

Kinetic Analysis of Pepsin Digestion of Chicken Egg White Ovomucoid and Allergenic Potential of Pepsin Fragments

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Key Words

Ovomucoid · Allergen · Digestion · Simulated gastric fluid · Fragment, pepsin-digested · Human serum IgE

Abstract

Background: The allergenic potential of chicken egg white ovomucoid (OVM) is thought to depend on its stability to heat treatment and digestion. Pepsin-digested fragments have been speculated to continue to exert an allergenic potential. OVM was digested in simulated gastric fluid (SGF) to examine the reactivity of the resulting fragments to IgE in sera from allergic patients. **Methods:** OVM was digested in SGF and subjected to SDS-PAGE. The detected fragments were then subjected to N-terminal sequencing and liquid chromatography/mass spectrometry/mass spectrometry analysis to confirm the cleavage sites and partial amino acid sequences. The reactivity of the fragments to IgE antibodies in serum samples from patients allergic to egg white was then determined using Western blotting (n = 24). **Results:** The rate of OVM digestion depended on the pepsin/OVM ratio in the SGF. OVM was first cleaved near the end of the first domain, and the resulting fragments were then further digested into smaller fragments. In the Western blot analysis, 93% of the OVM-reactive sera also bound to the 23.5- to 28.5-kDa fragments, and 21% reacted with

the smaller 7- and 4.5-kDa fragments. **Conclusion:** When the digestion of OVM in SGF was kinetically analyzed, 21% of the examined patients retained their IgE-binding capacity to the small 4.5-kDa fragment. Patients with a positive reaction to this small peptide fragment were thought to be unlikely to outgrow their egg white allergy. The combination of SGF-digestibility studies and human IgE-binding experiments seems to be useful for the elucidation and diagnosis of the allergenic potential of OVM.

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Introduction

Chicken egg white is one of the strongest and most frequent causes of food allergies among young children [1–5]. Egg white contains several allergens, including ovalbumin, ovotransferrin, lysozyme and ovomucoid (Gal d 1, OVM). OVM accounts for about 11% of all egg white proteins [6] and has a molecular weight of 28 kDa, containing a carbohydrate content of 20–25% [7]. OVM is known to be stable to digestion and heat, and cooked eggs can cause allergic reactions in OVM-specific allergic patients [8–11]. One possible reason for this is that OVM contains linear epitopes that are only slightly affected by conformational changes induced by heat denaturation.

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1018–2438/05/1361–0023\$22.00/0

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OVM consists of 186 amino acids divided into three domains of about 60 amino acids each; the third domain has been reported to be the most important domain with regard to allergenicity [12]. In a previous report, N-glycans in the third domain were suggested to be essential for allergenicity [13]; however, a recent report found that the deletion of the N-glycans did not affect the allergic reactivity.

We previously reported the digestibility of 10 kinds of food proteins in simulated gastric fluid (SGF) [8, 14]. OVM was digested relatively rapidly, but several fragments were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie blue (CBB) staining. The reactivity of these fragments with IgE antibodies from the sera of patients with egg white allergy is very important to understanding the mechanism of OVM allergy.

A few previous reports have described the reactivity of IgE in sera from patients with egg white allergies with OVM-derived fragments. Kovacs-Nolan et al. [15] separated pepsin-digested fragments of OVM using high-performance liquid chromatography (HPLC) and examined the IgE-binding activities of each fragment using an enzyme-linked immunosorbent assay (ELISA). Besler et al. [16] investigated the reactivity of pepsin-digested fragments with patient IgE using Western blotting and showed that the fragments retain their binding capacity to human IgE in some serum samples from OVM-allergic patients. However, little attention has been paid to the digestive conditions, and the number of serum samples has been somewhat small in these studies. Urisu et al. [17] reported that the sera of subjects that tested positive or negative during an oral egg white challenge exhibited a significant difference in their reactivity with pepsin fragments.

In the present report, kinetic data for different generations of SGF-stable OVM fragments were obtained, and the reactivity of the fragments with serum IgE from patients with egg white allergies was investigated using Western blotting.

Materials and Methods

Pepsin (catalog number P6887) and chicken egg white OVM (T2011, Trypsin Inhibitor, Type III-O) were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). The concentration of the OVM test solution was 5 mg/ml of water. The gels and reagents used for the SDS-PAGE analysis were purchased from Invitrogen (Carlsbad, Calif., USA).

