Cloning and Characterization of the Hakata Antigen, a Member of the Ficolin/Opsonin p35 Lectin Family*

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The Hakata antigen is a novel, thermolabile β₂-macroglycoprotein that reacts with sera from patients suffering from systemic lupus erythematosus. In this study we present the structure and the function of the Hakata antigen. We have identified cDNA clones encoding the Hakata antigen and analyzed its function. The cDNA included a possible open reading frame of 897 nucleotides, encoding 299 amino acids. The Hakata antigen consisted of a collagen-like domain in the middle section and a fibronogen-like domain in the COOH terminus, both of which are homologous to human ficolin-1 and opsonin P35, indicating that these three molecules form a distinct family. The molecular mass of the Hakata antigen expressed in transfected cells was 35 kDa under reduced conditions, and it formed ladder bands under nonreducing conditions compatible with the previous result that the Hakata antigen exists in serum as homopolymers. Purified Hakata antigen sustained lectin activity, showing affinity with N-acetylglucosamine, N-acetylgalactosamine, D-fucose as mono/oligosaccharide, and lipopolysaccharides from Salmonella typhimurium and Salmonella minnesota. These results suggest that the Hakata antigen, a new member of the ficolin/opsonin P35 family, plays a role in the serum exerting lectin activity under physiological conditions.

Inaba and Okochi (1) reported that sera from patients with systemic lupus erythematosus (SLE) contained an antibody that reacted with normal sera. The antibody was shown to react against a novel thermolabile β₂-macroglycoprotein, designated the “Hakata antigen” (2). A similar thermolabile substance had been reported by Epstein and Tan (3), but it was not known whether the two proteins are the same. The molecular mass of the Hakata antigen in serum was 650 kDa as determined by gel filtration. The antigen was thermolabile because it lost antigenicity upon heating to 56 °C for 1 min. The Hakata antigen was separated as a single band of 35 kDa by SDS-PAGE under reducing conditions. However, under nonreducing conditions it separated as ladder bands from 35 kDa to nearly the top of the gel, suggesting that the Hakata antigen exists in serum as homopolymers consisting of the 35 kDa subunit (2). All sera from 10,050 Japanese healthy blood donors, 99.99% of 751,352 Japanese patients’ sera, and 99.98% of 41,430 Swedish patients’ sera contained the Hakata antigen (4), thus implying that the Hakata antigen is a normal serum protein. The reference range of the Hakata antigen was 7–23 μg/ml (2). The antibody against the Hakata antigen was possessed by 4.3% of 349 SLE patients and 0.3% of 703 patients with other autoimmune diseases (4). Among patients with other autoimmune diseases who possessed the antibody against the Hakata antigen, one patient was found among those with chronic glomerulonephritis and another in the group with primary biliary cirrhosis.

In this study, we have cloned and characterized cDNA clones encoding the Hakata antigen revealing that the Hakata antigen is a novel serum protein that has Ca²⁺-independent lectin activity. The primary structure of the Hakata antigen is partially homologous to those of human ficolin-1 (5) and opsonin P35 (6), both of which contain collagen- and fibronogen-like domains.

MATERIALS AND METHODS

Purification of Human Hakata Antigen—The Hakata antigen was purified according to Yae et al. (2) with minor modifications as follows. In brief, Hakata antigen in serum was affinity-purified on a CNBr-activated Sepharose 4B column conjugated with a monoclonal antibody (4H5) produced against the Hakata antigen that had been purified from the propositus SLE patient serum (2). Fractions of the Hakata antigen were passed through a Hi trap Protein G column (Amersham Pharma Biotech) equilibrated with Buffer A (20 mM Tris- HCl, pH 7.5, 0.5 mM NaCl) and then applied to a Zn²⁺ column. Hakata antigen was eluted with 0–0.4 mM glycine gradient in Buffer A. Hakata antigen-rich fractions were collected and dialyzed against Buffer A. The dialyzed Hakata fraction was applied to a lentil lectin-agarose column and eluted with Buffer A, containing 0.2 mM methylmannoside. Purified Hakata antigen was dialyzed against Buffer A and its identity was confirmed by the reactivity with the autoantibody of the propositus SLE serum.

Purification of Human Hakata Antibody—Anti-Hakata human IgG was obtained from serum of the propositus patient with SLE by 35% ammonium sulfate precipitation and DEAE-Sepharose ion-exchange chromatography as described previously (2).

