

**Original:****Identification of IgE-Binding Peptides Derived from Chemical and Enzymatic Cleavage of Ovomuroid (Gal d 1)**

Matthias BESLER (a), Arnd PETERSEN (b), Hans STEINHART (a), Angelika PASCHKE (a)

(a) Institute of Biochemistry and Food Chemistry, University of Hamburg, Hamburg, Germany

(b) Division of Biochemical and Molecular Allergology, Forschungszentrum Borstel, Germany

**SUMMARY**

*Ovomucoid (OM), a major allergen of hen's egg white, consists of three trypsin inhibitory domains comprising 186 amino acids. In order to identify IgE-binding sites of its protein backbone OM was cleaved chemically and enzymatically. In SDS-PAGE/immunoblot experiments 10 peptides showing IgE-binding from human sera of egg allergic individuals were identified by amino acid sequencing. Up to five and six out of six sera reacted with peptides derived from trypsin and cyanogen bromide cleavage, respectively. Peptides derived by pepsin and thermolysin cleavage were detected by one and two sera, respectively. A total of five different N-terminal sequences of IgE-binding peptides from OM were determined. At least two distinct epitopes located on regions OM 90-121 and OM 134-186 were identified. The comparison of known antigenic regions from OM with three prediction algorithms revealed 37-46% probability for correct prediction.*

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**KEYWORDS**

**food allergy  
egg white  
ovomuroid  
chemical and enzymatic  
cleavage  
epitope mapping  
IgE-binding  
SDS-PAGE/immunoblot  
amino acid sequencing  
antigenicity prediction**

[Introduction] [Material&Methods] [Results] [Discussion] [Conclusion] [References] [Abbreviations]



Matthias Besler



Angelika Paschke

**Correspondence:**

Dr. Angelika Paschke

Institute of Biochemistry and Food Chemistry

University of Hamburg

Grindelallee 117, D-20146 Hamburg, Germany

Phone: +49-40-42838-4353

Fax: +49-40-42838-4342



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## INTRODUCTION

Hen's egg is one of the most frequent causes of adverse reactions to food in children (Bernhisel-Broadbent et al. 1994, Crespo et al. 1995) and adults (Nørgaard & Bindslev-Jensen 1992, Wüthrich 1993). Symptoms are often due to accidental ingestion of hidden food allergens (Yman et al. 1994, Moneret-Vautrin & Kanny 1995). Elucidation of allergic reactions has shown to be more frequently caused by egg white than egg yolk (Wüthrich 1981, Anet et al. 1985). Ovomuroid (OM), ovalbumin (OA), ovotransferrin (OT) and lysozyme (LY) were identified as major allergens in egg white protein (Langeland 1982, Langeland & Harbitz 1983, Hoffman 1983, Holen & Elsayed 1990). Most studies of the last few years have focused on allergenic structures of OA (Kahlert et al. 1992, Elsayed 1993, Honma et al. 1996), which is the main protein of egg white making up 54% of total protein content.

Recently OM has been reported to be the dominant allergen of egg white in egg-allergic children (Bernhisel-Broadbent et al. 1994, Urisu et al. 1997). OM is a glycoprotein with a molecular mass (Mr) of approximately 28.0 kDa and comprises about 11 % of total egg white proteins (Awade 1996, Lin & Feeney 1972). It consists of 186 amino acids forming three tandem domains, each providing trypsin inhibitory activity (Kato et al. 1987). The carbohydrate moieties of OM present up to 22-29% of the Mr. Recently it was demonstrated that the carbohydrate moieties have no impact on the IgE-binding of OM (Besler et al. 1997). Cooke & Sampson (1997) identified 5 IgE binding regions using synthetic peptides of OM. Several immunogenic studies of peptides derived by chemical or enzymatic fragmentation of OM have been done by different groups using specific mouse IgE or rabbit anti-OM sera (Kurisaki et al. 1981, Matsuda et al. 1985a, 1985c). However, detailed studies of human IgE reactivity to peptides derived from native OM are still missing.

The aim of the present study was to identify IgE binding sites on the protein backbone of OM by means of chemically and enzymatically derived peptides

## MATERIAL & METHODS

### *Human sera*

All human sera were RAST class  $\geq 3$  when tested for specific IgE against egg-white extract by CAP-RAST (Pharmacia, Uppsala, Sweden). The 6 sera and the pooled serum showed strong IgE-binding to purified native OM in SDS-PAGE / immunoblot experiments as previously described (Besler et al. 1997). The pooled serum was collected using equal aliquots of 10 sera. A serum from a non-atopic individual with no history of food hypersensitivity (RAST class 0) was used as negative control.

