Recombinant bovine conglutinin, lacking the N-terminal and collagenous domains, has less conglutination activity but is able to inhibit haemagglutination by influenza A virus

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Conglutinin is a bovine serum protein which was first described as a vertebrate lectin. This protein belongs to the family of C-type lectins. These lectins are composed of four characteristic domains: (1) an N-terminal cysteine-rich domain, (2) a collagen-like domain, (3) a neck domain and (4) a carbohydrate recognition domain (CRD). Recently lectins have been shown to function as immunoglobulin-independent defence molecules due to a complement-mediated mechanism or opsonization. Our previous study showed that bovine conglutinin can inhibit haemagglutination by influenza A viruses and act by directly neutralizing them due to its lectin properties. In order to elucidate the biological role of the collagen-like domain, a recombinant partial conglutinin lacking this collagen-like domain was produced in an *Escherichia coli* system and its biological activities were examined. A 497 bp sequence, consisting of a short collagen region (two repeats of G-X-Y amino acid sequences), the neck domain, and the CRD of conglutinin cDNA, was amplified by

the reverse-transcriptase PCR technique. The cDNA was transferred to a bacterial expression vector system (pRSET-A) and stable transfectants with a high level of conglutinin production were obtained. SDS/PAGE and Western blotting analyses showed a recombinant fusion protein of 27 kDa. Results of a cross-linking study and gel-filtration assay indicated that the recombinant conglutinin can form a trimeric structure and that it has sugar binding activity and specificity similar to that of native conglutinin. The recombinant conglutinin was also found to inhibit haemagglutination caused by influenza A virus as well as to possess less conglutination activity. These results suggest that in order for conglutinin to inhibit haemagglutination caused by the influenza virus, as well as to have sugar binding activity or to form trimers, it does not require the N-terminal and collagenous domains; however, they are essential for full conglutination activity.

INTRODUCTION

Bovine conglutinin (bovine conglutinin) is a member of the mammalian C-type lectin family [1], which includes mannan-binding proteins (MBPs) [2], surfactant protein A (SP-A) [3] and surfactant protein D (SP-D) [4]. These lectins are now known as 'collectins' and are characterized by a collagen-like domain and a globular carbohydrate recognition domain (CRD) [5]. In vertebrates, mechanisms dealing with cell-mediated immune responses and specific antibody responses are considered to be the major means of defence against invading pathogens. Recently, however, non-specific immune mechanisms in which these lectins play a role have been shown to be important in the neutralization and elimination of various micro-organisms in young children who do not have maternal antibody or have not developed efficient systems for specific protection [6]. Evidence for the role of these lectins in host defence emerged from a study showing that a decrease in the MBP level due to mutations in the MBP gene is associated with an increased susceptibility to infection [7]. We have previously found that conglutinin and MBP are able to inhibit the infectivity and haemagglutinating activity of influenza A viruses of the H1 and H3 subtypes [8,9]. We then succeeded in obtaining a cDNA clone encoding bovine conglutinin and demonstrated a strong relationship between conglutinin and several SP-D genes [10]. Recombinant bovine SP-D in mammalian cells has been shown to be capable of binding

directly to and agglutinating *Escherichia coli* [11]. The recombinant fusion SP-D in *E. coli*, composed of the neck domain and the CRD, was found to be a homotrimer composed of an α -helical bundle [12] and was able to bind to lipopolysaccharide from several bacteria [13]. However, there was no evidence of biological activities in them, such as agglutination of bacteria or inhibition of haemagglutination by viruses. In this report, we first produced recombinant conglutinin lacking the collagen domain in a bacterial expression system and characterized this lectin by comparing it with native conglutinin. The recombinant conglutinin in *E. coli* showed a similar sugar binding specificity to native conglutinin and it unexpectedly exhibited the same inhibition of haemagglutination by influenza A virus, which was dependent on calcium, as well as having less conglutination activity. Here we have elucidated the role of the N-terminal and collagen-like domains in the conglutination activity and haemagglutination-inhibitory activity against influenza A virus.

