Binding Activities of Pronase-Treated Fragments from Egg White Ovomucin with Anti-ovomucin Antibodies and Newcastle Disease Virus

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The prepared gel-like ovomucin and its β-subunit were treated with Pronase at various ratios (1/25600−1/6.25) to the sample weight at 37 °C for 24 h. The concentration, chemical composition, and SDS−polyacrylamide gel electrophoretic patterns of the obtained soluble fractions and their abilities to bind to anti-ovomucin antibodies and Newcastle disease virus (NDV) were measured. At a ratio of 1/6400 the highest soluble fraction (solubility: nearly 100%) was obtained. At a ratio of 1/800 the fragment with the highest binding activity to the antibodies was obtained, and at a ratio of 1/50 the fragments with the disulfide bonds intact (apparent molecular masses, AMMs: 55, 45, and 40 kDa) which showed binding to the antibodies were prepared and partially characterized. Fragments (AMMs: 220, 120, and 100 kDa) at ratios of 1/3200−1/800 and the final Pronase-resistant fragment (AMM: 120 kDa) at ratios of 1/12.5−1/6.25 with a binding activity to NDV could then be prepared. From the analysis of the fragments of Pronase-treated β-subunit, the AMM 120-kDa fragment was demonstrated to be a part of the AMM 220-kDa fragment.

Keywords: *Ovomucin; anti-ovomucin antibodies; Newcastle disease virus; Pronase treatment*

INTRODUCTION

Ovomucin (OVM) is a glycoprotein which accounts for approximately 3.5% of egg white proteins. From electron microscopic observations, it is reported to be fibrous with a polydisperse distribution of molecular lengths of 600 nm (Rabouille et al., 1990). It consists of an α-subunit (apparent molecular mass, AMM: 220 kDa) containing 10−15% carbohydrate and a β-subunit (AMM: 400 kDa) containing 50−65% carbohydrate, with a macromolecular structure from disulfide bonds between the subunits (Donovan et al., 1970; Kato et al., 1973; Hayakawa and Sato, 1978; Itoh et al., 1987). Thick egg white can be separated by ultracentrifugation into precipitate and liquid. Insoluble OVM can then be prepared from the precipitate and soluble OVM from the liquid (Kato et al., 1970; Hayakawa and Sato, 1977; Sato et al., 1976). Soluble OVM can also be obtained from thin egg white. Using light scattering measurements, the molecular weight of insoluble OVM is 2.3×10^7 Da in the presence of 6.5 M guanidine hydrochloride (Tomimatsu and Donovan, 1972), while that for soluble OVM is calculated to be 8.3×10^6 Da (Hayakawa and Sato, 1976).

Insoluble OVM that has been freeze-dried and then redissolved exhibits a gel-like consistency. Thus, it has been used after either reduction in the presence or absence of a denaturant (David et al., 1975; Kato et al., 1977) or solubilization by sonication (Hayakawa and Sato, 1977, 1978). However, these procedures are accompanied by cleavage of the disulfide bonds and the release of carbohydrate chains. In an earlier investigation, we reported that the carbohydrate chains and disulfide bonds between the subunits must be important due to the appearance of binding activities of OVM to viruses and that the binding activity of OVM to anti-OVM antibodies completely disappeared by the reduction of disulfide bonds in OVM (Tsuge et al., 1996b, 1997b). In those reports, we thought enzyme treatment of OVM would be a good method for solubilizing OVM with no great loss of biological activities.

We have reported that OVM exhibits a higher binding activity to viruses than do other egg white proteins and that the β-subunit moiety greatly contributes to its activity. Also, when OVM was individually digested with pepsin, trypsin, and Pronase, solubility and low molecularization were promoted mainly by Pronase. Furthermore, in a detailed analysis of fragments obtained from this Pronase-treated OVM, it was revealed that the regions containing sialic acid-rich carbohydrate chains in the β-subunit moiety participated most in binding to Newcastle disease virus (NDV), while fragments containing many disulfide bonds derived from the α-subunit were the main participants in binding to anti-OVM antibodies. Moreover, it was assumed that carbohydrate-rich regions in the β-subunit were made from fragments with molecular weights of about 220, 120, and 100 kDa (Tsuge et al., 1996a,b, 1997a).