### *Purification of ovomuroid*

OM (trypsin inhibitor from hen's egg white) was purchased from Fluka (Buchs, Switzerland) and purified by size exclusion chromatography (SEC) following ion exchange chromatography (IEC) as described before (Besler et al. 1997). Briefly, IEC was performed using a Resource Q column (1 ml, Pharmacia) applying 10 mg of crude OM per run. Eluent was 20 mM Tris / HCl (pH 8.0) with a salt gradient from 0 to 0.5 M NaCl. Detection wavelength was 280 nm. Collected fractions were further purified by SEC in 50 mM  $\text{Na}_2\text{HPO}_4$  / 0.15 M NaCl / HCl (pH 7.0) on a Sephacryl S-100 HR column (C 26/100, Pharmacia). Eluted fractions were desalted and concentrated by ultrafiltration using Centriplus Concentrators (Amicon, Beverly, MA, USA) with 10 kDa cut off membrane. Resulting solutions (final volume of 300 - 500  $\mu\text{l}$  in double distilled water) were stored until use at  $-20^\circ\text{C}$ . The protein contents of purified OM solutions were determined by the method of Lowry et al. (1951). Purity was controlled by SDS-PAGE / immunoblot and capillary zone electrophoresis (Besler et al. 1997).

### *Cleavages of ovomuroid*

For each of the following cleavage methods 625  $\mu\text{g}$  (22 nmol) of purified OM were used. Cyanogen bromide cleavage of OM was performed according to Kahlert et al. (1992) in 70 % (v/v) formic acid (750  $\mu\text{l}$ ) for 15 h at room temperature. The applied OM / cyanogen bromide ratio was 1:40 (w/w) using cyanogen bromide dissolved in acetonitrile (0.5 g/ml). The enzymatic cleavages were carried out with enzyme / substrate ratios of 1:10 (w/w) in volumes of 500  $\mu\text{l}$  for 15 h at  $37^\circ\text{C}$ . OM cleavage with pepsin from porcine stomach mucosa (Merck, Darmstadt, Germany) was performed in 5 % (v/v) acetic acid. Prior to hydrolysis with tosyl-L-phenylalanine-chloromethylketon-treated trypsin (Typ XIII from bovine pancreas, Sigma) and thermolysin (from *Bacillus thermoproteolyticus*, Merck) the enzymes were activated in 100 mM ammonium bicarbonate buffer containing 24 mM calcium chloride for 2 h at room temperature. Both cleavages were performed in 100 mM ammonium

bicarbonate buffer (Wilkinson 1986). All cleavage reactions were stopped by freezing the reaction vials in liquid nitrogen. Then cleavage products were lyophilized and stored at -20°C. Prior to electrophoresis cleavage products were dissolved in 100 µl sample buffer.

### **SDS-PAGE**

SDS-PAGE was performed in slab gels (200 mm high, 120 mm wide, 1.0 mm thick). Stacking gel (pH 6.8): 5% T, 3% C, 0.125 M Tris, 0.1 % SDS and 30 % (w/v) glycerol. A linear acrylamide gradient was utilized for the resolving gel modified according to Fling & Gregerson (1986). Resolving gel (pH 8.8): 20-30% T, 3% C, 0.75 M Tris, 0.1% (w/v) SDS and 20-0% (w/v) glycerol. Electrode buffers (pH 8.3) contained 0.05 M Tris, 0.1 % (w/v) SDS and 0.192 M glycine. Samples dissolved in Tris-HCl / SDS sample buffer (pH 6.8) containing 2 % (w/v) b-mercaptoethanol and 0.01 % (w/v) Coomassie blue R-250 as migration marker were boiled for 5 min. Applying 125 µg sample/cm, electrophoresis was performed at 200 V for 0.5 h and 400 V constant voltage for 18 h at 15°C on a Multiphor II horizontal slab electrophoresis unit (Pharmacia LKB) with a MultiDrive XL power supply (Pharmacia LKB). Molecular mass marker proteins in the range of 2.5 to 17 kDa (protein standard mixture I, Merck, Darmstadt, Germany) and 14 to 94 kDa (LMW electrophoresis calibration kit, Pharmacia) were used. Gels were silver stained according to Morissey (1981) or peptides were electrotransferred to blotting membranes.