MATERIALS AND METHODS

Buffers

Tris-buffered saline (TBS) contained 20 mM Tris/HCl, 140 mM NaCl, pH 7.4. TBS/NT was TBS containing 0.05 % (w/v) NaN_3 and 0.05 % (v/v) Tween 20. TBS/NTC was TBS/NT containing 5 mM CaCl_2 . TBS/C was TBS containing 5 mM CaCl_2 . SOB

Abbreviations used: SP-A, surfactant protein A; SP-D, surfactant protein D; MBP, mannan-binding protein; CRD, carbohydrate recognition domain; SRBC, sheep red blood cell.

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medium contained 2 % (w/v) bacto-tryptone, 0.5 % (w/v) bacto-yeast, 8.5 mM NaCl and 50 μ g/ml ampicillin. Coating buffer for the ELISA system was 15 mM Na₂CO₃, 35 mM NaHCO₃, 0.05 % (w/v) NaN₃, pH 9.6. The lysis buffer (buffer A) contained 6 M guanidine hydrochloride, 20 mM sodium phosphate, 500 mM NaCl, pH 7.8.

Preparation of the conglutinin DNA fragment by reverse-transcriptase PCR

PCR primers were designed and synthesized based on the bovine conglutinin cDNA sequence [10]: 5'-GGCTCGAGGGGGA-GAGTGGGCTTGACAGA-3' and 5'-GGGAATTCTCAAAAC-TCGACATCACAA-3', which contain *Xho*I and *Eco*RI sites respectively. Each reaction volume (50 μ l) contained 1 $\times$ reaction buffer as supplied by the manufacturer, 1 μ M of each primer, 200 μ M dNTPs, 1 unit of Deep Vent DNA polymerase (New England Biolabs), and 10 ng of cDNA. PCR was carried out in a Zymoreactor (Atto Co.) for 35 cycles consisting of denaturation at 92 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min. PCR products were digested with *Eco*RI and *Xho*I and inserted into the bacterial expression vector pRSET-A (Invitrogen Co.) using a DNA Ligation Kit (Takara Shuzo Co.). The ligation mixture was transfected into *E. coli* JM109.

Expression and purification of recombinant protein

A single colony of the transformed *E. coli* which carried the proper insert was incubated with SOB medium at 37 °C overnight. A 1.2 ml aliquot of this overnight culture was inoculated into 200 ml of SOB medium and cells were allowed to grow to a D_{600} of around 0.3. After addition of isopropylthiogalactoside to a final concentration of 1 mM, the culture was grown for an additional 1 h. The cells were infected at a multiplicity of infection (MOI) of 5 plaque-forming units (p.f.u.)/cell with an M13 phage containing the T7 RNA polymerase gene driven by the *E. coli lac* promoter and incubated for another 3 h. They were then harvested by centrifugation at 3000 g for 15 min. The bacterial pellets were suspended in 20 ml of buffer A (6 M guanidine hydrochloride, 20 mM sodium phosphate, 500 mM NaCl, pH 7.8) and lysed by sonication (15 s, 70 % output, 10 times). After centrifugation at 43000 g for 30 min, the supernatant was incubated with nickel-nitrilotriacetic acid-agarose (Qiagen) for 15 min. The nickel-agarose was poured into a column and thoroughly washed with TBS/NT [20 mM Tris/HCl, 140 mM NaCl, 0.05 % (w/v) NaN₃, 0.05 % (v/v) Tween 20, pH 7.4] and then washed with TBS/NTC (TBS/NT containing 5 mM CaCl₂). The fusion protein was eluted with TBS/NTC containing 0.5 M imidazole. The eluant was dialysed against 1000 vol. of TBS/NTC with three changes. After dialysis and centrifugation, the supernatant was applied to a mannan-Sepharose column. After washing with TBS/NTC, bound protein was eluted with TBS/NTC containing 5 mM *N*-acetylglucosamine. Purification of the recombinant conglutinin was confirmed by SDS/PAGE and Western blotting analyses.

