

HUMAN IgE ANTIBODY TO THE CARBOHYDRATE-CONTAINING
THIRD DOMAIN OF CHICKEN OVOMUCOIDTsukasa Matsuda^{*1}, Ryo Nakamura¹, Izumi Nakashima²,
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SUMMARY: Two kinds of the third domain, either with or without a carbohydrate chain, were prepared from chicken ovomucoid. The immunoreactivity of the domain preparations to human IgE antibody directed against ovomucoid was examined by using the sera from patients of egg allergy. About 30% of the serum antibody to ovomucoid reacted with the carbohydrate-containing domain, whereas little or no antibody with reactivity to the carbohydrate-free domain was detected, suggesting that the carbohydrate chain attached to the third domain played an important role in antigenic determinants of the carbohydrate-containing third domain against the human IgE antibody.

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Chicken ovomucoid is a glycoprotein with MW of 28,000 daltons, containing 186 amino acid residues and 4 or 5 carbohydrate chains (1,2). The ovomucoid polypeptide chain consists of three domains cross-linked by three intradomain disulfide bonds (2). There are two kinds of the third domain (131-186 residues), either with or without a carbohydrate chain; about half the ovomucoid molecules have the carbohydrate-containing domain and the remainder the carbohydrate-free one (2,3). The third domains can be cut apart from ovomucoid, and isolated by enzymatic hydrolysis and gel filtration (2,3).

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; RAST, radioallergosorbent test; PBS, 0.15 M NaCl/0.01 M phosphate buffer, pH 7.2; Ig, immunoglobulin; SDS, sodium dodecylsulfate.

Ovomucoid, which occupies about 10% of egg white proteins, has been reported to be a typical allergenic protein which causes hypersensitive reactions against egg allergy patients (4), and specific IgE antibody to ovomucoid often is found in sera from allergic patients (5). Little is known about ovomucoid antigenic determinants against human IgE antibody.

In the present study, the immunoreactivity of the carbohydrate-containing and -free third domains with human IgE antibody to ovomucoid was investigated by ELISA to determine whether the specific antibody to ovomucoid recognized the carbohydrate moiety of ovomucoid third domain.

MATERIALS AND METHODS

Ovomucoid and its third domains: Ovomucoid was isolated from egg white of White Leghorn hens by the method of Waheed and Salahuddin (6), and the third domains were prepared from ovomucoid by hydrolysis with *Staphylococcus aureus* V8 protease and gel filtration of Bio-Gel P-10 (7).

Patient sera: The sera with high RAST-score against egg white were selected from sera of patients with known clinical histories of egg allergy, and the sera with high titer of IgE antibody to ovomucoid were further selected by ELISA using purified ovomucoid.

ELISA: The reactivity of antibodies to ovomucoid and domains was examined by ELISA (8). Flat-bottomed microtiter plates were coated with an appropriate concentration (1-10 $\mu\text{g/ml}$) of protein antigens, and the human IgE and mouse IgG antibodies which reacted with the plate-binding antigens were determined by using peroxidase-coupled anti-human IgE and anti-mouse IgG antibodies (Cappel), respectively, and o-phenylenediamine as the enzyme substrate (9).

Mouse antisera to the third domains: The antisera to the domains were prepared from 6 week-old female mice of C3H. The mice were immunized by subcutaneous injection of 100 μg of antigen in 0.1 ml of PBS with capsular polysaccharide of *Klebsiella pneumoniae* (10). The mice received a booster injection of 100 μg of the antigen in 0.1 ml of PBS subcutaneously 30 days after the first immunization. Blood was collected 8 days after the booster injection by puncturing of the retro-orbital venous plexus. The sera were separated from pooled blood of 4 mice, and stored at -80°C before use.

Polyacrylamide gel electrophoresis: SDS-gel electrophoresis (15% acrylamide) was performed according to the method of Laemmli (11). Gel sheets were stained with Coomassie Brilliant Blue R-250.

Analytical method: Amino acid composition was determined with amino acid analyser (JLC-6AH, Jeol, Tokyo) after hydrolysis at 110°C for 24 h in 6 N HCl. Hexosamine was determined also with the amino acid analyser after hydrolysis with 2 N HCl for 18 h at 100°C (12), and neutral hexose and protein were determined by the phenol-sulfuric acid method (13) and the method of Lowry et al. (14), respectively.