Preparation of Mononclonal and Polyvalent Antibodies Against the Hakata Antigen—Nonreduced, purified Hakata antigen (native form) and reduced, pyridylethylated Hakata antigen (denatured form) were used for preparation of antibodies. The antigen (200 μg) and complete Freund’s adjuvant (Difco Laboratories) were injected into BALB/c mice.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D88587.

† Dr. Yae passed away in August 1996. This paper is dedicated to Dr. Yae’s collaborative efforts in scientific research in a career spanning nearly a quarter of a century.

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‡ The abbreviations used are: SLE, systemic lupus erythematosus; TBS, Tris-buffered saline; LPS, lipopolysaccharide; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; PCR, polymerase chain reaction.
Amino Acid Sequence Analysis of Fragmented Hakata Antigen—

The amino-terminal end of the Hakata antigen was blocked, and thereafter, partial amino acid sequences of the Hakata antigen were determined after fragmentation of the antigen with CNBr or proteinases; pyridylethylated Hakata antigen was fragmented by CNBr, chymotrypsin, and amino-terminal end of the Hakata antigen was blocked, and thereafter, dylethylated according to the procedure described previously (2, 7). The 22-mer designed from the peptide sequence LGEVDHYQ in Fig. 1) and PCR (9), several oligonucleotides were synthesized as primers for the sequencing of the antigen with CNBr or proteinases; pyridylethylated Hakata antigen were sequenced on a gas chromatography—mass spectrometry system containing 0.1% trifluoroacetic acid (8). Purified peptides fragmented from pyridylethylated Hakata antigen were sequenced on a gas phase sequencer (Applied Biosystems division, model 492, Urayasu, Chiba, Japan), and the phenylthiohydantoins were identified by an Applied Biosystems 120A phenylthiohydantoin analyzer on-line system.

cDNA Cloning of the Hakata Antigen—To generate a cDNA probe by PCR (9), several oligonucleotides were synthesized as primers for the cDNA cloning of the Hakata antigen, based on analysis of the amino acid sequences of the Hakata antigen. One primer, the sense primer (a 22-mer designed from the peptide sequence LGEVDHYQ in Fig. 1) and the antisense primer (a 21-mer designed from the peptide sequence GRAYVSE in Fig. 1), was successfully used. PCR was performed using a human lung agt11 cDNA library (CLONTECH, Palo Alto, CA), and bands of the expected size were subcloned to TA cloning vector and sequenced. One of the PCR clones contained a unique sequence that encoded the partial amino acid sequence of the Hakata antigen. Using the primer 1 as a probe (probe 1), a Hakata antigen cDNA clone lacking a portion of the 3'-end (clone 11-9) was isolated from the lung agt11 cDNA library. By further screening using the cDNA clone as a probe (probe 2), two positive clones, B-1 and D-1, were obtained (Fig. 2A). These positive clones were subcloned into pBluescript II SK+ (TOYOBO Co., Osaka, Japan) and sequenced by dyeoxy method with a Thermo Sequenase kit (Amersham Pharmacia Biotech). By overlapping the sequences of these clones, a stretch of Hakata antigen cDNA was constructed. The 3'-end was determined by rapid amplification of cDNA ends (3'-RACE) using a RACE PCR kit (CLONTECH).

Transient Expression of the Hakata Antigen—A transient expression plasmid for the Hakata antigen was constructed by subcloning the Hakata cDNA insert into the pCI (Promega Corp., Madison, WI). PLC(3'-end) cells were used for transient expression according to Östman et al. (10) with minor modifications. Briefly, cells were seeded into a 15-mm culture plate at a density of 5 x 105 cells/well, and 1 μg of plasmid was transfected by the lipofection method of Tfx™-50 (Promega Corp.) on the following day. After overnight incubation, cells were washed three times with a phosphate-buffered saline (10 mM sodium phosphate, pH 7.5, 0.14 M NaCl) and incubated with Dulbecco's modified Eagle's medium supplemented without fetal calf serum for 3 more days with medium being changed everyday. The medium was then used for immunoprecipitation with the autoantibody of the propositus SLE serum.