Serum Specimens

Sera from 24 patients with egg white allergies and a healthy volunteer were used after obtaining informed consent from the patients and ethical approval by the Institutional Review Board of the National Institute of Health Sciences. Twenty-two of the patients had been diagnosed as having an egg white allergy at hospitals in Japan, based on their clinical histories and positive IgE responses to egg white proteins by radioallergosorbent test (RAST), while the remaining 2 allergen-specific sera were purchased from Plasma Lab International (Everett, Wash., USA); the commercial sera originated from adult Caucasians who had been diagnosed as having several food allergies, including egg white, based on their clinical history and skin tests. The commercial sera also showed positive IgE responses to egg white proteins when examined using RAST.

Preparation of SGF

Pepsin (3.8 mg; approximately 13,148 units of activity) was dissolved in 5 ml of gastric control solution (G-con; 2 mg/ml NaCl, pH adjusted to 2.0 with distilled HCl), and the activity of each newly prepared SGF solution was defined as the production of a ΔA_{280} of 0.001/min at pH 2.0 and 37°C, measured as the production of trichloroacetic acid-soluble products using hemoglobin as a substrate. The original SGF was prepared at a pepsin/OVM concentration of 10 unit/ μ g, and this solution was diluted with G-con for the experiments performed at pepsin/OVM concentrations of 1 and 0.1 unit/ μ g. The SGF solutions were used within the same day.

Digestion in SGF

SGF (1,520 μ l) was incubated at 37°C for 2 min before the addition of 80 μ l of OVM solution (5 mg/ml). The digestion was started by the addition of OVM. At each scheduled time point (0.5, 2, 5, 10, 20, 30, and 60 min), 200 μ l of the reaction mixture was transferred to a sampling tube containing 70 μ l of 5 $\times$ Laemmli buffer (40% glycerol, 5% 2-mercaptoethanol, 10% SDS, 0.33 M Tris, 0.05% bromophenol blue, pH 6.8) and 70 μ l of 200 mM Na₂CO₃. For the zero-point samples, the OVM solution (10 μ l) was added to neutralized SGF (190 μ l of SGF, 70 μ l of 5 $\times$ Laemmli buffer, and 70 μ l of 200 mM Na₂CO₃). All neutralized samples were then boiled at 100°C for 3 min and subjected to SDS-PAGE.

SDS-PAGE Analysis and Staining Procedure

Samples (15 μ l/lane) were loaded onto a 10–20% polyacrylamide Tris/Tricine gel (Invitrogen, Carlsbad, Calif., USA) and separated electrophoretically. The gels were fixed for 5 min in 5% trichloroacetic acid, washed for 2 h with SDS Wash (45.5% methanol, 9% acetic acid), stained for 10 min with CBB solution (0.1% Coomassie Brilliant blue R, 15% methanol, 10% acetic acid), and destained with 25% methanol and 7.5% acetic acid. The stained gel images were then analyzed using Image Gauge V3.1 (Fuji Film, Tokyo, Japan), and the density of each band was quantified. Periodic acid-Schiff (PAS) staining [18] was used to detect the glycosylated fragments.

N-Terminal Sequence Analysis

OVM (1.5 mg) was digested in SGF containing 1 unit/ml pepsin, concentrated by centrifugation using Centriprep YM-3 (Millipore Corporation, Bedford, Mass., USA) and subjected to SDS-PAGE followed by electrical transblotting to a 0.2- μ m polyvinylidene difluoride membrane (Bio-Rad, Richmond, Calif., USA) and CBB staining. The detected fragment bands were then cut out and sequenced using a Procise 494HT Protein Sequencing System (Applied Biosys-

tems, Foster City, Calif., USA) or an HP G1005A Protein Sequencing System (Hewlett-Packard, Palo Alto, Calif., USA); each fragment was analyzed for 5 cycles.

Carboxymethylation and Peptide Mapping Using Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS)

The digested OVM sample was separated electrophoretically as described above, stained with CBB, and the stained bands were cut out. The gel pieces were homogenized in 20 mM Tris-HCl (pH 8.0) containing 0.1% SDS and the proteins were extracted. The extracts were concentrated and purified by acetone precipitation. The acetone precipitates were incubated with 2-mercaptoethanol (92.5 mM) in 72 µl of 0.5 M Tris-HCl buffer (pH 8.6) containing 8 M guanidine hydrochloride and 5 mM EDTA at room temperature for 2 h. To this solution, 1.5 mg of moniodoacetic acid was added, and the mixture was incubated at room temperature for 2 h in the dark. The reaction mixture was desalted using a MicroSpin G-25 column (Amersham Bioscience, Uppsala, Sweden) and lyophilized. Reduced and carboxymethylated proteins were digested with trypsin (50 ng/µl in 50 mM NH₄HCO₃).