### **Immunostaining**

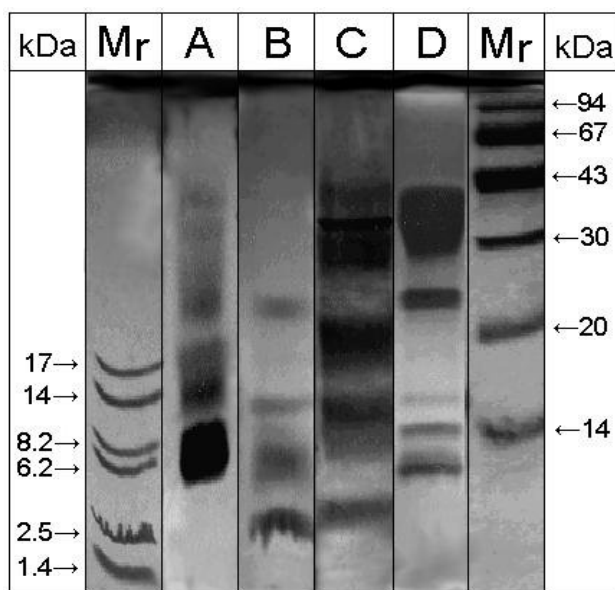
Immunostaining was carried out after electrotransfer of peptides from slab gels onto nitrocellulose (NC membrane 0.2 µm, Schleicher & Schuell, Dassel, Germany) at 0.8 mA/cm<sup>2</sup> for 75 min using a NovaBlot semidry blotting apparatus (Pharmacia). The discontinuous electrotransfer buffer system described by Vieths *et al.* (1992) was used. Afterwards the membrane was dried for 30 min and cut into strips. Immunostaining of IgE was performed as described previously by Möller *et al.* (1997). Briefly, dried membrane strips were blocked twice (15 min) in phosphate buffered saline (PBS) containing 0.3 % (v/v) Tween 20 and incubated overnight with 1 ml of each serum diluted 1:10 in PBS containing 0.3 % (w/v) bovine serum albumin (BSA) and 0.1 % (v/v) Tween 20 at room temperature. Then strips were incubated consecutively with rabbit anti-human-IgE (1:4000, DAKO, Copenhagen, Denmark), biotinylated goat anti-rabbit-IgG (1:6000, DAKO) for 1 h each and with streptavidin-horseradish-peroxidase (1:20000, Medac, Hamburg, Germany) for 20 min. 3,3',5,5'-tetramethylbenzidine and diethylsodiumsulfosuccinate were used as substrates for staining. Protein staining was performed using India Ink according to Hancock & Tsang (1983).

### **Amino acid sequence analysis**

Automated Edman degradation of peptides was performed using a 473 A pulse liquid sequencer (Applied Biosystems, CA, USA). 250 µg (9 nmol) cleavage products of OM were separated by SDS-PAGE. After electrophoresis, gels were equilibrated in CAPS-electrotransfer buffer (10 mM 3-[cyclohexylamino]-1-propanesulfonic acid, 10 % (v/v) methanol, pH 11) for 15 min. Prior to use PVDF-membranes (Immobilon-P-membrane, Serva, Heidelberg, Germany) were rinsed with methanol, water (5 min) and electrotransfer buffer (15 min) (Ward *et al.* 1990). Electrotransfer conditions were the same as described above. Proteins were stained by 0.1 % (w/v) Coomassie blue R-250 in 50 % (v/v) methanol and 10 % (v/v) acetic acid for 5 min and destained with 50 % (v/v) methanol in 10 % (v/v) acetic acid. Blotting membranes were air-dried and stored at room temperature under dry conditions. Stained peptides were cut out and arranged in the cartridge block of the sequencer. Since the amino acid sequence of OM is known, the analysis of 5 to 11 amino acids was sufficient for identification of the peptides.

### **Antigenicity prediction of sequential epitopes**

Computer analysis for antigenicity prediction was performed using ANTHEPROT V4.0 software by G. Deleage (Lyon, France, <http://www.ibcp.fr>). Three different algorithms using the primary amino acid sequence data were applied: a) the hydrophilicity method as described by Hopp & Woods (1981), b) combined antigenicity profile by Parker *et al.* (1986) and, c) antigenicity method by Welling *et al.* (1985). The averaged propensities were calculated onto a sliding window of 7 amino acids.



**Figure. 1: SDS-PAGE / silver staining of OM peptides derived from different cleavages.** Lane A: Cyanogen bromide cleavage, lane B: pepsin cleavage, lane C: thermolysin cleavage, lane D: trypsin cleavage, Mr: molecular mass marker proteins.