In this series of studies aimed at clarifying the relation between OVM structure and function, we have come to realize the necessity of experiments on the time-course digestion of OVM. In the present study, a method of preparing various Pronase-treated OVM fragments to anti-OVM antibodies and NDV and the characterization of those fragments were investigated as a followup to the previous paper (Tsuge et al., 1997a).

MATERIALS AND METHODS

Materials. Mouse anti-OVM antibodies prepared for a previous study (Tsuge et al., 1997a) were also used in this study. NDV (strain Ishii) and anti-NDV chicken serum were purchased from the Kitasato Institute.

Preparations of OVM and Reduced and Alkylated β-Subunit. OVM from fresh egg white (White Leghorn hens) was prepared as a gel-like precipitate by the method of Kato

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et al. (1970) and in part freeze-dried. The prepared gel-like OVM (about 1 g) was dissolved in 10 mL of 10 mM carbonate buffer (pH 11.0), dialyzed against phosphate-buffered saline (pH 7.0), and used as the solubilized OVM. To prepare the reduced and alkylated β-subunit, the freeze-dried OVM was solubilized, reduced with 2-mercaptoethanol (2-ME) in the presence of SDS, alkylated with iodoacetamide, and then applied to a gel filtration column (Sephacryl S-400, 2.0 × 75 cm; Pharmacia LKB Products). These procedures were carried out according to the method described in the previous paper (Tsuge et al., 1997a).

Pronase Treatment of Samples. Three milliliters of 10 mM K-phosphate buffer (pH 8.5) was added to 3 mg of freeze-dried OVM and reduced and alkylated β-subunit, respectively, with swelling by incubation at 37 °C for 10 min. Pronase dissolved in the same buffer was added to each swollen liquid at ratios of 1/25600−1/6.25 (w/w) to OVM and to reduced and alkylated β-subunit solution at 1/12800, 1/400, and 1/12.5 (w/w). After each incubation at 37 °C for 24 h, the Pronase was inactivated by heating at 100 °C for 5 min and then centrifuged at 6000 rpm for 30 min. The supernatants obtained in this process were used for samples.

Enzyme-Linked Immunosorbent Assay (ELISA) with Anti-OVM Antibodies and NDV. ELISA with anti-OVM antibodies and NDV was also carried out by the method described in the previous paper (Tsuge et al., 1997a). Each sample of Pronase-treated OVM was diluted 20-fold with 50 mM carbonate buffer (pH 9.6), and each solubilized solution and its serially diluted solutions were coated on flat-bottomed microtiter plates. Each experiment was done in triplicate, and values were expressed as mean ± SD ($n = 3$).

SDS−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was performed using 7.5% or 12.5% gels according to the method of Laemmli (1970). Portions of 20 μL of the samples (0.1%), which were prepared in Tris-glycine buffer with and without 2-ME and heated at 100 °C for 3 min, were applied to each slot of gel and electrophoresed at a constant current of 17 mA. The gels were stained with Coomassie Brilliant Blue R-250 (CBB) for the detection of protein and with periodate−Schiff (PAS) reagent for the detection of carbohydrate.

Western Blot Analysis. After SDS−PAGE in the presence and absence of 2-ME of samples treated with Pronase at the ratio of 1/50, the separated proteins in the gel were transblotted to PVDF membranes (polyvinylidene difluoride; Bio-Rad) using the transfer buffer systems described by Matsudaira (1987) by the semidry electroblotting method. The membranes were incubated with 100-fold diluted anti-OVM antibodies, and the bound antibodies were visualized with chicken peroxidase-conjugated antibody to mouse IgG (Chemicon International Inc.) according to the manufacturer's protocol.

Analytical Methods. Protein was determined by the method of Lowry et al. (1951), in which BSA was used as the standard. Hexose was determined by the phenol−sulfuric acid method (Dubois et al., 1956), in which galactose was used as the standard. Amino acid analyses were carried out by high-performance liquid chromatography (LC module 1) of phenyl isothiocyanate derivatives which had been previously hydrolyzed with 6 N HCl containing 1% phenol for 24 h at 110 °C. Hexosamine was determined by the modification of Elson−Morgen's method (Neuhaus and Letzring, 1957), in which galactosamine was used as the standard. Sialic acid was determined by the thiobarbituric acid method (Warren, 1959), in which *N*-acetylneuraminic acid (NeuAc) was used as the standard.