Tandem electrospray mass spectra were recorded using a hybrid quadrupole/time-of-flight spectrometer (Qstar Pulsar i; Applied Biosystems, Foster City, Calif., USA) interfaced to a CapLC (Magic 2002; Michrom BioResources, Auburn, Calif., USA). Samples were dissolved in water and injected into a C18 column (0.2 × 50 mm, 3 µm, Magic C18, Michrom BioResources). Peptides were eluted with a 5–36% acetonitrile gradient in 0.1% aqueous formic acid over 60 min at a flow rate of 1 µl/min after elution with 5% acetonitrile for 10 min. The capillary voltage was set to 2,600 V, and data-dependent MS/MS acquisitions were performed using precursors with charge states of 2 and 3 over a mass range of 400–2,000.

Western Blotting of Digested Fragments with Human Serum IgE

The digested OVM samples were applied to a 10–20% polyacrylamide Tris/Tricine 2D gel, followed by electrical transfer to a nitrocellulose membrane. The membrane was then blocked with 0.5% casein-PBS (pH 7.0) and cut into 4-mm strips. The strips were incubated with diluted human serum (1/4 to 1/5) in 0.2% casein-PBS (pH 7.0) at room temperature for 1 h and then at 4°C for 18 h. After washing with 0.05% Tween 20-PBS, the strips were incubated with rabbit anti-human IgE (Fc) antibodies (Nordic Immunological Laboratories, Tilburg, The Netherlands) at room temperature for 1 h, and then with horseradish peroxidase-conjugated donkey anti-rabbit Ig antibodies (Amersham Biosciences, Little Chalfont, UK) at room temperature for 1 h. Finally, the strips were reacted with Konica ImmunoStain HRP-1000 (Konica, Tokyo, Japan), according to the manufacturer's protocol.

Results

Kinetics of OVM Digestion by Pepsin

OVM was digested in SGF containing various concentrations of pepsin, and the fragments were separated by SDS-PAGE and stained with CBB (fig. 1). The molecular weight of OVM, based on its amino acid sequence, is about 20 kDa, but a broad band representing intact OVM

appeared at about 34–49 kDa in the SDS-PAGE gel because of the presence of five N-linked sugar chains. The pepsin band was detected at 39 kDa, overlapping with the intact OVM band, and lysozyme (14 kDa) contamination was detected in the OVM sample that was used. Intact OVM rapidly disappeared within 0.5 min in SGF (pepsin/OVM = 10 unit/µg), and a fragment band was detected at 7 kDa. When the pepsin content in SGF was reduced to 1 and 0.1 unit/µg, the digestion rate markedly decreased. Intact OVM was still detected after 30 min when the pepsin/OVM ratio was 0.1 unit/µg. The fragment bands were clearer (fig. 2) when a concentrated SGF-digested OVM solution (pepsin/OVM = 1 unit/µg, digestion times 5 and 30 min) was used, followed by SDS-PAGE. As shown in figure 2, a strong 23.5- to 28.5-kDa band (FR 1) was detected at 5 min, while 10- (FR 2), 7- (FR 3) and 4.5- to 6-kDa (FR 4) bands were detected after 30 min. FR 1 and FR 2 were both positively stained by PAS, suggesting that the FR 1 and FR 2 fragments have high carbohydrate contents. The time courses for the amounts of intact OVM and the four fractions are plotted in figure 3, where the pepsin/OVM ratio is 1 unit/µg. FR 1 rapidly increased but slowly disappeared after 2 min. FR 2 and FR 3 also rapidly reached maximum values at 5 min and then slowly disappeared. On the other hand, FR 4 gradually increased throughout the entire period of the experiment.

Preheating (at 100°C for 5 or 30 min) of the OVM solution (5 mg/ml in water) did not influence the digestion pattern (fig. 1).