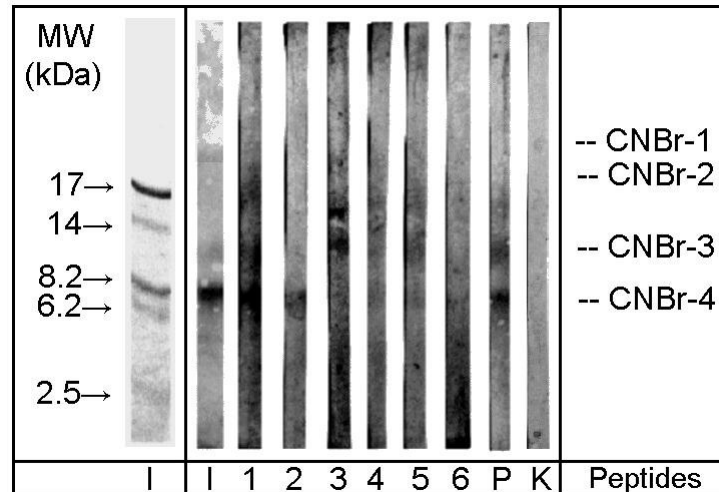
## RESULTS

### *Cyanogen bromide cleavage of Ovomuroid*

Four cleavage products were obtained by cyanogen bromide cleavage of OM as determined by SDS-PAGE silver staining (Fig.1). These peptide bands were broad shaped and migrated according to Mr of approximately 23, 18, 14 to 16 and 6 to 10 kDa, respectively (Tab.1). All four fragments were detected by immunostaining. Four and six out of six patients' sera from egg allergic individuals showed strong IgE-binding to fragments CNBr-3 and CNBr-4, respectively. Whereas only one and three sera showed weak binding to fragments CNBr-1 and CNBr-2, respectively (Tab.1, Fig.2). The N-termini of three peptides (CNBr-2 to CNBr-4) turned out to be identical as determined by amino acid sequencing (Tab.1). The identified amino acids were consistent with the internal sequence 85-90 (V-L-C-N-R-A) of OM. Cleavage occurred at Met-84.

### *Pepsin cleavage of Ovomuroid*

According to SDS-PAGE/silver staining OM was totally degraded by cleavage with pepsin (Fig.1). However, besides the detection of all four cleavage products by silver and immunostaining OM was detected in immunostaining by one serum (Tab.2, immunostains only visible on originals). The amino acid sequence of fragment PE-1 corresponded to the internal sequence 50-55 (F-G-T-N-I-S) (Tab.2). The occurring substitution of N by D may be due to a desamidation of N. The fragment PE-3 corresponded to the internal sequence 134-139 (V-S-V-D-C-S) (Tab.2). The amino acid sequencing of PE-2 resulted in two overlapping N-termini. The internal sequences 1-11 (AEVDCSRFPNA) and 134-143 (VSVDCSEYPK) could be identified by the different concentrations and comparison with the known primary structure of OM, respectively (Tab.2).



**Figure 2: SDS-PAGE / immunoblot of OM peptides derived from cyanogen bromide cleavage.** Immunostaining: 1-6 patients' sera, P pooled serum, K negative control serum. Protein staining: I indian ink, Mr: molecular mass marker proteins. CNBr-1 to CNBr-4: OM peptides derived by cyanogen bromide cleavage.

**Table 1: Cyanogen bromide cleavage of Ovomuroid.** Results of SDS-PAGE separated cleavage products including remaining OM in comparison with native OM.

Protein / Peptide	Mr (kDa)	Silver staining <sup>a</sup>	Immuno-staining <sup>b</sup>	Amino acid sequence <sup>c</sup>
native OM	30-45	+++	6	n.d.
remaining OM	30-45	+	1	n.d.
CNBr-1	~ 23	+	1	n.d.
CNBr-2	~ 18	+	3	V-L-?-N-R-A
CNBr-3	14-16	++	4	V-L-?-N-R-A
CNBr-4	6-10	+++	6	V-L-?-N-R-A

**Table 2: Pepsin cleavage of OM.** Results of SDS-PAGE separated cleavage products including remaining OM in comparison with native OM.

Protein / Peptide	Mr (kDa)	Silver staining <sup>a</sup>	Immuno-staining <sup>b</sup>	Amino acid sequence <sup>c</sup>
native OM	30-45	+++	6	n.d.
OM	30-45	-	1	n.d.
PE-1	23.5	+	2	F-G-T-D-I-S
PE-2	14	+++	2	1) AEVD?SRFPNA 2) VSVD?SEYPK
PE-3	6-8	++	2	V-S-V-D-?-S
PE-4	3.5	++	1	n.d.

<sup>a</sup> **Intensity:** none (-), weak (+), strong (++), very strong (+++) detection

<sup>b</sup> **Number of sera** with IgE binding in immunoblot (total 6 sera)

<sup>c</sup> **N-terminal sequence** (n.d. not determined)

**Table 3: Thermolysin cleavage of OM.** Results of SDS-PAGE separated cleavage products including remaining OM in comparison with native OM.

Protein / Peptide	Mr (kDa)	Silver staining <sup>a</sup>	Immuno-staining <sup>b</sup>	Amino acid sequence <sup>c</sup>
native OM	30-45	+++	6	n.d.
remaining OM	30-45	++	4	n.d.
TH-1	19-22	++	2	I-S-K-E-H
TH-2	~13.5	++	1	n.d.
TH-3	~3.5	++	0	n.d.

**Table 4: Trypsin cleavage of OM.** Results of SDS-PAGE separated cleavage products including remaining OM in comparison with native OM.