RESULTS AND DISCUSSION

The SDS−PAGE patterns of the prepared gel-like OVM and OVM treated for 24 h at differing Pronase-added ratios (PARs) are shown in Figure 1. OVM was stained with a band showing the α-subunit in CBB stain in the presence of a reductant (Figure 1A, lane 2) and that showing the β-subunit in PAS stain (Figure 1B, lane 2). With no reductant present, OVM could not invade even the stacking gel due to its macromolecular structure, and therefore no bands were detected whatsoever. No other egg white proteins such as ovalbumin, ovotransferrin, or lysozyme were detected in the prepared OVM, making it clear that the prepared OVM was highly purified (Figure 1C,D, lane 2).

As for Pronase-treated samples, in CBB stain with reductant present (Figure 1A), broad bands were generated for each AMM of about 100, 65, and 48 kDa in the low-PAR region (PARs: 1/25600−1/1600, lanes 3−7). When the PAR was raised (1/800−1/6.25, lanes 8−15), the AMMs of bands were 55, 40, and less than 29 kDa and finally degraded to small fragments that could not be separated through electrophoresis of the used gel. Because these bands could not be detected with PAS stain, they are thought to be mainly fragments derived from the α-subunit. For PAS stain (Figure 1B), a broad band with AMM in the vicinity of 300 kDa was first generated with PARs of 1/25600 and 1/12800 (lanes 3 and 4). At a ratio of 1/6400 (lane 5), main bands were generated at 250, 220, and 200 kDa and minor bands at 120 and 100 kDa. At ratios of 1/3200−1/800 (lanes 6−8), the 250- and 200-kDa bands were virtually lost, but thicker bands could be detected at 220, 120, and 100 kDa. If PAR was raised further (higher than 1/400, lanes 9−15), the 220- and 100-kDa bands were diminished or disappeared, but almost no change was seen in the 120-kDa band. Then, at a ratio of 1/6.25 (lane 15), only a broad band of 120 kDa could be detected. As these bands were barely detected with CBB stain, they were thought to be fragments derived from β-subunit.

The SDS−PAGE patterns with no reductant present are shown in Figure 1C,D. With CBB stain (Figure 1C) in regions of low PAR (lower than 1/1600, lanes 3−7), the broad bands for AMMs above 200 kDa that were not detected in the presence of reductant could be detected. From this it is presumed that several fragments are bridged with disulfide bonds, and one can further conjecture that there are comparatively many disulfide bonds within the α-subunit. With PAS stain, a nearly identical pattern is exhibited at PARs of 1/3200−1/6.25 regardless of the presence or absence of reductant (Figure 1C,D), but the mobility of those fragments at ratios of 1/25600−1/6400 is decreased compared to that for each of the fragments when a reductant is present. This indicates that each of the fragments obtained in that region of low PAR contains disulfide bonds between the fragments. From the above description, it can be seen that the α-subunit regions containing disulfide bonds can be maintained comparatively well even when treated with Pronase. It was also found that disulfide bonds almost never exist in carbohydrate-rich regions in the β-subunit because the PAS-stained SDS-PAGE patterns in regions of high PAR are nearly coincident regardless of the presence or absence of reductant (Figure 1B,D).

Subsequently, the concentration and chemical composition of soluble fractions from each Pronase-treated OVM were investigated (Table 1). The concentration of the soluble fractions first rises as PAR becomes higher and then decreases greatly. There is an approximately 1.8-fold difference produced between the highest concentration of soluble fractions at a ratio of 1/6400 and the lowest at 1/6.25. This is presumed due to the formation of insoluble aggregates among fragments because of excess fragmentation of the α-subunit in high-PAR regions. In regions of low PAR (1/25600 and