Table 1. N-Terminal sequences of pepsin fragments

Digestion period	Fraction	Fragment	Residues	Sequence	Ratio % ^a
5 min	FR 1	1-1	50–54	FGTNI	73.1
		1-2	51–55	GTNIS	11.6
		1-3	1–5	AEVDC	6.9
5 min	FR 2	2-1	1–5	AEVDC	68.8
		2-2	134–138	VSVDC	28.2
5 min	FR 3	3-1	1–5	AEVDC	48.4
		3-2	134–138	VSVDC	24.3
		3-3	104–108	NECLL	9.6
30 min	FR 4	3-4	85–89	VLCNR	6.5
		4-1	134–138	VSVDC	30.6
		4-2	104–108	NECLL	24.0
		4-3	19–23	VLVCN	20.6

^a Molar ratios of the fragments to the total amount in each fraction.

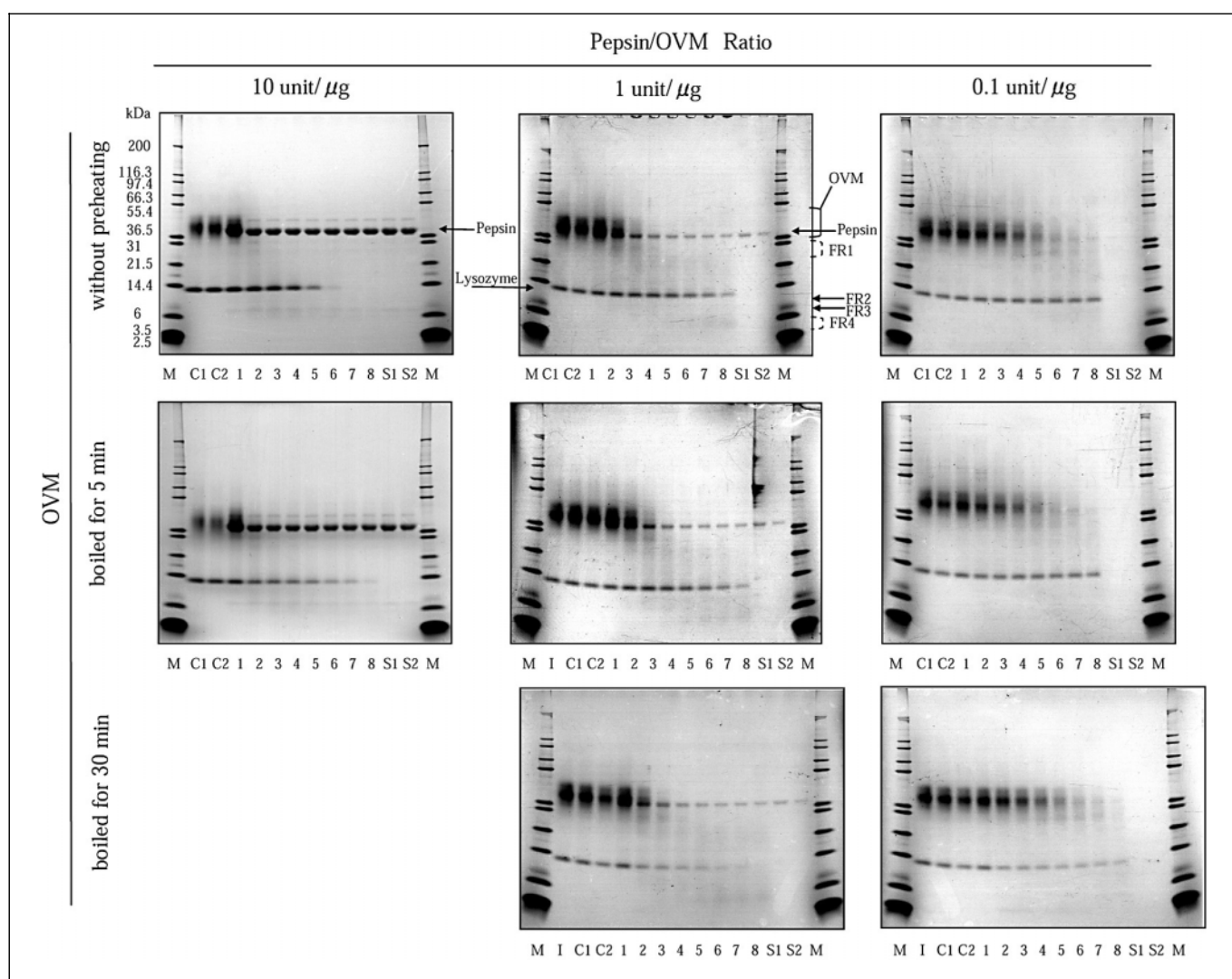


Fig. 1. Kinetic patterns of OVM digestion in SGF-containing pepsin. Digested samples were analyzed by SDS-PAGE followed by CBB staining. The digestion patterns of OVM without preheating (upper panels), preheated at 100°C for 5 min (middle panels), and preheated at 100°C for 30 min (lower panels) are shown. The ratio of pepsin to OVM was 10 unit/1 μ g (left), 1 unit/1 μ g (middle), and 0.1 unit/1 μ g (right). Lane M = Molecular weight markers; lanes C1 and

