51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

PHOSPHOGLUCOMUTASE AS A NOVEL MEAT ALLERGEN

Soichi Tanabe*[1], Yuki Nakaguma[1], Yoshihisa Takahata[2], Fumiki Morimatsu[2], And Toshihide Nishimura[1]

[1] *Graduate School of Biosphere Science, Hiroshima University, Higashi-hiroshima,*
[2] *Research and Development Center, Nippon Meat Packers, Inc., Tsukuba, JAPAN*

Key Words: meat safety, meat allergy, phosphoglucomutase, serum albumin, cross-reactivity

Introduction

The prevalence of chicken allergy ranges from 0.6 to 5% in food allergic subjects (Table 1). Food allergy is triggered by allergic proteins (allergens) that bind to the patients IgE antibodies on mast cell surfaces. Although there are several allergens, chicken serum albumin has been implicated as the major chicken allergen. However, we detected unknown allergic band migrating at 58 kDa.

Objectives

The aim of this study was to identify the 58 kDa allergen and to clarify its properties. As a result, in this study, phosphoglucomutase (PGM) was identified as a novel chicken allergen. Thus, we evaluate whether bovine and porcine PGMs were also recognized by the patients IgE antibodies or not. The possible cross-reactivity between serum albumin and PGM was also investigated.

Materials & methods

Patients

Serum samples were obtained from 8 patients allergic to meat with atopic dermatitis. Meat allergy was proved by clinical symptoms and a positive radioallergosorbent test. Informed consent was obtained from all donors. Sera from two non-allergic adults were also used as negative controls. Serum samples were used after appropriate dilution with 0.1 % Tween 20 containing PBS (PBS-T).

Meat extract

Chicken, beef, and pork thigh meat were obtained from market source. Five grams of each meat was homogenized in 15 mL of 40 mM potassium phosphate buffer (pH 7.2) using a polytron homogenizer (Kinematica, Switzerland). The homogenate was centrifuged at 10,000 *g* for 20 min at 4 ^{o}C and the supernatant was filtered through filter paper (extract). Protein concentration of the extract was measured using a commercial kit (Bio-Rad, USA).

Western-blotting

Meat (chicken, beef, and pork) extract or fractions on DEAE-cellulose column chromatography (described below) were electrophoresed in 10 % polyacrylamide gels by the usual method and blotted onto polyvinylidenedifluoride (PVDF) membranes (Immobilon-P, Millipore, USA). Since patient IgE antibodies reacted strongly with BSA but not with human serum albumin (HSA), the membrane was soaked in a 1 % HSA (Sigma, USA)-PBS-T solution for blocking, instead of using BSA, which is frequently chosen. The membrane was then incubated with five-fold diluted pooled sera for 1 hr at room temperature and sequentially with biotinylated anti-human IgE (Kirkegaard & Perry Lab. Inc., USA) for 2 hrs at room temperature. The membrane was further incubated with avidin-DH and biotin-conjugated HRP (VECTORSTAIN ABC-PO kit, Vector Laboratories, USA) according to the manufacturer's protocol, and was finally stained with a diaminobenzidine substrate kit (Vector Laboratories). Separately, proteins were stained with coomassie brilliant blue (CBB; Bio-Rad).

For inhibition western-blotting analysis, the patient sera (2 ml) were preincubated with 2 mg of phosphoglucomutase (PGM, Sigma, USA) for 1 hr at room temperature, and then applied to the same analysis.

DEAE cellulose column chromatography

The chicken extract was put on a column (5x18 cm) of DEAE-cellulose (Wako Pure Chemical Industries, Japan) that had been equilibrated with 10 mM Tris-HCl buffer (pH 7.2). Elution with a linear gradient of 1 M NaCl in the same buffer was performed. Proteins were monitored by the UV absorbance at 280 nm. Fractions 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, and 36-40 were collected and applied to western-blotting as described above.

Determination of N-terminal amino acid sequence

The electroblotted proteins on PVDF membrane were stained with CBB. The bands of interest at 58 kDa were excised, and then the N-terminal amino acid sequences were analyzed by a G1005A Protein Sequencer (Hewlett Packard, USA).