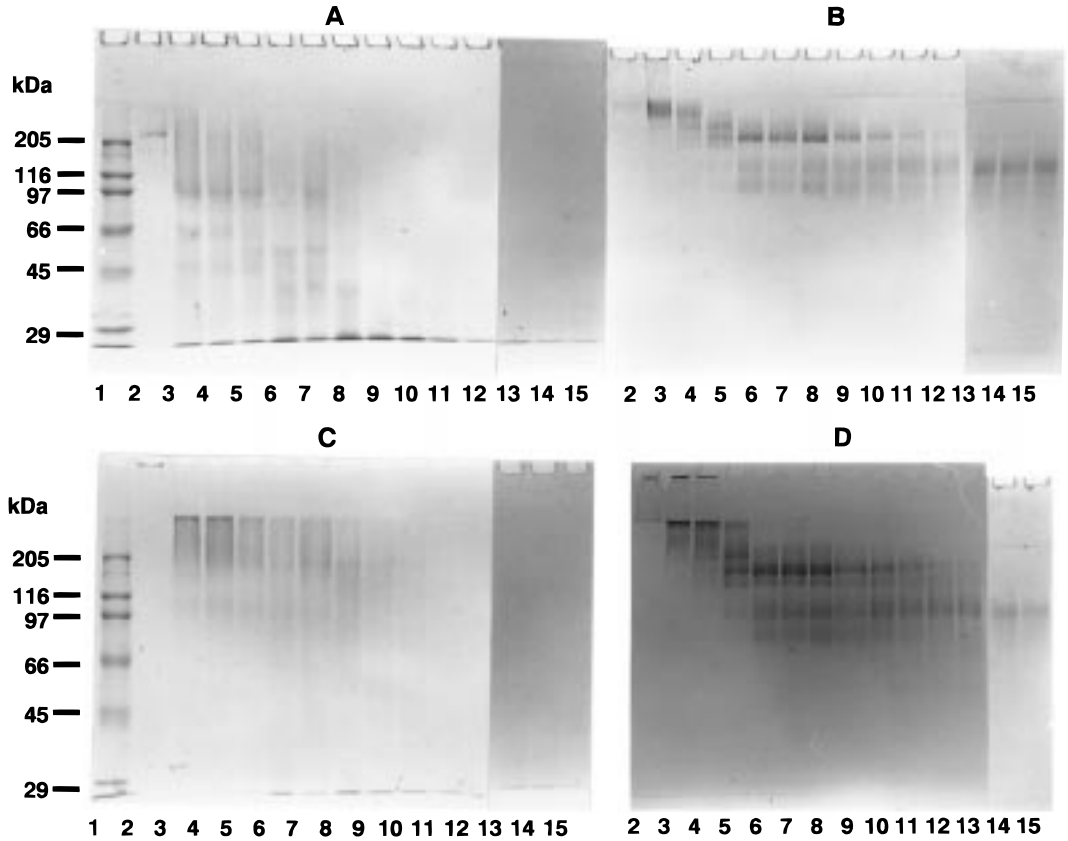

Figure 1. SDS−PAGE patterns of OVM treated with Pronase at ratios of 1/25600−1/6.25 to the sample weight at 37 °C for 24 h. Electrophoresis was performed in the presence of 2-ME (A and B) or in the absence of 2-ME (C and D), and gels were stained by CBB (A and C) and PAS reagent (B and D). Lanes: 1, molecular weight markers [myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa); molecular weights of fragments of >205 kDa were estimated from their migration by using α-subunit (220 kDa) and β-subunit (400 kDa)]; 2, prepared gel-like OVM; 3−15, Pronase-treated ovomucin (3, PAR = 1/25600; 4, 1/12800; 5, 1/6400; 6, 1/3200; 7, 1/1600; 8, 1/800, 9, 1/400 10, 1/200; 11, 1/100; 12, 1/50; 13, 1/25; 14, 1/12.5; 15, 1/6.25].