C2 = OVM without pepsin at 0 (C1) and 60 (C2) min; lanes 1–8 = SGF-digested OVM at 0, 0.5, 2, 5, 10, 20, 30 and 60 min, respectively; lanes S1 and S2 = SGF alone at 0 (S1) and 60 (S2) min; lanes I = OVM without preheating; FR 1 = fraction 1 containing a fragment at 23.5–28.5 kDa; FR 2 = fraction 2 containing a 10-kDa fragment; FR 3 = fraction 3 containing a 7-kDa fragment; FR 4 = fraction 4 containing 4.5- to 6-kDa fragments.

Sequence Analysis of OVM Fragments

The sequences of the five N-terminal residues in each fragment were analyzed, and the data are summarized in table 1. Figure 4 schematically depicts the identified fragments; the arrows in the upper panel indicate the sites of pepsin cleavage.

The internal sequences of the FR 1, FR 3, and FR 4 fragments were also identified by LC/MS/MS and are shown in table 2 and in the upper panel of figure 4.

Reactivity of the Fragments with Serum IgE from Patients with Egg White Allergy

Western blot analysis using patient sera as the source of the primary antibodies was performed to identify sera that reacted with intact OVM and the SGF fragments. Representative blotting data are shown in figure 5, and all the results are listed in table 3. Ninety-two percent of the serum samples from allergic patients reacted with OVM, and 93% of the OVM-positive sera reacted with FR 1

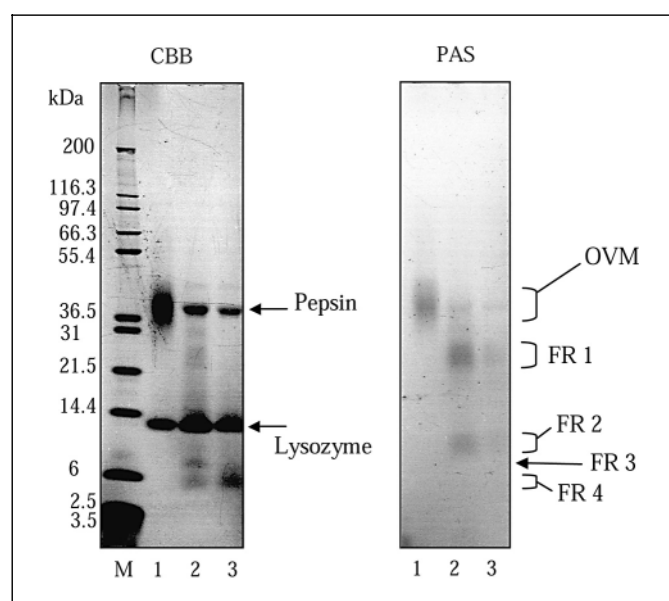


Fig. 2. CBB and PAS staining of OVM fragments following digestion in SGF (pepsin/OVM = 1 unit/ μ g) for 5 and 30 min. Lane M = Molecular weight markers; lane 1 = original OVM (2.5 μ g/lane); lanes 2 and 3 = OVM digested for 5 and 30 min, respectively, and concentrated (12 μ g, equivalent to the original OVM/lane). Samples were applied to two SDS-PAGE gels and electrophoresed. One plate (left panel) was stained with CBB reagent, and the other (right panel) was stained with PAS reagent.