Protein / Peptide	Mr (kDa)	Silver staining <sup>a</sup>	Immuno-staining <sup>b</sup>	Amino acid sequence <sup>c</sup>
native OM	30-45	+++	6	n.d.
remaining OM	30-45	+++	5	n.d.
TR-1	24	+++	4	n.d.
TR-2	14.5	+	3	A-F-N-P-V
TR-3	10.5	++	5	A-F-N-P-V
TR-4	7-8	++	4	A-F-N-P-V
TR-5	2.5	-	2	A-F-N-P-V

<sup>a</sup> **Intensity:** none (-), weak (+), strong (++), very strong (+++) detection

<sup>b</sup> **Number of sera** with IgE binding in immunoblot (total 6 sera)

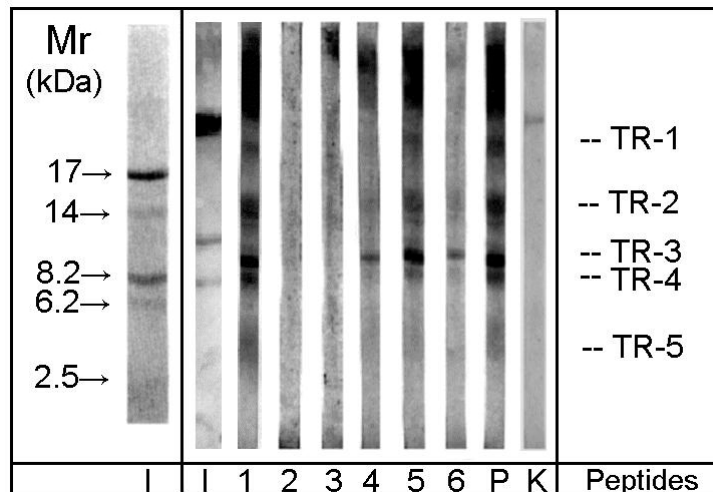
<sup>c</sup> **N-terminal sequence** (n.d. not determined)

### *Thermolysin cleavage of Ovomuroid*

As estimated by SDS-PAGE/silver staining OM was degraded only partially by thermolysin (Fig.1). Three fragments were detected at Mr of ~3.5, ~13.5 and 19-22 kDa (Tab.3). Fragments TH-1 and TH-2 showed weak IgE binding from two and one patients' sera in SDS-PAGE/immunoblot, respectively. TH-3 did not bind IgE from any serum (Tab.3, immunostains only visible on originals). The N-terminal sequence of TH-1 matched the internal sequence 54-58 (I-S-K-E-H) of OM. Thermolysin cleaved OM behind Asp-53.

### *Trypsin cleavage of Ovomuroid*

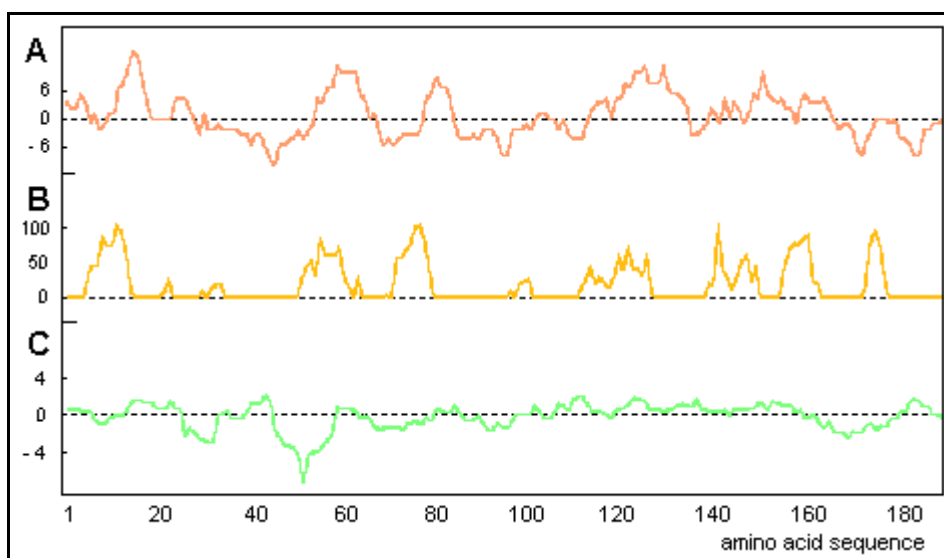
OM was cleaved incompletely by trypsin as shown by SDS-PAGE/silver staining (Fig.1). Nevertheless five cleavage products were obtained. Four of them were detected by silver staining, while the fifth could only be visualized by immuno staining (Fig.3). All fragments bound IgE from patients' sera. Five out of six sera reacted with TR-3 (Mr 10.5 kDa), four sera with TR-1 (24 kDa) and TR-4 (7-8 kDa), three sera with TR-2 (14.5 kDa) and two sera with TR-5 (2.5 kDa). All analyzed fragments (TR-2 to TR-5) showed the same N-terminal sequence (Tab.4). They corresponded to the internal sequence 90-94 (A-F-N-P-V) of OM. The cleavages occurred at Arg-89.



**Figure 3: SDS-PAGE / immunoblot of OM peptides derived from trypsin cleavage.** Immunostaining: 1-6 patients' sera, P pooled serum, K negative control serum. Protein staining: I Indian ink, Mr molecular mass marker proteins. TR-1 to TR-5: OM peptides derived from trypsin cleavage.