SDS/PAGE, native PAGE and Western blotting

SDS/PAGE and native PAGE were carried out with a 4–20 % gradient polyacrylamide gel. The polypeptides were stained with 1 % Coomassie Blue or were silver-stained with a 2D-Silver Stain II Kit (Daiichi Pure Chemical Co., Tokyo, Japan). Proteins were transferred on to a nitrocellulose membrane and the blot was incubated with rabbit anti-conglutinin serum. This was followed by incubation with anti-(rabbit IgG)-conjugated biotin (Vector)

and a final incubation with alkaline phosphatase-conjugated streptavidin (BRL). Nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (BRL) was used as the alkaline phosphatase substrate.

Chemical cross-linking study

The recombinant protein was dissolved at a concentration of 22.8 μ g/ml in 0.1 $\times$ PBS containing 10 mM CaCl₂. Samples were treated with bis(sulphosuccinimidyl)suberate for 20 min at 37 °C. They were then diluted 1:1 with double-strength SDS/PAGE sample buffer and heated to 100 °C for 5 min.

Gel-filtration chromatography

Chromatography of purified recombinant conglutinin was performed on a Superose 6 column at a flow rate of 0.5 ml/min with TBS containing 10 mM EDTA at pH 8.0. Then, 40 μ g of recombinant conglutinin was applied to the column. Fractions were monitored at 280 nm. The recovery of recombinant protein was also monitored with a Coomassie protein assay reagent (Pierce). Fractions were assayed with anti-(bovine conglutinin) rabbit serum using the sandwich ELISA system described below. Calibration of the column was carried out using a Pharmacia standard molecular-mass-marker kit containing thyroglobulin, ferritin, catalase, aldolase, albumin, ovalbumin, chymotrypsinogen A and ribonuclease A.

Binding of conglutinin proteins to mannan

Microtitre plates were coated with mannan (10 μ g/ml) in 100 μ l of coating buffer [15 mM Na₂CO₃, 35 mM NaHCO₃, 0.05 % (w/v) NaN₃, pH 9.6] at 4 °C overnight. The plates were washed three times with TBS/NTC [20 mM Tris/HCl, 140 mM NaCl, 0.05 % NaN₃ (pH 7.4), 5 mM CaCl₂, 0.05 % (v/v) Tween-20] after each step. After coating, the plates were blocked with 1 % BSA in TBS/NTC for 1 h at room temperature. Dilutions of lectins (recombinant or native conglutinin) alone or together with dilutions of various saccharides, rabbit anti-conglutinin serum and goat anti-(rabbit IgG) horseradish peroxidase conjugate (Bio-Rad) were added sequentially and incubated for 1 h at 37 °C at each step. Finally, 100 μ l of TMB substrate solution was applied to each well (TMB Microwell Peroxidase Substrate System, KPL). Before and after the addition of 100 μ l of 1 M phosphoric acid, the A_{450} or A_{655} was read on a Titertek Multiskan Plus MKII plate reader (Flow Laboratories). A sugar inhibition assay was used for this ELISA system. The microtitre plates were coated with yeast mannan (1 μ g/well) (Sigma) and the recombinant conglutinin was incubated with an increasing concentration of sugars. The concentration of sugar which inhibited 50 % of binding was defined as the I_{50} [14].

Conglutinin assay

Sheep red blood cells (SRBCs) with iC3b on their surface were prepared by incubation of 1 % SRBCs sensitized with anti-SRBC antibody (Sigma) with an equal volume of 10-fold diluted fresh equine serum as complement source for 10 min at 37 °C. Conglutination activity was detected by incubation at 37 °C of 50 μ l of the recombinant conglutinin or native conglutinin with 50 μ l of 1 % SRBCs, prepared as described above, in veronal buffer only or in buffer containing 30 mM *N*-acetylglucosamine. The conglutination titre was taken as the dilution containing the lowest protein concentration showing agglutination.