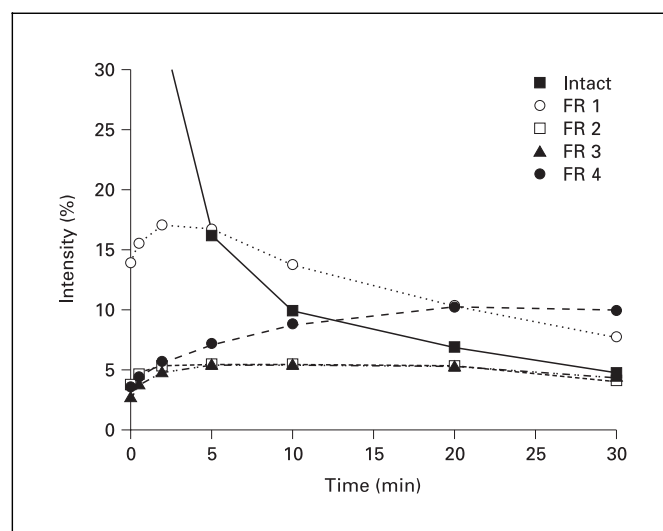


Fig. 3. Quantification of the SGF-digestion pattern of intact OVM and the digestion fragments at a pepsin/OVM ratio of 1 unit/ μ g. The intensity of each band was calculated using the ratio of the band's density to the total density of the originally detected band at $t = 0$. Values are the mean of duplicate analyses. Similar results were observed in another set of experiments.

Table 2. Identified inside sequences in pepsin- and trypsin-digested OVM

Pepsin digestion	Fraction	Residues	Sequence
5 min	FR 1	83–89	VMVLCNR
		90–103	AFNPVCGTDGVTYD
		90–112	AFNPVCGTDGVTYDNECLCAHK
		90–122	AFNPVCGTDGVTYDNECLCAHKVEQGASVDKR
		113–122	VEQGASVDKR
5 min	FR 3	90–112	AFNPVCGTDGVTYDNECLCAHK
		90–122	AFNPVCGTDGVTYDNECLCAHKVEQGASVDKR
		104–111	NECLCAH
		104–112	NECLCAHK
		104–121	NECLCAHKVEQGASVDK
		104–122	NECLCAHKVEQGASVDKR
		113–122	VEQGASVDKR
		134–159	VSVDCSEYKPDCTAEDRPLCGSDNK
30 min	FR 4	90–112	AFNPVCGTDGVTYDNECLCAHK
		104–111	NECLCAH
		104–112	NECLCAHK
		104–122	NECLCAHKVEQGASVDKR
		112–122	KVEQGASVDKR
		113–121	VEQGASVDK
		113–122	VEQGASVDKR
		165–185	CNFCNAVVESNGTLTLSHFGK

after 5 min of digestion. Three of the serum samples also reacted with FR 2, FR 3, and FR 4 after 30 min of digestion.

The three samples that react with FR 2, FR 3, and FR 4 were obtained from patients who exhibited persistent allergies to egg white. One of these serum samples, No.4, was obtained from a 3-year-old girl who is presently 6 years old; her total IgE level has decreased slightly to 4,450 IU/ml, but the specific IgE level for egg white remains at more than 100 IU/ml, and the patient has not outgrown her hypersensitivity to eggs. Another patient, No. 13, was a 1-year-old boy; 7 years later, his total and egg white-specific IgE levels had been reduced to 947 and 6.85 IU/ml, respectively, but eating raw eggs still caused allergic symptoms. The third FR 4-positive patient, No. 19, was an 11-year-old boy whose total IgE level decreased to 3,940 IU/ml and whose egg white-specific IgE decreased to 13.5 IU/ml after a period of about 2 years; however, this patient has also not outgrown his allergies. These cases and our previously reported data [17] indi-

cate that the induction of egg white tolerance may be difficult in patients whose serum IgE exhibits binding activity to digested small fragments of OVM.

Discussion

In the SGF-digestion system, preheating the OVM (100°C for 5 or 30 min) did not affect the OVM digestion pattern (fig. 1), consistent with the results of previous reports [9] in which heat treatment did not markedly decrease the allergenicity of OVM. On the other hand, a decrease in the pepsin/OVM ratio dramatically reduced the digestion rate, suggesting that digestibility may vary depending on the amount of OVM intake and the conditions of the individual's digestion system. In its native state, OVM possesses serine protease inhibitor activity. Fu et al. [11] and our group [10] previously reported that intact OVM was stable for 60 min in simulated intestinal fluid. Kovacs-Nolan et al. [15] also reported that pepsin-