RESULTS

The purity of the domain preparations was tested by SDS-gel electrophoresis and the electrophoretogram is shown in Fig. 1. Molecular weight

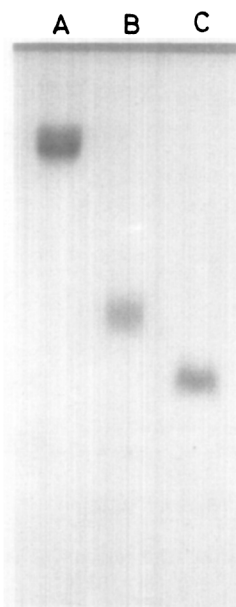


Figure 1: SDS polyacrylamide gel electrophoresis of ovomucoid (A), the third domains with (B) and without (C) carbohydrate.

of the carbohydrate-free domain is calculated to be about 5,400 daltons from its amino acid composition (2), and the molecular weight of the carbohydrate-containing domain was estimated to be about 10,000 daltons from its electrophoretic mobility by using intact ovomucoid (28,000 daltons), lysozyme (14,300 daltons), and the carbohydrate-free domain (5,400 daltons) as standard.

Amino acid composition of the carbohydrate-containing domain was identical with that of the carbohydrate-free domain and also with the theoretical value calculated from the amino acid sequence of residues 131-186 (2) within experimental error. The N-acetylglucosamine and hexose contents of the third domain with carbohydrate were about 7 and 4 mole per mole of polypeptide, respectively.

A typical ELISA pattern of the reaction between human IgE antibody and ovomucoid or its domains is shown in Fig. 2. The patient serum showed definitely high titer of IgE against ovomucoid compared with the normal serum. About 30% of the antibody to ovomucoid reacted with the carbohydrate

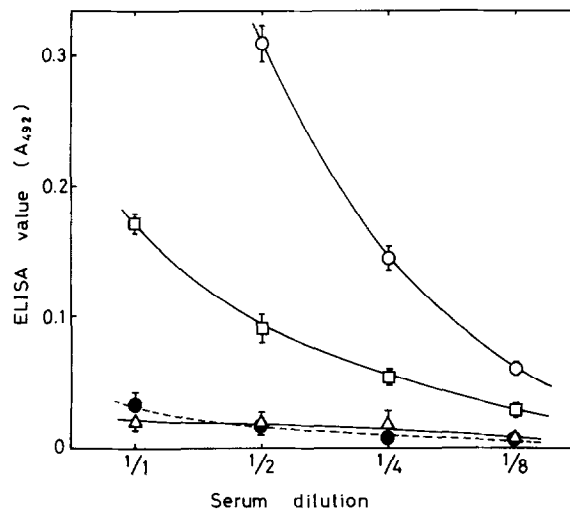


Figure 2: ELISA for measuring the human IgE antibody with reactivity to ovomucoid (—○—), the third domains with (—□—) and without (—△—) carbohydrate in the serum from an allergic patient. The antibody to ovomucoid in the control serum (---●---) was also examined for comparison. The protein concentrations of coating antigens were 1 μ g/ml and 10 μ g/ml for ovomucoid and the domains, respectively, and the diluted sera (1/300) were diluted further serially. The incubation time for enzyme reaction was 40 min. Each point represents the means of 2 determinations.

-containing third domain, whereas the antibody with reactivity to the carbohydrate-free third domain was not detected in this experimental condition

The reactivity of IgE antibody in the sera from 3 patients was examined for the domains with and without carbohydrate. As shown in Fig. 3-A, all of the three sera contained the IgE antibody with reactivity to the carbohydrate-containing domain, whereas little or no antibody was detected against the carbohydrate-free domain.

Figure 3-B shows the reactivity of the carbohydrate-containing and -free domains with the mouse IgG antibodies directed against each domain. Most of the antibodies to the carbohydrate-free domain reacted with the carbohydrate-containing domain, whereas a part of the antibodies to the carbohydrate-containing domain did not react with the carbohydrate-free domain. Both of the antibodies to the domains reacted well with intact ovomucoid.