Virus

A/Ibaraki/1/90 (H3N2), which had been passed less than 10 times and is sensitive to animal sera, was used for the haemagglutination inhibition test. The virus was grown in chorio-allantoic membranes (CAMs) of embryonated hen eggs, according to standard procedures, and stored at -70°C until use.

Haemagglutination inhibition test

Haemagglutination inhibition tests were carried out in 96-well microtitre plates with 1% (v/v) chicken erythrocytes using a standard microtitre assay method [15]. The ether-treated egg-derived viral antigens for the haemagglutination inhibition test were prepared in our laboratory. The incubation mixture in TBS/C (TBS with 5 mM CaCl_2), had (i) nothing additional added, (ii) 30 mM *N*-acetylglucosamine added, or (iii) 5 mM EDTA added. Inhibition of virus-mediated agglutination of chicken erythrocytes by conglutinins was observed after a 1 h incubation at room temperature.

RESULTS

Expression of recombinant conglutinin in *E. coli*

cDNA sequencing of bovine conglutinin [10] and extensive amino acid analyses [16] have shown a sequence of 371 amino acid residues with a signal peptide of 20 residues. The 497 bp PCR product was digested and ligated into bacterial expression vector pRSET-A. Sequencing of this fragment confirmed correct amplification including that of the short collagen region, the neck domain and the CRD, corresponding to amino acid residues 191 to 351 of the mature conglutinin protein, and no PCR errors were noted (results not shown). Stable transfectants with a high level of partial conglutinin production were obtained. However, we could not obtain stable clones capable of a high production level of whole conglutinin (results not shown). Purification of the

Figure 1 SDS/PAGE analysis of recombinant fusion conglutinin

Aliquots of recombinant proteins at each purification step were analysed on an SDS/polyacrylamide gel (4–20% gradient gel) under reducing conditions and stained with Coomassie Blue. Lane M, protein standards; lane 1, total cell lysate; lane 2, soluble fraction with guanidine hydrochloride; lane 3, insoluble fraction with guanidine hydrochloride; lane 4, fusion protein eluted from the nickel column; lane 5, fusion protein eluted from the mannan-Sepharose column.

Figure 2 Chemical cross-linking and native PAGE of recombinant fusion conglutinin

Aliquots were treated with 0 mM, 0.5 mM, 1.0 mM, 2.5 mM, 5 mM and 10 mM bis-(sulphosuccinimidyl)suberate for 1 h at 22°C . Following electrophoresis on an SDS/polyacrylamide gel (4–20% gradient gel under reducing conditions), cross-linked complexes were visualized by Coomassie Brilliant Blue staining (A). A native PAGE of recombinant conglutinin in polyacrylamide gel (4–20% gradient gel) was performed and the proteins were visualized by silver staining (B).

recombinant conglutinin is shown in Figure 1. When treated with guanidium, the recombinant protein with a histidine tag was able to bind to the nickel column. Following binding, the column was washed thoroughly with TBS/NT and then with TBS/NTC. The recombinant protein which had bound to the column was eluted with imidazole buffer. After the eluted protein had been dialysed with TBS/NTC, it may have been altered to an active form capable of binding to the mannan-Sepharose column. After 5 days dialysis, we used the 5 mM *N*-acetylglucosamine eluate from that column for further study. SDS/PAGE showed a recombinant fusion conglutinin of 27 kDa, although it had a molecular mass of 22.5 kDa according to the deduced amino acid sequence (Figure 1). The N-terminal sequence of this fusion conglutinin has been deduced from the amino acid sequence from plasmid, and digestion of the recombinant conglutinin with enterokinase showed an amino acid sequence identical to that of mature conglutinin (results not shown). These results indicate that this system could produce a proper recombinant fused conglutinin protein and that the difference of molecular mass of a recombinant fusion conglutinin was dependent on the assay system. Western blotting analysis shows identification of the protein as a partial conglutinin, which was confirmed using rabbit polyclonal antibody raised to native conglutinin (results not shown). The partial conglutinin was eluted with 5 mM *N*-acetylglucosamine only, as was native conglutinin, indicating that this fusion protein has a high affinity for mannan. The final purified yield of the recombinant conglutinin was 2.8 mg from 1 litre of *E. coli* culture.