ELISA

ELISAs were performed by the usual method. Briefly, 100 μl of the meat extracts (0.025 mg/ml) or protein (PGM, BSA or GAPDH, Sigma) solutions (0.05-0.1 mg/ml) were coated on the well. After blocked with 1 % HSA in PBS, the appropriately (10-40 fold) diluted serum samples were applied to the well. For inhibition ELISA assay, proteins (BSA or PGM, 0.05-0.1 mg/ml) were added to serum samples as inhibitors prior to applying to the well. The binding of IgE-antibody to plate-coated antigin was determined by sequential incubations with biotinylated anti-human IgE, streptavidin-peroxidase conjugate (Boehringer Mannheim, Germany), and *o*-phenylenediamine (Wako). The values in Fig. 4 are expressed as relative ELISA values based on the absorption at 492 nm. Percent inhibition in Fig 3 and Table 2 was calculated as (1-A/B)×100, where A is the ELISA value of serum preincubated with inhibitor, and B is that of the control.

Results & Discussion

1. Phosphoglucomutase (PGM) as a novel chicken allergen

Chicken extract was applied to SDS-PAGE, and the separated proteins were stained with CBB (Fig. 1A). Western-blotting analysis was also performed using patients IgE-antibodies (Fig. 1B). Several IgE-positive bands were detected; among them, previously-known allergens, such as chicken serum albumin (CSA), fructose-bisphosphate aldolase (FBPA), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), were identified according to their molecular masses. In addition, as shown in Fig. 1B, we detected unknown allergic band migrating at 58 kDa. Regretfully, we failed to reveal the N-terminal amino acid sequence by using a protein sequencer because of the low yield and/or the impurity of the sample.

Thus, chicken extract was first applied to DEAE-cellulose column chromatography as indicated in Fig. 2A. Fractions 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, and 36-40 on this chromatography were collected and applied to western-blotting. Since the 58 kDa protein was eluted mainly in fraction 11-15 (Fig. 2B, arrow), it was excised, and then the N-terminal amino acid sequence was analyzed again. As a result, the sequence was revealed to be VHIETVKTKA that is completely identical to the N-terminal amino acid sequence of chicken phosphoglucomutase (PGM). Thus, we identified PGM as a novel chicken allergen.

2. Bovine, porcine and rabbit PGMs are also allergen for meat allergic patients

Rabbit PGM was also recognized by the patient IgE antibodies; PGM was judged to be a major allergen, since, among eight patients, six were reactive to rabbit PGM (Fig. 4, *left*). For further studies, we used rabbit PGM, since, only rabbit one was available from the market source.

By the same western-blotting analysis of beef and pork extract, we demonstrated that bovine and porcine 58kDa proteins (probably PGMs) were also allergic to meat allergic patients. In inhibition ELISA, the binding of IgE-antibody to beef, pork, and chicken was inhibited by the preincubation of serum with rabbit PGM (Fig. 3). Thus, we concluded that bovine and porcine PGMs were also allergen for meat allergic patients. This result also supported by the result of inhibition western-blotting analysis (*data not shown*).

3. The cross-reactivity between PGM and serum albumin

Next, we compared the IgE-binding abilities of PGM and previously-known allergens such as bovine serum albumin (BSA) and glyceraldehydes-3-phosphate dehydrogenase using eight individual serum samples (Fig. 4). The pattern of recognition by individual IgE antibodies was very similar between PGM and BSA; that is, patients who recognized PGM strongly, reacted also BSA strongly. Then, inhibition ELISA was performed to clarify whether there would exist the cross-reactivity between PGM and BSA. It was proven that the binding of IgE-antibody to PGM or BSA was inhibited by the preincubation of serum with BSA or PGM each other (Table 2). This fact clearly indicated that there surely exist the cross-reactivity between these two allergens.

Conclusions

We first identified phosphoglucomutase as a novel meat allergen and demonstrated its cross-reactivity with serum albumin, the major meat allergen. This finding would contribute greatly to the elucidation of meat allergy.

References

Tanabe S. et al.: Hypoallergenic and T cell rreactive analogue peptides of bovine serum albumin, the major beef allergen. Molecular Immunology, 41: 885–890, 2004.

Tanabe S. et al.: Some Human B and T cell Epitopes of bovine serum albumin, the major beef allergen. Biochem. Biophys. Res. Commun., 293: 1348–1353, 2002.

Quirce S. et al.: Chicken serum albumin (Gal d 5) is a partially heat-labile inhalant and food allergen implicated in the bird-egg syndrome. Allergy, 56: 754− 762, 2001.