Immunoprecipitation with Anti-Hakata Antigen Monoclonal Antibody 4H5 and Western Blot Analysis—The medium was incubated with the autoantibody of the propositus SLE serum for 1 h at 4 °C. Immunoprecipitation was performed using Protein G-Sepharose (Amersham Pharmacia Biotech) for 1 h at 4 °C, washed three times with Tris-buffered saline (TBS: 10 mM Tris·Cl, pH 7.2, 150 mM NaCl) containing 1% Triton X-100, 0.1% SDS, and 1% Trasylol. The immune complexes were eluted by boiling for 5 min in SDS-PAGE sample buffer (100 mM Tris·Cl, pH 6.5, 0.01% bromophenol blue, 38% glycerol, 4% SDS) in the presence or absence of 10 mM β-mercaptoethanol and analyzed by SDS-PAGE (10% acrylamide). After electrophoresis, the samples were transferred to polyvinylidene fluoride membrane (Millipore Corp., Bedford, MA) and probed with the rabbit polyclonal anti-Hakata antigen antibodies. After washing with TBS, the membrane was incubated with alkaline phosphatase–labeled anti-IgG and detected with an ECL chemiluminescent kit (Tropix Inc., Bedford, MA).

Hemagglutinating Activity—Fresh erythrocytes from human and sheep were washed and resuspended in TBS to yield a hemocrit of 10%. The erythrocyte suspensions were treated with trypsin (1 mg/ml) for 30 min at 37 °C and washed extensively with TBS. Hemagglutinating activity was determined in microtiter 96-well plates (U-shaped). Each well contained 25 μl of the cell suspension and 25 μl of serial dilutions of the Hakata antigen (highest concentration of Hakata antigen, 250 μg/ml) in TBS with or without 20 mM CaCl2 at a hemocrit of 1%. The inhibitory activity of lipopolysaccharides (LPSs) and mono/oligosaccharides was tested by adding the corresponding LPS and mono/oligosaccharides with serial dilutions from 0.5 μ M in a similar system. After 3 days of culture, the supernatant was collected (2H2), reacted with anti-Hakata antigen antibodies, and analyzed by ELISA. The results are shown in Fig. 2B.

RESULTS

Amino Acid Sequence Analysis of Fragmented Hakata Antigen—The amino-terminal end of the Hakata antigen was blocked, and thereafter, partial amino acid sequences of the pyridylethylated Hakata antigen were determined after fragmentation of the antigen with CNBr or proteinases. Fig. 1 shows the summary of amino acid sequences determined from the major fragmented peptides. The yield of individual peptides was 2–16% of the pyridylethylated Hakata antigen. Although it has been previously reported that the Hakata antigen contained five residues of hydroxyproline per molecule (2), peptide 3 in Fig. 1 contained six residues of hydroxyproline and also 11 GXY repeats in which X and Y residues were frequently proline and hydroxyproline residues, suggesting the collagen-like characteristics of the Hakata antigen.

cDNA Cloning of the Hakata Antigen—Based on the amino acid sequence of peptide 2 in Fig. 1, a pair of sense and anti-sense primers was designed, and PCR was performed to generate probe 1 (Fig. 2A) using a human lung cDNA library, as described under “Materials and Methods.” Primary screening

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Amino Acid Sequence Analysis of Fragmented Hakata Antigen—The summary of amino acid sequences of the purified peptides fragmented from pyridylethylated Hakata antigen is shown. The bold P represents hydroxyproline.

(1) A5KVLGDFCQGAQPCGILGKPQDGPGPCQKPDPGSRPQKPCQKLPYKRLQGQGPRNCKLPCPP
(2) RLGKEDVRQGKSESTGGLSLISGQFRTPDDDDDEDSHHCSAVIVGBAWYASCYR
(3) GRYAVSEAAWYQDGLFNASNGRIVVYPRVRWML
(4) RAGQFQGGEFPLWHNLGLQELQWQNLVELEDLWGN
(5) GRALFVFTCMDDGQWGNPLYQRQGGSDFPR
(6) TFNATYFR

Fig. 1. Amino acid sequence analysis of the fragmented Hakata antigen. The summary of amino acid sequences of the purified peptides fragmented from pyridylethylated Hakata antigen is shown. The bold P represents hydroxyproline.

Acrylamide. After electrophoresis, the samples were transferred to polyvinylidene fluoride membrane (Millipore Corp., Bedford, MA) and probed with the rabbit polyclonal anti-Hakata antigen antibodies. After washing with TBS, the membrane was incubated with alkaline phosphatase–labeled anti-IgG and detected with an ECL chemiluminescent kit (Tropix Inc., Bedford, MA).