#### *Antigenicity prediction of sequential epitopes*

Computer analysis using the method of Hopp & Woods (1981) demonstrated at least five putative antigenic regions on the primary structure of OM, while the antigenicity profiles by Parker et al. (1986) and by Welling et al. (1985) predicted at least six antigenic regions (Fig. 4). The best agreement with each other was found for the algorithms of Hopp & Woods (1981) and Parker et al. (1986). Hydrophobicity and antigenicity peaks showed an overlap of 44%. The methods of Hopp & Woods (1981) and Welling et al. (1985) agreed to an extent of 36%. 25% agreement was shown by the methods of Parker et al. (1986) and Welling et al. (1985).



**Figure 4: Antigenicity prediction profiles of OM.** A: Hydrophilicity method by Hopp & Woods (1981). B: Antigenicity method by Parker et al. (1986). C: Antigenicity method by Welling et al. (1985).

## DISCUSSION

### *Cleavage Pattern of OM fragments in SDS-PAGE*

OM contains two Met-residues, therefore three fragments of OM could be expected cleaving with cyanogen bromide which is specific for Met. The three fragments are referring to the sequences 1-68 (Mr 7453 Da), 69-84 (Mr 1705 Da) and 85-186 (Mr 10954 Da), respectively (calculated Mr of the peptide chain without carbohydrate moieties). Beeley (1976a) obtained three fragments with Mr of 12, 11 and, 4.7 kDa, respectively. Whereas Kurisaki et al. (1981) identified two fragments after cyanogen bromide cleavage of OM referring to the sequences 1-68 and 69-186. In the present study four fragments were obtained by cyanogen bromide cleavage. The fragments CNBr-2, CNBr-3 and CNBr-4 showed the same N-terminus cleaved behind Met-84, but had different electrophoretical mobilities in SDS-PAGE experiments. Due to the absence of Trp-residues (which is reported to cause the only known side reaction of cyanogen bromide cleavage, Fontana & Gross 1986) in OM and the absence of Met-residues in the sequence 85-186 it was concluded, that all three fragments represent the sequence 85-186. Acidic hydrolysis was excluded by control without cyanogen bromide. The different mobilities of the fragments could be due to by conformational differences e.g. variable glycosylation sites or only partial reduction of the five disulfide bonds located on the sequence 85-186.

The pattern of OM cleavage with pepsin was in good agreement with the results described by Matsuda et al. (1985a, 1985c). They found four fractions separated by SEC with Mr of 25, 18, 13 and, 2 kDa, respectively. Differences in Mr of the fragments are due to the different methods used for Mr estimation (SEC and SDS-PAGE, respectively). Beeley (1976b) separated nine fractions after trypsin cleavage of OM by SEC, while Yet et al. (1988) isolated four fractions. None of the five trypsin cleavage fragments we characterized in this study matched the N-terminus of one of the fractions found by Beeley (1976b) and Yet et al. (1988), respectively. In contrast the fragments identified by Kato et al. (1987) after trypsin cleavage of OM contained the sequences 1-89 and 90-186, which were also present in the fragments TR-1 and TR-2 to TR-5, respectively.

### *IgE-binding Epitopes*

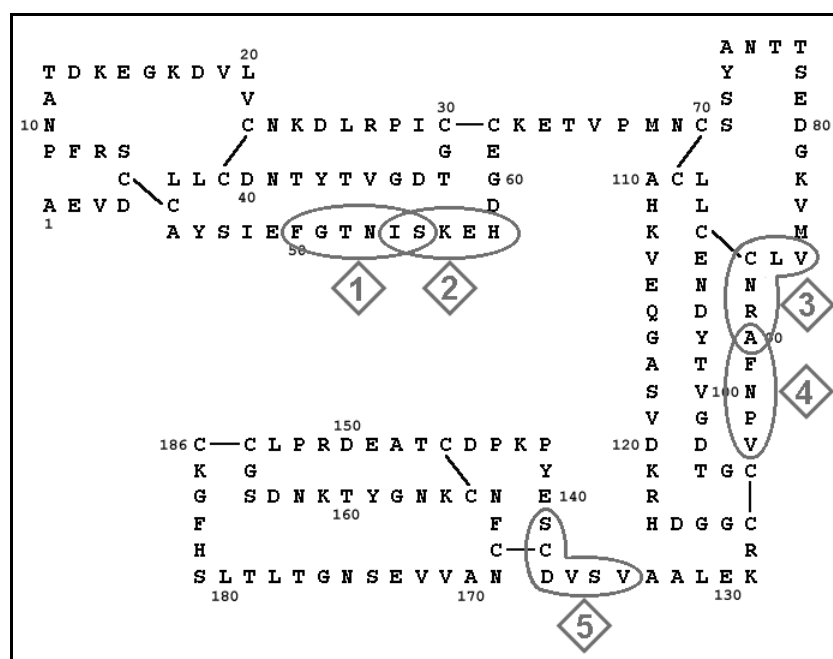
A total of 10 IgE-binding peptides derived from OM cleavage were identified by their N-terminal amino acid sequence. Three fragments obtained after cyanogen bromide cleavage and four fragments after trypsin treatment revealed identical N-termini, respectively. Thus in total five different N-terminal sequences of IgE-binding fragments of OM could be identified (Tab.5). Three N-termini were located on domains I (1-68) and II (65-130), while two N-termini were located on domain III (131-186) (Tab.5 and Fig.6). All five sequences were in agreement with antigenic fragments described by Beeley (1976 a), Kurisaki et al. (1981), Konishi et al. (1982) and Matsuda et al. (1985 a, c) using antibodies of animal origin. Binding of human IgE was described for the three isolated domains only (Matsuda et al.1985b, 1986, Cooke & Sampson 1997), but not for chemical or enzymic peptide mapping of OM.