Table 1. Concentration and Chemical Composition of Soluble Fractions Obtained from Ovomucin Digested with Pronase at Ratios of 1/25000−1/6.25 to the Sample Weight at 37 °C for 24 h

Pronase-added ratio	concn of soluble fraction[a] (μg/mL)	chemical composition (%)				
		protein	carbohydrate[b]	hexose	hexosamine	NeuAc
1/25600	942.1	69.9	30.1	10.9	13.9	5.3
1/12800	944.4	68.9	31.1	10.8	15.1	5.2
1/6400	1011.0	70.2	29.8	12.0	13.7	4.0
1/3200	1001.1	69.9	30.1	11.5	13.8	4.8
1/1600	978.3	67.5	32.5	12.1	15.5	4.9
1/800	968.2	67.4	32.6	11.9	15.7	5.0
1/400	890.1	64.3	35.7	13.3	17.2	5.3
1/200	851.5	60.6	39.4	14.3	19.6	5.6
1/100	811.9	61.9	38.1	14.6	18.0	5.6
1/50	782.0	53.8	46.2	16.9	22.4	7.0
1/25	615.8	53.4	46.6	19.7	19.0	7.8
1/12.5	606.0	49.2	50.8	21.2	22.2	7.4
1/6.25	575.8	46.6	53.4	22.9	22.1	8.45

[a] Concentration of supernatant obtained by centrifugation of Pronase-digested ovomucin. [b] Hexose + hexosamine + NeuAc.

1/12800), gel-like precipitates remained. Prior to enzyme treatment, swollen OVM solutions were adjusted to 0.1% (w/v), and at 1/6400−1/800 the concentration of soluble fractions approached values of 0.1%, or very close to 100% solubilized. From the fact that no precipitate can be seen even by centrifuging each sample, it was thought that the chemical composition of soluble fractions in these PARs is very nearly the same as that of prepared OVM (Tsuge et al., 1997a). It may be resonable that the proportions of carbohydrate to protein moieties were different in each soluble fraction and that the carbohydrate moiety in soluble fractions increased with increased PARs.

The binding of each Pronase-treated OVM to anti-OVM antibodies was also measured by ELISA (Figure 2). One group (1/12800−1/800) had a higher binding activity than untreated OVM, and another group (1/200−1/12.5) had a lower activity. The reason for the higher binding activity in the former group is thought to be that an antigenic determinant inherent in the OVM without leading to smaller fragments is more exposed to the outside due to the enzyme treatment. In the latter group, these antigenic determinants are thought to be partially reduced to smaller fragments by Pronase treatment. Even with SDS−PAGE (Figure 1C) in a system containing no reductant, the fact that at a

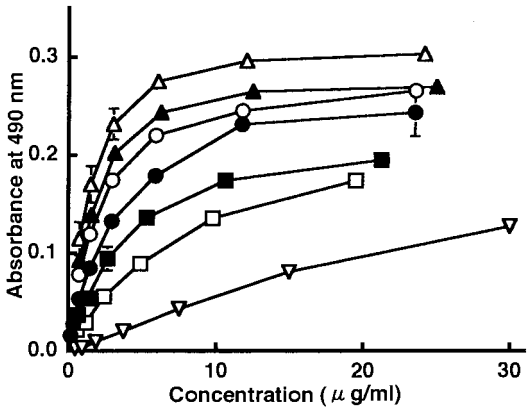

Figure 2. Bindings of OVM treated with Pronase at various added ratios to anti-OVM antibodies: ●, solubilized OVM; Pronase-treated OVM (○, PAR = 1/12800; ▲, 1/3200; △, 1/800; ■, 1/200; □, 1/50; ▽, 1/12.5). Values are expressed as the mean ± SD (n = 3).

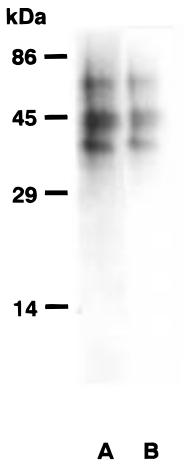

Figure 3. Binding of Pronase-treated OVM (PAR = 1/50) to anti-OVM antibodies. The sample was dissolved with buffer without 2-ME, electrophoresed on 12.5% acrylamide gel (lane A, stained with CBB), and blotted on PVDF membrane (lane B, visualized with anti-OVM antibodies). Molecular weight markers: lactoferrin (86 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), and lysozyme (14 kDa).

PAR of 1/200 all of the high-molecular-weight broad bands seen in regions of low PAR virtually disappeared suggests that fragments containing antigenic determinants are further digested to smaller peptides. The prepared gel-like OVM was used as the antigen in this study. The antibodies against the nonglycosylated or low-glycosylated part of the polypeptide rather than the carbohydrate chain moiety in OVM would be generally easier to produce. However, the little glycosylated polypeptides would be digested according to their susceptibility to degradation by Pronase, as described above, even though they contained antigen determinant regions.