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Electron Microscopy—Hakata antigen was diluted to 20 μg/ml in TBS containing 50% glycerol. Rotary shadowed specimens were prepared by quickly spraying the diluted antigen onto freshly cleared mica discs and shadowing with a carbon platinum at an angle of 3°. All of the specimens were examined with a Jeol 2000 EX electron microscope operated at 100 kV.
Cloning and Characterization of the Hakata Antigen

**Primary Structure of the Hakata Antigen**—Fig. 2B shows the complete nucleotide sequence and the deduced amino acid sequence of the Hakata antigen. Underlined amino acid sequences consisted of the sequences of peptides from purified Hakata antigen (listed in Fig. 1). The peptides could be aligned in order of peptide 1, 5, 4, 6, 2, and 3 (Fig. 2A). The Hakata antigen is a plasma protein (1, 2) and should have a hydrophobic signal sequence, according to the rules of von Heijne (12). A hydrophobic stretch from Met-1 to Gln-24 may be the signal sequence (Fig. 2). Cleavage at the Gln-24/Glu-25 bond with a typical motif for the recognition of signal peptidase (13) could yield a mature protein with 275 amino acids. However, the secreted Hakata antigen was blocked at the NH₂-terminal end, and thus the NH₂-terminal residue could not be confirmed by peptide sequencing. One potential N-glycosylation site was found at Asn-189 (Fig. 2). A collagen-like domain, recognized beginning at Gly-48, contained 11 G-rich repeats and six residues of hydroxyproline at positions 50, 53, 59, 65, 68, and 77 in the COOH terminus, which was homologous with human fibrinogen. A collagen-like domain, recognized beginning at Gly-48, contained 11 G-rich repeats and six residues of hydroxyproline at positions 50, 53, 59, 65, 68, and 77 in the COOH terminus, which was homologous with human fibrinogen and was recognized by C-type lectin motifs (14) in the COOH terminus. A collagen-like domain, recognized beginning at Gly-48, contained 11 G-rich repeats and six residues of hydroxyproline at positions 50, 53, 59, 65, 68, and 77 in the COOH terminus, which was homologous with human fibrinogen and was recognized by C-type lectin motifs (14) in the COOH terminus.

**Lectin Activity of the Hakata Antigen**—The Hakata antigen

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**Fig. 2. cDNA cloning of the Hakata antigen.** A, cDNA cloning strategy of the Hakata antigen is shown. The box depicts cDNA covering the whole Hakata antigen. The numbers in the box represent those of peptides from purified Hakata antigen listed in Fig. 1. The two lines of arrows denote the probes used for cDNA cloning. 11–9, B-1, and D-1 indicate the clones containing the nucleic acid sequences of the Hakata antigen. kb, kilobases. B, the first and second lines indicate the deduced amino acid sequence of the Hakata antigen and the nucleotide sequence, respectively. Small letters in the amino acid sequence depict the possible signal sequence. Underlined portions correspond with those from the purified peptides in Fig. 1. The bold N represents the possible glycosylated aspartic acid. C, schematic model of the Hakata antigen is shown.
agglutinated nontreated and trypsin-pretreated sheep erythrocytes at antigen concentrations of 125 and 15 μg/ml, respectively. However, the antigen did not agglutinate human erythrocytes at the antigen concentration of 250 μg/ml, no matter what cells were pretreated with trypsin (Fig. 5A). The agglutination activity was lost when the antigen was dissociated to monomer by reducing reagents (data not shown). The activity was resistant to heating at 56 °C for 10 min but not at 100 °C for 5 min. The agglutination activity was Ca²⁺-independent (data not shown).

The LPS binding activity of the Hakata antigen was determined by measuring its potential to agglutinate human erythrocytes coated with LPS purified from *Salmonella typhimurium*, *Salmonella minnesota*, and *Escherichia coli* (strain O111). Hakata antigen strongly agglutinated the sensitized human erythrocytes with LPS from *S. typhimurium* at a concentration of 8 μg/ml but weakly agglutinated the sensitized erythrocytes with LPS from *S. minnesota* and *E. coli* (O111) at the concentration of 125 μg/ml (Fig. 5B). Free LPS from *S. typhimurium* and *S. minnesota* inhibited the agglutination of erythrocytes coated with LPS from *S. typhimurium* at 8 and 125 μg/ml, respectively (Fig. 5C). Free LPS from a Ra mutant of *S. typhimurium* did not inhibit the agglutination of erythrocytes coated with LPS from *S. typhimurium* at as high a concentration as 500 μg/ml (Fig. 5C), suggesting that the Hakata antigen binds to the oligosaccharide chain. Mono/oligosaccharides of GalNAc, GlcNAc, and D-fucose inhibited the agglutination at 38, 9, and 2 mM, respectively (Table I). The Hakata antigen...  