As discussed above all three fragments of the cyanogen bromide cleavage represented the sequence 85-186 of OM. Thus smaller IgE-binding regions could not be identified by cyanogen bromide cleavage (Tab.5, No.3). For the location of IgE-binding epitopes on trypsin derived peptides the smallest peptide TR-5 (N-terminus 90-94) was considered to be most important (Tab.5, No.4). Assuming the C-terminus of TR-5 resulted by the most probable trypsin cleavages at Lys-113 and Lys-122 the Mr of the sequences 90-112 or 90-121 were calculated to be 2468 Da and 3399 Da, respectively (Fig.5, No.4). It could not be deduced which of both sequences was obtained, because SDS-PAGE is not sufficient for exact Mr determination. Therefore it was concluded that an important IgE-binding epitope is certainly located on the protein region 90-121.



**Table 5: Overview of IgE binding fragments of OM derived by different cleavages**

No. in Fig. 5	N-Termini	Fragments	Mr (kDa)	Cleavages
1	50-55	PE-1	23.5	Pepsin
2	54-58	TH-1	19-22	Thermolysin
3	85-90	CNBr-2, -3, -4	6-18	Cynogen bromide
4	90-94	TR-2, -3, -4, -5	2.5-14.5	Trypsin
5	134-139	PE-3	6-8	Pepsin

**Figure 5: Amino acid sequence of OM: N-termini of IgE binding peptides.** Identity of peptides 1 to 5 referring to Tab.5.

The fragments PE-1 (N-terminus 50-55) and TH-1 (N-terminus 54-58) with 23.5 and 19-22 kDa in SDS-PAGE, respectively, correspond to the regions 50-186 and 54-186. With regard to the glycosylation sites Asn-53, -69, -75 and, -175 (each accounting an average Mr of 1.8 kDa according to Yet et al. 1988) the sequence 50-186 had a calculated Mr of 22.0 kDa, while the sequence 54-186 had a Mr of 19.8 kDa. The Mr of 6-8 kDa of the fragment PE-3 corresponded to the sequence 134-186 with calculated Mr about 4.2 kDa and 6.0 kDa (taking the glycosylation site Asn-175 into account). Therefore we assumed no further cleavages between Val-134 and the C-terminus of OM. In summary at least two distinct IgE-binding epitopes located on the protein regions 90-121 and 134-186 were identified. In contrast to IgE binding studies utilizing synthetic or recombinant peptides these regions represent the native structure of OM. This is important regarding posttranslational modifications, particularly the high degree of glycosylation. Furthermore chemically and enzymatically derived peptides should represent the 3D-structure of the native protein better than shorter synthetic peptides.