Bindings of the prepared fragments to anti-OVM antibodies were evaluated by immunoblotting analysis using the anti-OVM antibodies. Figure 3 shows the SDS−PAGE pattern in the absence of 2-ME of the concentrated sample which was treated at a PAR of 1/50 and the immunoblotting pattern. The fragments corresponded to AMMs of 55, 45, and 40 kDa (these fragments could scarcely be detected due to the nonconcentrating procedure on the sample in Figure 1C, lane 12). These fragments, which were stained in SDS−PAGE patterns (Figure 3A), could be recognized with antibodies (Figure 3B). In the SDS−PAGE pattern in the presence of 2-ME of the same sample as described above, the fragments corresponding to AMMs of 30, 20, 15, and 10 kDa were detected. However, they could not be recognized with antibodies (data not shown), suggesting that the conformation of the polypeptide formed by disulfide bonds and the resistance to degradation by Pronase were important for the recognition.

Table 2. Amino Acid Compositions of 55-, 45-, and 40-kDa Fragments from Ovomucin Digested with Pronase at a Ratio of 1/50[a]

	fragments (kDa)		
	55	45	40
Asx	10.5	10.5	11.0
Glx	12.5	10.7	11.2
Ser	8.4	8.7	7.5
Gly	7.8	8.8	10.9
His	3.2	1.6	2.4
Arg	3.2	2.8	3.3
Thr	5.6	6.1	5.8
Ala	6.5	5.6	5.5
Pro	5.9	8.8	6.4
Tyr	5.1	4.9	3.9
Val	7.9	5.6	6.9
Met	1.5	0.6	1.4
1/2Cys	3.9	6.8	4.8
Ile	4.4	5.1	5.4
Leu	6.3	5.3	5.2
Phe	3.6	3.9	4.0
Lys	3.5	4.1	4.4

[a] Values are represented in mol %. Tryptophan was not determined.

Table 2 shows the amino acid compositions of fragments (AMMs: 55, 45, and 40 kDa), which contained a certain amount of half-cystine residues. Although the contents of disulfide bonds and free thiol groups in the three fragments were not directly obtained from the half-cystine residues, the fact that each of the fragments was reduced to smaller fragments in the SDS−PAGE patterns in the presence of 2-ME indicated the presence of at least two segments bridged by disulfide bonds in the fragments. These results showed that disulfide bonds in the fragments, which surely derived from the α-subunit, were needed for reaction with antibodies. The AMMs of the three fragments that reacted with anti-OVM antibodies were relatively higher for determinations of the antigen structure using the fragments containing antigen regions. Under the Pronase-treated conditions used, antigen regions which had digested into much smaller fragments could not be found. Therefore, proteases other than Pronase should be used for the preparation of molecules containing antigen regions smaller than the fragment of AMM 400 kDa, if necessary.

The binding of each Pronase-treated OVM to NDV was measured using ELISA (Figure 4), which showed the binding to be greatly reduced compared to untreated OVM. As shown in our previous report (Tsuge et al., 1996b), this is because the protein moiety of OVM plays an important role in binding to NDV. In general, the binding of carbohydrate chains that are receptors to viruses is extremely low in their free forms (Yolken et al., 1992; Kawasaki et al., 1993). When such carbohydrate chains attached to a peptide moiety are clustered, as in OVM, it is thought that binding to NDV is more stable because they can bind with NDV ligands at many locations. These Pronase-treated OVM samples can be separated into one group with comparatively high binding (PARs: 1/12800−1/800) to NDV and one with

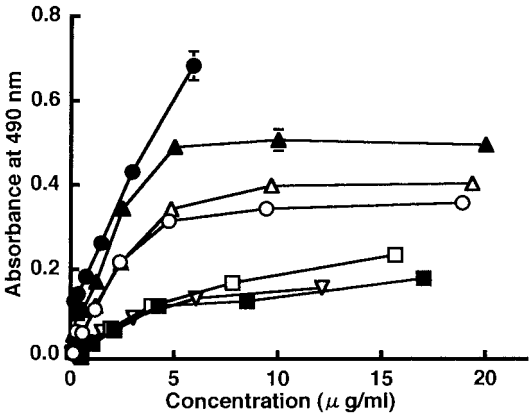

Figure 4. Bindings of OVM treated with Pronase at various added ratios to NDV. Symbols are the same as in Figure 2. Values are expressed as the mean ± SD (n = 3).