**Fig. 3.** Alignment of the amino acid sequences of the Hakata antigen, opsonin P35, human ficolin-1, fibrinogen β, and fibrinogen γ. The amino acid sequences of the Hakata antigen, opsonin P35, human ficolin-1, fibrinogen β, and fibrinogen γ are shown. The bold letters represent conserved amino acids.  

**Fig. 4.** Expression of the Hakata antigen in transfected cells. The pCIneo expression plasmid only (lane 1) or one containing the cDNA of the Hakata antigen (lanes 2–4, 6) was transfected to PLC cells. In lanes 5 and 7, human serum was applied. The cell lysate (lanes 1, 2, 6) or either the supernatant (lane 3) or the precipitates (lane 4) by anti-Hakata antigen antibody, respectively, were applied to SDS-PAGE under nonreducing (lanes 1–5) or reducing (lanes 6 and 7) conditions.

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antigen bound to GalNAc- and GlcNAc-agarose columns was eluted with 0.5 mM GalNAc and 0.5 mM GlcNAc, respectively, whereas neither mannose-agarose nor lactose-agarose sustained binding to the Hakata antigen (Fig. 6).

Binding Activity of the Hakata Antigen to Molecules Bound by Collectins and Ficolins—We examined binding activity of the Hakata antigen to elastin, fibronectin, and zymosan, which had been reported to bind collectins and ficolins (14–16). The Hakata antigen did not bind to any of these proteins (data not shown).

Electron Microscopy of the Hakata Antigen—Electron microscopy of rotary shadowed Hakata antigen is shown in Fig. 7. The rough image of the Hakata antigen resembled the electron micrographs of ficolin, having globular domains on the ends of thin rods (17). In the electron micrographs, we could visualize two configurations of octadecamer, Form 1 and Form 2, as indicated in Fig. 8. Interpretive drawings of the images are given in Fig. 8.

**DISCUSSION**

The Hakata antigen is a novel, thermolabile β2-macroglycoprotein that reacts with sera from patients suffering from SLE (1, 2). The Hakata antigen is a normal serum protein with concentrations of 7–23 μg/ml. Transient Hakata antigen deficiency was found in 15 patients suffering from autoimmune diseases including SLE (4). The serum concentration of the Hakata antigen correlated with those of albumin and cholinesterase among chronic liver diseases, and the serum concentration decreased with increasing severity of cirrhosis, suggesting that hepatocytes produce the Hakata antigen (18).

The Hakata antigen was cloned and characterized. The amino acid sequences determined from purified Hakata antigen were completely adjusted to the deduced amino acid sequence from cloned cDNA (Figs. 1 and 2). The cDNA included 1592 nucleotides with a possible open reading frame of 897 nucleotides (Fig. 2). The molecular mass of the Hakata antigen in human serum was determined to be 35 kDa on SDS-PAGE analysis under reduced conditions, but under nonreducing conditions it was determined to be 650 and 520 kDa by Sepharose 4B gel filtration and analytical ultracentrifugation, respectively (2), indicating that Hakata antigen in serum is a homopolymer of the 35 kDa-subunit. The transfected product of a cloned cDNA in PLC cells reacted with the human anti-Hakata antibody obtained from serum of the propositus patient with SLE (Fig. 3) and formed ladder bands from the gel top to the position of 35 kDa molecular mass under nonreducing conditions, whereas the ladder bands migrated to a single band of 35 kDa under reducing conditions on SDS-PAGE (Fig. 3). This indicates that the cloned cDNA codes the Hakata antigen a novel thermolabile β2-macroglycoprotein.

