occupational Allergen in a Pharmaceutical Industry: Serratia Peptidase and Lysozyme Chloride. *Ann. Allergy Asthma Immunol.* **1997**, *78*, 225–229.
5. Eloy, L.; Miguel, H.; Santiago, Q.; Maneo, I. Occupational Asthma Caused by  $\alpha$ -Amylase Inhalation: Clinical and Immunologic Findings and Bronchial Response Patterns. *J. Allergy Clin. Immunol.* **1992**, *89*, 118–125.
6. Zentner, A.; Jeep, S.; Wahl, R.; Kunkel, G.; Kiethe-Tebbe, J. Multiple IgE-Mediated Sensitizations to Enzymes After Occupational Exposure: Evaluation by Skin Pricks Test, RAST, and Immunoblot. *Allergy* **1997**, *52*, 928–934.
7. Brisman, J.; Belin, L. Clinical and Immunological Responses to Occupational Exposure to  $\alpha$ -Amylase in the Baking Industry. *Br. J. Ind. Med.* **1991**, *48*, 604.
8. Chai, H.; Farr, R.S.; Froelich, L.A.; Mathison, D.A.; McLean, J.A.; Rosenthal, R.R.; Sheffer, A.L.; Spector, S.L.; Townly, R.G. Standardization of Bronchial Challenge Procedure. *J. Allergy Clin. Immunol.* **1977**, *56*, 323–327.
9. Aiken, T.C.; Ward, R.; Peel, E.T.; Hendrick, D.J. Occupational Asthma Due to Porcine Pancreatic Amylase. *Occup. Envir. Med.* **1997**, *54*, 762–764.
10. Neewhous, M.L.; Tagg, B.; Popck, S.L.; McEdan, A.C. An Epidemiologic study of workers Producing Enzyme-Washing Powders. *Lancet* 1970; *i*, 689–693.
11. Davies, G.E.; McArdle, L.A. Allergy to Laboratory Animals: A Survey by Questionnaire. *Int. Arch. Allergy Appl. Immunol.* **1981**, *64*, 302–307.
12. Block, G.; Tse, K.S.; Kijek, K.; Chan, H.; Chan-Yeung, M. Baker's Asthma: Clinical and Immunological Studies. *Clin. Allergy* **1983**, *13*, 359–370.
13. Lee, S.J.; Kim, H.Y.; Suh, Y.J.; Kim, S.S.; Lee, S.K.; Nahm, D.H.; Park, H.S. Occupational Asthma in Medical Personnel Caused by Inhalation of Pancreatic Extracts. *J. Asthma Allergy Clin. Immunol.* **2000**, *20*, 184–192.
14. Wiessmann, K.J.; Baur, X. Occupational Lung Disease Following Long-Term Inhalation of Pancreatic Extracts. *Eur. J. Respir. Dis.* **1985**, *6*, 13–20.