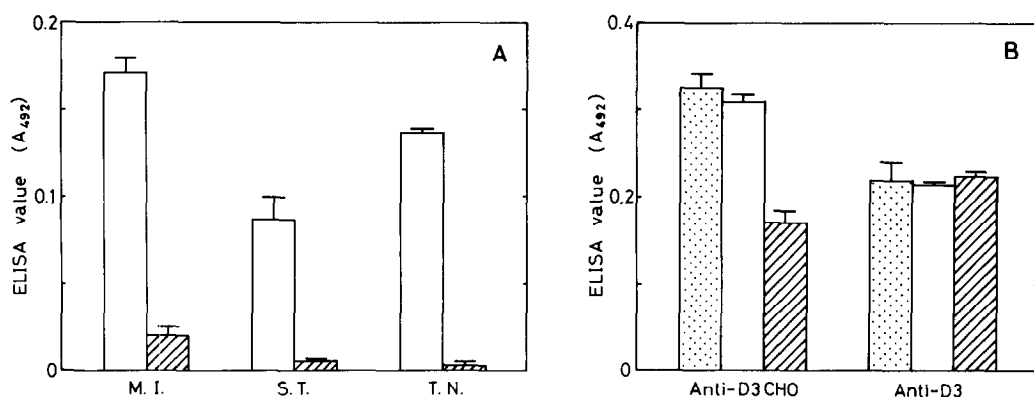


Figure 3: A — Reactivity of IgE antibody in the sera from 3 patients (M.I., S.T. and T.N.) to the third domains with (□) and without (▨) carbohydrate. B — Reactivity of mouse IgG antibodies directed against the third domains with (Anti-D3CHO) and without (Anti-D3) carbohydrate to intact ovomucoid (▤) and the third domains with (□) and without (▨) carbohydrate. The serum dilutions for human and mouse sera were 1/300 and 1/8000, respectively. The protein concentration of coating antigen was 10 $\mu\text{g/ml}$, and the incubation time for enzyme reaction was 40 min. Each point represents the means of 2 determinations.

DISCUSSION

The carbohydrate-containing third domain of ovomucoid has one asparagine-linked carbohydrate chain, with Asn¹⁷⁵ as its binding site (2). The major components of ovomucoid carbohydrate chains were reported to be a series of novel penta antennary complex-type (15), but the carbohydrate chain of the third domain has not been clarified yet.

The authors have already reported that polypeptide secondary structure of the carbohydrate-containing third domain was almost identical to that of the carbohydrate-free domain, and that the domain conformation was maintained after cutting apart from the parent molecule (7). In addition to these findings, the good reactivity between the antibody to the carbohydrate-free domain and the carbohydrate-containing domain (Fig. 3-B) indicated that there were little or no differences in the antigenic property of polypeptide moiety between the third domains with and without carbohydrate chain. Some of the mouse antibodies to the carbohydrate-containing domain did not react with the carbohydrate-free domain (Fig. 3-B). This suggested that the carbohydrate moiety and/or its attachment site could be recognized as antigenic determinants of the carbohydrate-containing domain. Thus,

the difference in immunoreactivity to human IgE antibody between the domains with and without carbohydrate would be ascribed to the binding carbohydrate chain. The carbohydrate chain might play an important role as the antigenic determinants of the ovomucoid against human IgE antibody.

It is interesting to note that little or no antibody to the carbohydrate-free third domain was contained in the human antibody to ovomucoid (Fig. 3-A). It is uncertain why no antibody was produced against the carbohydrate-free domain in the allergic patient. The carbohydrate-free domain was immunogenic by itself (Fig. 3-B), and a considerable part of the mouse antibodies to the carbohydrate-containing domain reacted with the carbohydrate-free domain (Fig. 3-B). Also, the specific antibody was produced against the carbohydrate-free domain in rabbits or mice immunized with intact ovomucoid (unpublished data). Thus, this characteristic property of the antibody reactivity to the ovomucoid third domains might be specific to the antibody in the sera from allergic patients.

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