Collectins are a group of oligomeric Ca$^{2+}$-dependent (C-type) animal lectins consisting of three domains: an NH$_2$-terminal domain containing cysteine residues, a collagen-like domain containing Gly-X-Y triplet repeats, and a COOH-terminal domain containing a carbohydrate recognition domain (19–22). Mannose-binding protein, conglutinin, and pulmonary surfactant proteins (SP-A and SP-D) are included in the collectin group. Collectins bound to carbohydrates with the COOH-terminal domain in a Ca$^{2+}$-dependent manner (23–25). Human opsonin P35 is also an oligomeric serum lectin containing a collagenous domain similar to collectins. However, the COOH-terminal region of opsonin P35 contains a fibrinogen-like domain instead of a carbohydrate recognition domain, although opsonin P35 has Ca$^{2+}$-dependent and GlcNAc-binding lectin activity (6). The overall sequence of opsonin P35 is highly homologous to porcine ficolin and human ficolin-1, which binds to transforming growth factor-β1 and elastin (5, 6, 15, 26). The Hakata antigen was homologous to human ficolin-1 and opsonin P35 and also contained three structural domains consisting of an NH$_2$-terminal domain, a collagen-like domain, and a fibrinogen-like domain (Fig. 2), although the homology was not so high as that between human ficolin-1 and opsonin P35 (Fig.

![Fig. 6. Binding activity of the Hakata antigen with mono/oligosaccharide.](Image)

**TABLE I**

| Mono/Oligosaccharide | mM |
|----------------------|----|
| d-Galactose          | >300 |
| GalNAc               | 38  |
| d-Glucose            | >300 |
| GlcNAc               | 9   |
| d-Fucose             | 2   |
| L-Fucose             | >300 |
| Mannose              | >300 |
| Lactose              | >300 |
| Maltose              | >300 |
3. The Hakata antigen shows 48% homology to human ficolin-1 and opsonin P35, suggesting that the antigen should have lectin activity. Actually, the antigen had lectin activity binding to GalNAc, GlcNAc, D-fucose, and LPSs from S. typhimurium and S. minnesota. The oligosaccharide chain of S. typhimurium, which is completely lacking in the Ra mutant, is composed of a trisaccharide repeating unit containing one residue each of D-mannose, D-galactose, and L-rhamnose, and a monosaccharide side chain, which is a residue of 3,6-dideoxy-\(\beta\)-xylohexose (abequose), 3,6-Dideoxy-\(\beta\)-xylohexose, whose structure is similar to \(\beta\)-fucose, would be important for binding of the Hakata antigen to the oligosaccharide chain of S. typhimurium. Unlike opsonin P35 (6), the lectin activity of the Hakata antigen was Ca\(^{2+}\)-independent, similar to ficolin (17). Lectin activity of the Hakata antigen was retained by 56 °C up to 10 min, although antigenicity of that is lost by heating for 56 °C for 1 min. The results indicate that Hakata antigen, human ficolin-1, and opsonin P35 shared common features of domain structure, polymerization tendency, images of electron micrographs, and lectin activities, and the primary structures indicate that the Hakata antigen forms a family with human ficolin-1 and opsonin P35.

Based on electronmicroscopy findings and on the analogy of the ficolin molecule (17), we propose that the Hakata antigen is an octadecamer consisting of an elementary trimer unit (Fig. 8). The elementary trimer unit has three fibrinogen-like globular domains connected to a collagen-like domain that terminate in a small, "triglobular" clustered NH\(_2\)-terminal domain. Given that each monomer has an approximate molecular mass of 35 kDa, the trimer represents a molecular mass of 105 kDa. Because the Hakata antigen has a molecular mass of roughly 650 kDa in serum, we could visualize two sets composed of three units of each trimer coalescing to form an octadecamiceric structure (18 monomers) with two clusters of the amino-terminal ends remaining free (Form 1 in Fig. 8). Alternatively, we could also visualize the amino-terminal clusters of the octadecamer coalescing as depicted in Form 2 (Fig. 8). The dimensions of the octadecamer Form 1 are 40 nm in height with a width of 54 nm. Form 2, although having the same dimensions for height as in Form 1, has a reduced width of 33 nm because of the coalescing of the two amino-terminal clusters. Evidence that the interaction of the octadecamer with a molecular mass of 650 kDa was because of the sulphydryl groups comes from the appearance of a single band corresponding to 35 kDa in SDS-PAGE under reducing conditions (Fig. 3) (2).

A histochemical study and the lower serum level in cirrhotic patients (18) indicated that the major production site in vivo was the liver. Although the fraction of SLE patients was small (4.3%), the Hakata antigen disappeared from the patients’ sera, and the antibody against the Hakata antigen was produced (4). Furthermore, a dysfunctional mannose-binding protein allele was a risk factor for developing SLE (27). The physiological and pathological significance of ficolin/opsonin P35 and collectins remains to be determined.

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