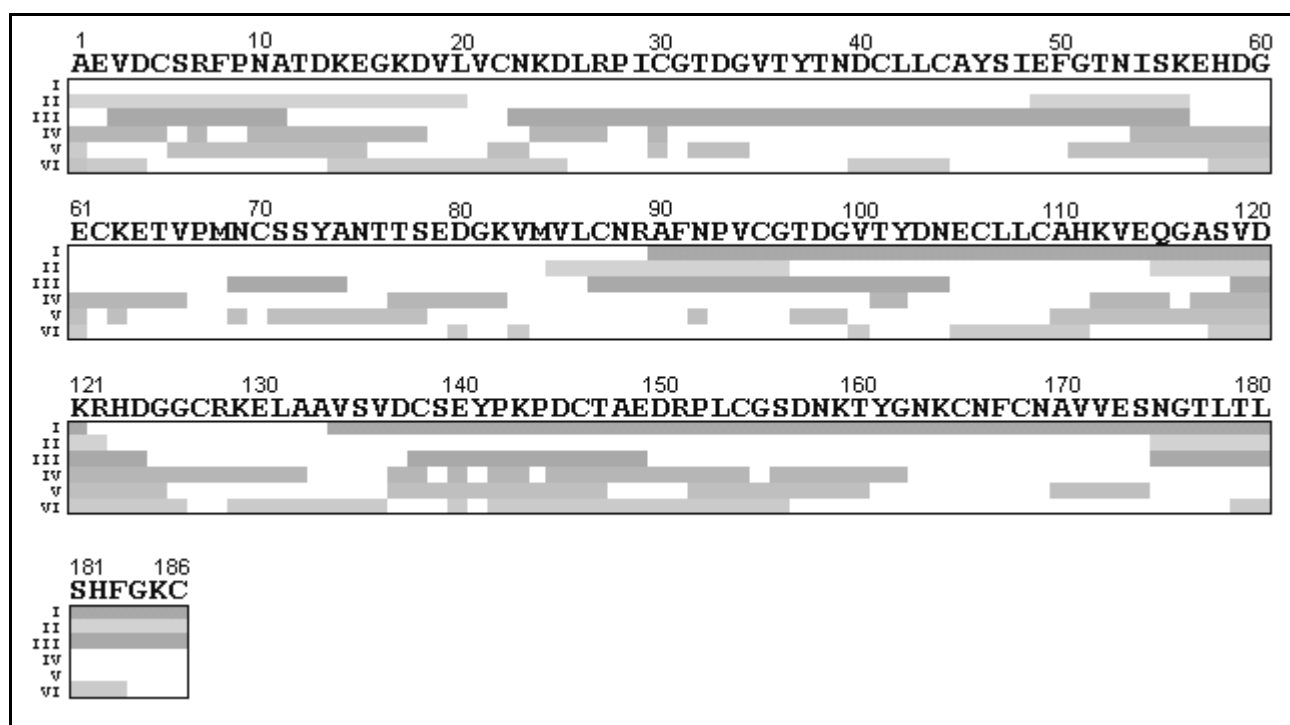
Cooke & Sampson (1997) used synthetic dodeca peptides with overlapping sequences of 10 amino acids in order to analyze the whole sequence of OM. They described five IgE and six IgG binding epitopes using a pooled serum from egg allergic patients in immunoblot experiments. Four of the N-terminal sequences determined in the present study were in agreement with IgE binding epitopes described by Cooke & Sampson (1997). The epitope 49-56 identified by Cooke & Sampson (1997) is represented in

our study by the fragment PE-1. The epitope 85-96 corresponds to the N-terminal sequence of fragments CNBr-2 to CNBr-4, while the fragment TR-5 overlaps with the epitope 115-122. Fragment PE-3 contains the epitope 175-186. The fifth IgE binding epitope 1-20 described by Cooke & Sampson (1997) could not be represented in our study.

### *Antigenicity prediction of sequential epitopes*

All three antigenicity prediction methods demonstrated peaks located inside the two smallest IgE binding regions presented in this study (Fig.6). The highest agreement was demonstrated by the combined antigenicity profile (Parker et al. 1986), which shared 50% of the region OM 90-121 and 47% of the region OM 134-186. The methods of Hopp & Woods (1981) and Welling et al. (1985) encompassed 34% and 38% of OM 90-121 as well as 42% and 45% of OM 134-186, respectively.

In comparison with the epitopes presented by Cooke & Sampson (1997) antigenicity peaks of the Parker et al. (1986) method were located inside four out of five IgE binding epitopes and inside all the six IgG binding epitopes. Whereas the peaks of the other two prediction algorithms were located inside three out of five IgE binding epitopes and inside five out of six IgG binding epitopes. Accounting the overlap of antigenicity peaks with IgE and IgG binding regions of OM correct prediction was achieved by 46% (Parker et al. 1986), 37% (Hopp & Woods 1981) and, 36% (Welling et al. 1985), respectively. False epitope prediction was about 28% (Parker et al. 1986, Welling et al. 1985) and, 38% (Hopp & Woods 1981), respectively. These results are in agreement with several other studies, who described probabilities for correct epitope prediction about 45% (Ferreira da Cruz et al. 1996) and 50-60% (Van Regenmortel & Pellequer 1994). Moreover these algorithms are based on the primary amino acid sequence taking no posttranslational modifications into account.



**Figure 6: Comparison of experimental data with predicted antigenic regions on the primary structure of OM.** I: IgE binding regions derived from chemical and enzymatic cleavages. II: IgE binding regions identified using synthetic peptides (Cooke & Sampson 1997). III: IgG binding regions identified using synthetic peptides (Cooke & Sampson 1997). IV: Antigenic regions determined by the method of Hopp & Woods (1981). V: Antigenic regions determined by the method of Parker et al. (1986). VI: Antigenic regions determined by the method of Welling et al. (1985).

## CONCLUSION

At least two distinct IgE binding epitopes located on peptides derived from native OM were identified. The results obtained by means of chemical and enzymic cleavage of OM are equivalent to epitope mapping using synthetic peptides. In summary OM is a well characterized allergen with regard to its B-cell reactivity. Future investigations should focus on the T-cell responsiveness of OM regions to provide further knowledge of the mechanisms of egg white allergy. The antigenicity prediction algorithms used in this study were of low value for correct epitope prediction.

## ACKNOWLEDGEMENT

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