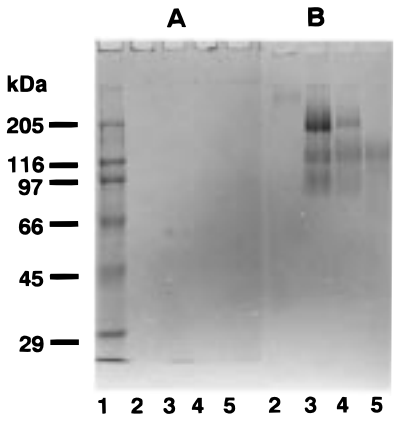

Figure 5. SDS−PAGE patterns of reduced and alkylated β-subunit treated with Pronase at various added ratios. The samples were dissolved with buffer with 2-ME and electrophoresed on 7.5% acrylamide gels. The gels were stained with CBB (A) and PAS reagent (B). Lanes: 1, molecular weight markers shown in Figure 1; 2, reduced and alkylated β-subunit; 3−5, Pronase-digested reduced and alkylated β-subunit (3, PAR = 1/12800; 4, 1/500; 5, 1/12.5).

low binding (PARs: 1/200−1/12.5) with a 2-fold difference between the groups. It is remarkable that the 120-kDa fragment as the smallest unit which shows the binding to NDV was more easily prepared at PARs of 1/12.5−1/6.25 without any other carbohydrate-containing fragments than at 1/50 in the previous paper (Tsuge et al., 1997a), in which the digest was applied to a gel-filtration column to separate it from other similar fragments. The chemical composition of the 120-kDa fragment after dialysis in this study was nearly equal to that described previously (Tsuge et al., 1997a).

The SDS−PAGE patterns of the reduced and alkylated β-subunit and its β-subunit treated for 24 h at three PARs are shown in Figure 5. The reduced and alkylated β-subunit was stained with a band in PAS stain (Figure 5B, lane 2) and not with CBB (Figure 5A, lane 2). No other bands were detected in the prepared β-subunit, making it clear that the prepared β-subunit was highly purified. When Pronase at a PAR of 1/12800 (w/w) to reduced and alkylated β-subunit was added to the β-subunit solution and treated for 24 h, bands in PAS stain were generated for each AMM of about 220 (main fragment), 120, and 100 kDa (minor fragments) (Figure 5B, lane 3). At 1/400 the bands of 220 and 100 kDa were diminished, and at 1/12.5 only one band of 120 kDa could be detected (Figure 5B, lanes 4, 5). The fact that

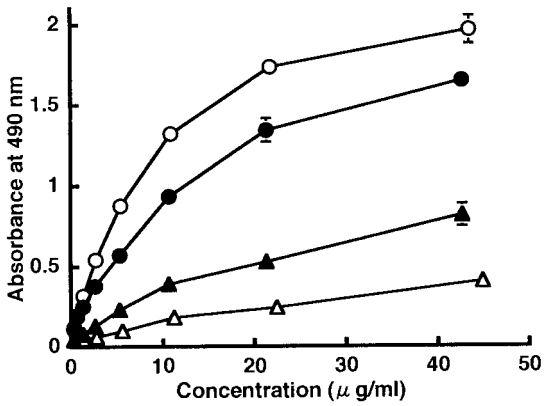

Figure 6. Bindings of reduced and alkylated β-subunit digested with Pronase at various added ratios: ●, reduced and alkylated β-subunit; Pronase-digested reduced and alkylated β-subunit (○, PAR = 1/12800; ▲, 1/400; △, 1/12.5).

the fragment of 120 kDa could not be digested to smaller fragments even at the higher Pronase ratio of 1/12.5 shows that the 120-kDa fragment was the final Pronase-resistant one. Moreover, the fragments having AMMs of 220, 120, and 100 kDa were demonstrated to be the main constituents of the reduced and alkylated β-subunit, because the other fragments were not detected in the SDS−PAGE patterns and the precipitate removed by centrifugation (6000 rpm × 30 min) in the process of the Pronase digestion of the reduced and alkylated β-subunit did not appear. On the other hand, no bands in the three Pronase-treated reduced and alkylated β-subunits were detected in the CBB stain (Figure 5A, lanes 3−5).