Structural characterization of recombinant conglutinin

Native conglutinin seems to be a 9–18-mer of the same constituent polypeptide [17]. The molecular mass of the largest oligomer was found to be approx. 1000 kDa and the typical oligomeric structure may consist of four trimeric building blocks with a criss-cross structure [18]. In the cross-linking study, the recom-

Figure 3 Gel-filtration chromatography of recombinant conglutinin

The recombinant fusion conglutinin was subjected to chromatography using a Superose 6 column. Each fraction (Fr.) was analysed using an ELISA system for detection of conglutinin proteins or monitored for protein concentration as described in the Materials and methods section. Arrows show the peak positions of the eluted standards: THY, thyroglobulin (669 000 Da); FER, ferritin (440 000 Da); ALD, aldolase (158 000 Da); BSA (67 000 Da); OVA, ovalbumin (43 000 Da); CHY, chymotrypsinogen A (25 000 Da).

binant fusion protein was found to consist of monomers of 27–30 kDa, dimers of 58–66 kDa and trimers of 90–100 kDa (Figure 2A). In complete cross-linking conditions, we found that there was a major trimer band and a trace of monomer band but there was no dimer band. A native PAGE analysis of recombinant conglutinin showed the major broad band of 60–100 kDa and a minor band of 18 kDa (Figure 2B). Gel-filtration analysis showed three major peaks consisting of 90–110 kDa (fraction 31), 34–39 kDa (fraction 34) and 4.6 kDa (fraction 39) components by UV monitoring at 280 nm and ELISA analysis (Figure 3). However, there was no protein found in fraction 39 by quantitative analysis. SDS/PAGE and silver staining analyses also failed to reveal a small protein of 4.6 kDa (results not shown). This small molecule (fraction 39) was confirmed not to be a peptide by UV absorbance at 200 nm or 280 nm. Fraction 31 of the first peak in native PAGE showed a broad band of trimers and fraction 34 of the second peak showed a minor band of monomers (results not shown). The cross-linking study, native PAGE and gel-filtration analyses indicate that this bovine conglutinin was capable of forming trimers without the collagenous domain. These results are not inconsistent with the fact

Table 1 Sugar specificities of recombinant fusion conglutinin and native conglutinin

Each I_{50} value shows the concentration required for 50% inhibition of binding. The values for native bovine conglutinin are taken from [14]. ∞ shows no inhibition.

Sugar inhibitors	I_{50} (mM)	
	Recombinant conglutinin	Native conglutinin
<i>N</i> -Acetyl-D-glucosamine	0.65	1.4
Fucose	—	41.5
L-Fucose	37.8	—
D-Fucose	55.3	—
D-Mannose	12.3	19.5
Maltose	25.8	49.0
<i>N</i> -Acetyl-D-mannosamine	26.6	—
Glucose	39.5	41.5
Galactose	46.8	> 100
<i>N</i> -Acetyl-D-galactosamine	∞	—
Lactose	> 100	∞

that recombinant human SP-A [19], human MBP [20] and bovine SP-D [12], which lack collagen-like domains, are able to form trimeric structures by association of the neck region.

Carbohydrate-binding activities of recombinant conglutinin

To examine the carbohydrate-binding specificity of the recombinant conglutinin, a sugar inhibition assay was conducted using an ELISA system (Table 1). Concentrations of sugar required for 50% inhibition were obtained from inhibition curves. The results showed that specificities of the recombinant fusion conglutinin were similar to those of native bovine conglutinin [14]. It was shown that the binding activity of the recombinant fusion conglutinin was Ca^{2+} -dependent as was that of the native conglutinin (Figure 4). In addition, *N*-acetylglucosamine abolished its binding activity to mannan. These results indicate that the histidine-tag fused to the recombinant protein did not affect its binding activity or its binding specificity to mannan.