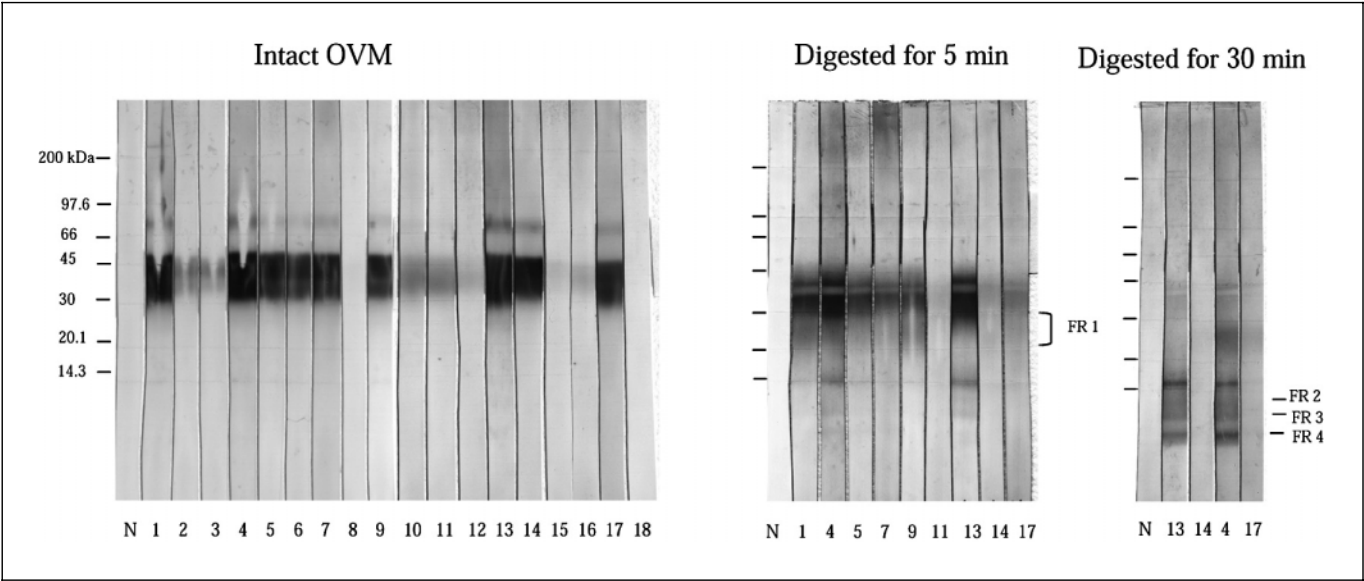


Fig. 4. Amino acid sequence and schematic representation of the SGF-digestion pattern of OVM. The amino acid sequence of OVM is shown in the upper panel. The arrows indicate the SGF-digested points according to the results of an N-terminal analysis of the OVM fragments (table 1), and the underlined regions indicate sequences identified by LC/MS/MS. Solid line = FR 1; dotted line = FR 3; dashed line = FR 4. Shaded areas represent reported human IgE epitopes [16]. The lower panel is a summary of the OVM digestion pattern according to N-terminal analysis.

Fig. 5. Western blot analysis of intact OVM and the fragments with serum IgE from egg white allergic patients and a normal volunteer. The fragments were prepared as described in the legend of figure 2. The number of each strip corresponds to the sample numbers in table 2.

Table 3. Reactivity of OVM and pepsin fragments with patient serum IgE

No.	IgE content, IU/ml		Reactivity with patient IgE ¹				
	total	egg white-specific	intact OVM	FR 1	FR 2	FR 3	FR 4
1	3,700	>100	+++	++	–	–	–
2	402	3.74	+	n.d.	n.d.	n.d.	n.d.
3	251	6.85	+	n.d.	n.d.	n.d.	n.d.
4	6,510	>100	+++	+++	+	+	++
5	2,060	>100	++	++	–	–	–
6	1,240	12.4	++	n.d.	n.d.	n.d.	n.d.
7	4,180	31.3	++	++	–	–	–
8	56	20.1	±	n.d.	n.d.	n.d.	n.d.
9	1,355	50.7	++	++	–	–	–
10	22,810	2.11	+	n.d.	n.d.	n.d.	n.d.
11	1,463	4.65	+	–	–	–	–
12	14,230	0.70–3.49	±	n.d.	n.d.	n.d.	n.d.
13	8,000	>100	+++	+++	+	+	++
14	22,490	1.05	+++	±	–	–	–
15	934	66.3	+	n.d.	n.d.	n.d.	n.d.
16	345	20.1	+	n.d.	n.d.	n.d.	n.d.
17	1,500	80	++	+	–	–	–
18	3,300	>10	–	n.d.	n.d.	n.d.	n.d.
19	20,500	26.8	+++	++	±	±	±
20	138	45.4	++	+	–	–	–
21	940	2.44	+	+	–	–	–
22	91	0.70–3.49	+	±	–	–	–
23	828	0.9	++	+	–	–	–
24	21	3.50–17.4	–	n.d.	n.d.	n.d.	n.d.
positive/tested			22/24 (92%) ²	13/14 (93%) ³	3/14 (21%) ³	3/14 (21%) ³	3/14 (21%) ³

n.d. = Not done.