The reduced and alkylated β-subunit did not show any binding to anti-OVM antibodies, but it did to NDV, as described in the previous paper (Tsuge et al., 1997b). The binding of each Pronase-treated reduced and alkylated β-subunit to NDV was measured using ELISA (Figure 6). At 1/12800 the binding of each Pronase-treated reduced and alkylated β-subunit was increased, and at 1/400 and 1/12.5 it was reduced compared to the untreated β-subunit. In connection with the results in Figures 2 and 4 described above, the 120-kDa fragment might be thought to be a basic unit to bind with NDV, although its binding activity was lower than that of the other larger fragments. Each of the 220- and 120-kDa fragments was separated, respectively, by gel-filtration chromatography as described in the previous paper (Tsuge et al., 1997a) and digested again with Pronase at a ratio of 1/12.5. Their SDS−PAGE patterns are shown in Figure 7. The major part of the 220-kDa fragment was digested to a 120-kDa fragment (Figure 7, lanes 1, 2). However, the 120-kDa fragment was not further digested to smaller fragments. These results indicated that the 120-kDa fragment was a constituent of the 220-kDa one and a Pronase-resistant one. The ordered manner of each fragment in the β-subunit needs further clarification.

On the basis of the foregoing, the most favorable condition to obtain fractions from OVY with comparatively higher binding activities to antibodies and NDV by Pronase treatment appears to be treatment for 24 h at PARs of 1/3200−1/800, judging from the results of ELISA (Figures 2 and 4). However, the PAR should be chosen in terms of the objective. For example, a higher ratio than 1/50 should be chosen for preparing the smaller fragments which reacted with the anti-OVM

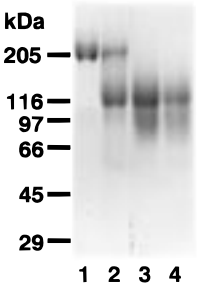

Figure 7. SDS−PAGE patterns of the isolated 120- and 100-kDa fragments digested with Pronase at a PAR of 1/12.5. The samples were dissolved with buffer with 2-ME and electrophoresed on 7.5% acrylamide gels. The gel was stained with PAS reagent. Lanes: 1, 220-kDa fragment; 2, Pronase-digested 220-kDa fragment; 3, 120-kDa fragment; 4, Pronase-digested 120-kDa fragment. Molecular weight markers shown in Figure 1 were used.

antibodies and for analyzing the antigen structure in the α-subunit. When analyzing the structure of the β-subunit, a ratio of 1/25600 for 24 h is effective to obtain fragments which are nearly equal to the molecular weight of the β-subunit. Ratios of 1/3200−1/50 for 24 h are effective to obtain three types of fragment (AMMs: 220, 120, and 100 kDa) from the β-subunit, and ratios of 1/12.5−1/6.25 with treatment times of 24 h are effective to obtain Pronase-resistant fragments (AMMs: 120 kDa) containing sialic acid-rich carbohydrate chains. OVM prepared from egg white by water dilution and 2% KCl wash is difficult to solubilize. Using the Pronase treatment method of the present study, large quantities of a high concentration of soluble fractions were more easily obtained from insoluble OVM than untreated OVM which was generally solubilized with the alkaline pH or surface-active agents with reducutant, and there is no cleavage of disulfide bonds or release of carbohydrate chains. Also, according to the choice of PAR, fragments with various binding abilities to NDV or anti-OVM antibodies can be obtained. This will be an effective method for analyzing the relations among a great many functions, beginning with the structure of OVM and its biological activity.

CONCLUSION

This study presented a method for preparing soluble fragments from gel-like OVM by digestion with Pronase in various concentrations in connection with the binding activities to antibodies and NDV. Some basic fragments having their 30 binding activities were also prepared and partially characterized. According to the choice of adequate PARs, this method will be effective for analyzing the relationship between the function and structure of OVM which is a macromolecular structure having carbohydrate-rich regions.

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