Conglutination activity of recombinant conglutinin

The conglutination activity of the recombinant fusion conglutinin or native conglutinin was determined using a microtitre plate assay system. Figure 5 shows that the conglutination pattern of the recombinant conglutinin was different from that of native conglutinin. The conglutination activity of native conglutinin seemed to occur through the agglutination and precipitation of SRBCs due to its lectin activity and oligomeric structure. However, the recombinant conglutinin, which consisted largely of the trimeric form, might have less ability to precipitate compared with whole native conglutinin. The conglutination titre of the recombinant conglutinin was 1.25–2.5 $\mu\text{g/ml}$, whereas that of native conglutinin was 0.15 $\mu\text{g/ml}$. The activity of the recombinant protein was completely abolished by 30 mM *N*-acetylglucosamine, as seen in lane C.

Haemagglutination inhibition activity of recombinant conglutinin

The recombinant fusion conglutinin exhibited haemagglutination inhibition activity against influenza A virus in the absence of

Figure 4 Binding of the recombinant fusion congrutinin and native bovine congrutinin to mannan

Mannan (10 mg/ml) was coated on to the plates. After blocking, recombinant protein at various concentrations (0, 1, 10, 100, 1000 ng/ml) was added in TBS/NTC or TBS/NTC with 20 mM *N*-acetyl-D-glucosamine (A) or 10 mM EDTA (B). Anti-(native congrutinin) rabbit serum and anti-(rabbit IgG)-conjugated horseradish peroxidase were diluted 1000-fold or 2000-fold, respectively, in TBS/NTC. The absorbance was read at 655 nm (■, recombinant fusion congrutinin in TBS/NTC; ●, recombinant fusion congrutinin in TBS/NTC with 20 mM *N*-acetyl-D-glucosamine; ▲, recombinant fusion congrutinin in TBS/NTC with 10 mM EDTA).

Figure 5 Conglutination assay of recombinant congrutinin or native congrutinin

The conglutination assay was performed using a 2-fold dilution of native congrutinin (lane A), or recombinant congrutinin in the absence (lane B) or presence of 30 mM *N*-acetyl-D-glucosamine (lane C).

EDTA or *N*-acetylglucosamine (Figure 6). This activity was lectin-dose-dependent and calcium-dependent. The lowest concentration of the recombinant congrutinin causing haemagglutination inhibition was 39–78 ng/ml. Compared with native congrutinin [9], rat SP-D, and human SP-D [21], the haemag-

Figure 6 Inhibition of haemagglutination of influenza A virus by incubation with recombinant congrutinin or native congrutinin in the presence of Ca^{2+} , GlcNAc, or EDTA

The haemagglutination inhibition test was performed using a 2-fold dilution of native congrutinin (lane A) or recombinant congrutinin in 96-well V plates to which 16 haemagglutinin units (final concentration) of the influenza A virus (A/Ibaraki/1/90-H3N2) were then added. The mixture of recombinant congrutinin was incubated with 0.5% chicken erythrocytes in TBS, pH 7.5, without any other additions (lane B), with 30 mM *N*-acetyl-D-glucosamine (lane C), or with 10 mM EDTA (lane D) at room temperature for 1 h.

glutination inhibition activity of this new recombinant lectin was similarly high against influenza A virus.

DISCUSSION

The new recombinant fusion congrutinin, composed of synthesized peptides with a histidine tag, the short 'collagen' region (two repeats of G-X-Y amino acid sequences), the neck domain, and the CRD, exhibited several biological properties: it was shown to be calcium-dependent with respect to its ability to bind to mannan; and to have less conglutination activity in agglutinating sensitized SRBCs conjugated to complement (EAiC3b) as well as having haemagglutination inhibition activity against influenza A virus. Although many recombinant collectins have been produced in *E. coli*, no inhibitory activity directed against infectious micro-organisms has been found [13]. Other recombinant collectins produced by eukaryotic systems have similar activities to native collectins [11,22]. However, levels of their production are frequently low, or incorrect post-transcriptional modifications can occur. We succeeded here in producing a recombinant fusion congrutinin with several biological activities.