Takahata Y.et al.: IgE-antibody specificities of the patients allergic to meat products. Anim. Sci. J. 71: 494–500, 2000.

Tables and Figures

Table 1. Prevalence of chicken allergy

Country (Subjects)	%	references
France (544 food allergic children)	0.6	Rance et al., 1999
USA (40 food allergic children with AD)	5	Sampson and Albergol 1984
South Africa (112 children with AD)	3.8	Steinman and Potter 1994
Switzerland (383 food allergic patients)	2.3	Etesamifar and Wuthrich 1998

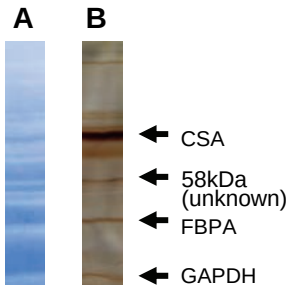

Fig. 1 SDS-PAGE analysis of chicken extract
(A) CBB staining, (B) imuuno-staining with patients IgE-antibodies

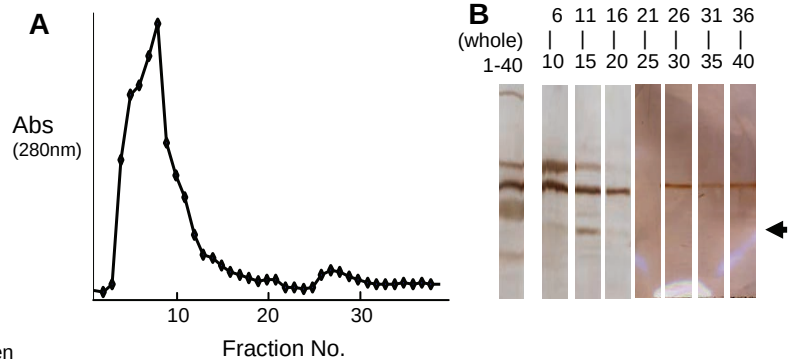

Fig. 2 DEAE−cellulose column chromatogram of extract and western-blotting analysis of its fractions using IgE-antibodies of chicken allergic patients
(A) Chicken extract was applied to DEAE cellulose column that had been equilibrated with 10 mM Tris-HCl buffer (pH 7.2). Elution with a linear gradient of 1 M NaCl in the same buffer was performed (Fr. No. 1-40). Proteins were monitored by the UV absorbance at 280 nm.
(B) The obtained fractions were applied to western-blotting. The 58 kDa band in Fr. 11-15 was excised, and then the N-terminal amino acid sequence was analyzed by a Protein Sequencer.

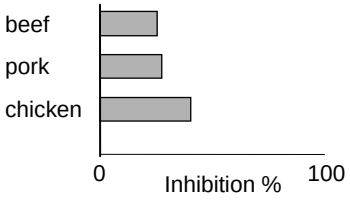

Fig. 3 Inhibition ELISA of beef, pork, and chicken extract using rabbit PGM as an inhibitor

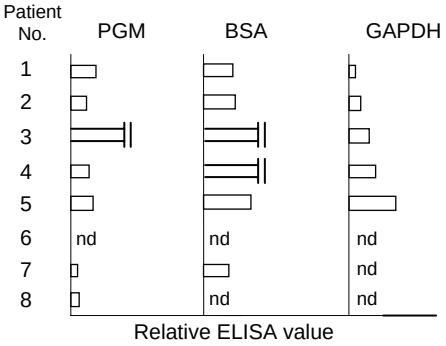

Fig. 4 IgE-binding abilities of PGM, BSA, and GAPDH
PGM, BSA, or GAPDH (10 μg/well) were coated on the well. After blocked with 1 % HSA in PBS, 10-fold diluted individual serum samples were applied to the well. The binding of IgE-antibody to coated antigen was determined.

Table 2 Crossreactivity between PGM and BSA

	antigen coated on the well	
	PGM	BSA
inhibitor		
PGM	96%	83%
BSA	92%	93%

PGM or BSA (0.1 mg/mL at a final concentration) was added to pooled serum samples diluted 10-fold in PBS-T. After 1 hr preincubation at 37°C, the PGM-serum or BSA-serum mixture was added to polystyrene microtiter plates coated with BSA or PGM (5 μg/well) and blocked with 1% HSA in PBS. Serum not treated with BSA or PGM was used as a control. The binding of IgE-antibody to PGM was determined.