¹ Intensity of the reactivity of each band was evaluated by the ratio to normal serum: – = <1; ± = 1–2; + = 2–5; ++ = 5–10; +++ = >10.² Percent of egg white-positive samples.³ Percent of intact OVM-positive samples.

digested OVM retains its trypsin inhibitor activity. Therefore, OVM and its pepsin-digested fragments were thought to be stable in the small intestine.

At a pepsin/OVM ratio of 1 unit/μg, FR 1 reached a maximum level after 2 min of digestion, while both FR 2 and FR 3 reached maximum levels after 5 min of digestion; thereafter, FR 1, FR 2, and FR 3 gradually decreased. However, FR 4 increased continuously throughout the 30-min period of digestion and the major fragments were seen after 30 min of digestion (fig. 3). FR 4 was mainly composed of three fragments whose N-terminals were 134V, 104N and 19V (table 1). A C-terminal sequence, 165N–185C, was also identified in FR 4 (table 2). These fragments contain known IgE epitopes [19] and therefore may cause allergic responses. Three of the

OVM-positive sera from patients with egg white allergy reacted positively with the FR 4 fragments (table 3).

The present results are consistent with the previous finding that pediatric subjects with a higher IgE-binding activity to pepsin-treated OVM were unlikely to outgrow their egg allergy [17]. For peanut allergies, differences in IgE-binding epitopes have been reported between the patients with clinically active peanut allergies and those who developed a tolerance, regardless of the presence of high or low peanut-specific IgE levels [20].

The N-terminal residue of the major fragment (4-1) of FR 4 was Val-134 (30%; table 1). This fragment retains most of domain III, which has been reported to have significantly higher human IgG- and IgE-binding activities than those of domains I and II [12]. A domain-III OVM

variant has also been reported to cause a reduction in immunogenicity and allergenicity [21].

Domains I, II, and III contain one, three, and one N-glycosylation sites, respectively [7]. The possible relation between the carbohydrate chain in domain III and allergenicity is interesting. One report suggested that this carbohydrate chain may play an important role in allergenic determinants against human IgE antibody [13], and another report suggested that the carbohydrate chains of OVM may protect against peptic hydrolysis [22]. However, the carbohydrate moieties have been shown to have only a minor effect on allergenicity [23]. As shown in figure 2, intact OVM, FR 1, and FR 2 fragments were detected using PAS staining, suggesting the presence of carbohydrate chains, but FR 4 was not stained with the PAS reagent, despite being clearly detected with CBB. Therefore, FR 4 might contain little or no carbohydrate chains. Since FR 4 seems to maintain its allergenic potential, as described above, the absence of the carbohydrate chains in FR 4 suggests that they are not necessary for OVM allergenicity. Since the minimum peptide size capable of eliciting significant clinical symptoms of allergic reactions is thought to be 3.1 kDa [24], FR 4 may be able to trigger mast cell activation and elicit clinical symptoms.

In this report, the SGF-digestion kinetic pattern of OVM was investigated in detail, and the partial sequences

of the fragments in the 4 fractions separated by SDS-PAGE were determined. Furthermore, the reactivity of the fragments with a number of serum samples from patients with egg white allergies was detected using Western blotting. The four fractions were separated according to their molecular weight and consisted of more than one fragment, as determined by N-terminal analysis. The identified sequences that started at Asn-104 and Val-134 in FR 3, as determined using LC/MS/MS (table 2), coincided with the 3-2 and 3-3 fragments in the N-terminal analysis (table 1), and the sequence that started at Asn-104 in FR 4 coincided with fragment 4-2. Moreover, the LC/MS/MS analysis indicated that FR 3 and FR 4 contained other parts of domain II and the C-terminal sequence N165–C185, which are thought to be minor components of these fractions. The combination of SGF digestion and patient IgE may provide useful information for the diagnosis and prediction of potential OVM allergenicity.

Acknowledgement

This study was supported by a grant from the Ministry of Health, Labor and Welfare, and the Cooperative System for Supporting Priority Research of Japan Science and Technology Agency.

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