It was previously reported that native bovine congrutinin with a deletion of 54 amino acids consisting of the N-terminal domain and short collagenous domain had neither conglutination activity nor binding activity to EAiC3b, although it retained binding activity to mannan-BSA [17,23]. Our preliminary data showed that this altered congrutinin was no longer capable of the inhibition of haemagglutination by or neutralization of influenza A virus. Therefore, we considered that the binding to EAiC3b or inhibition of haemagglutination by influenza A virus was dependent on the complete structure of congrutinin including the collagenous domain. However, recent reports with recombinant collectins [12,13,19,20] revealed that the neck domain adjacent to the CRD could form trimers and that its CRD had lectin activity in terms of binding to micro-organisms. In this paper, we first showed that another collectin (bovine congrutinin) with deletion of the N-terminal and collagen-like domains is capable of conglutination activity by binding to EAiC3b, as well as showing haemagglutination inhibition activity against the influenza virus, through its properties as a lectin. However, this recombinant congrutinin has less conglutination activity compared with the native congrutinin. This indicates that it is very important for full

conglutination activity to have the N-terminal and collagen-like domains present to provide further oligomerization and to be able to bridge large distances between clusters of CRDs.

Members of the collectin family almost always possess a collagen-like domain and a CRD which is a globular domain. These collectins act in host defence as opsonins which bind to targets via their globular portion and to collectin receptors via their collagenous domain [5,24,25]. The collagen-like domain is essential for the binding of collectin to the C1q receptor (collectin receptor) [26] and for complement activation via the 'lectin pathway' [27,28]. Mutations in the collagenous domain of MBP are known to cause immunodeficiencies in adults [29] as well as infants [6]. This collagenous region might be important for the assembly of the MBP homopolymer. Recently, human MBP and SP-A were also shown to bind to influenza virus and to have haemagglutination inhibition activity [30]. This interaction of MBP and SP-A with influenza virus was found to occur via neuraminidase of another envelope glycoprotein [30]. It is considered that the binding of MBP or SP-A to neuraminidase caused steric interference which was responsible for inhibition of haemagglutination of chicken erythrocytes [30]. The native conglutinin was shown to have haemagglutination inhibition activity due to binding to viral haemagglutinin [9]. The recombinant conglutinin with most of the collagen domain deleted was unable to form oligomers and was therefore incapable of steric interference. Another experiment showed that this recombinant conglutinin has neutralizing activity against influenza viruses (T. Kase, S. Eda, Y. Suzuki, T. Kawai, K. Ohtani, T. Sakamoto, T. Kurimura and N. Wakamiya, unpublished work). These results suggest that two patterns of haemagglutination inhibition by lectins were caused by their direct binding to haemagglutinin or/and the steric interference due to their binding to viral neuraminidase. Furthermore, we are led to wonder whether the recombinant MBP, which also lacks the collagen-like domain, has haemagglutination inhibition or neutralizing activity as well?

Taken together, these results suggest that collectins might play an important role in basic immunity against influenza viruses. Our species is very sensitive to influenza virus and has periodically suffered severely from this disease. Finally, the question remains as to why human MBP and SP-A bind to neuraminidase whereas bovine conglutinin appears to bind to haemagglutinin, even though the sugar specificities of all three are almost the same. We are now constructing other recombinant collectins lacking the collagen-like domain to elucidate the various ways in which this family of lectins can combat pathogenic micro-organisms.

We are grateful to Professors Takachika Azuma and Tsuguo Mizuochi for useful discussions and encouragement. We thank Dr. Yoshitaka Omata for kindly providing bovine plasma. This work was partially supported by the Sankyo Foundation of Life Science.

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