Ultrastructure and composition of bovine conglutinin

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Conglutinin binds in a Ca^{2+} -dependent manner to the carbohydrate portion of zymosan and cell-bound iC3b (complement subcomponent C3b cleaved by Factor I in the presence of factor H) similarly to lectin-like proteins that participate in the clearance of plasma glycoproteins. This carbohydrate-binding protein has been found to include both collagenous and non-collagenous domains. Electron micrographs of bovine conglutinin are presented in which conglutinin appears as a tetramer of four 'lollipop' structures emanating from a central hub. The stem region, linking each head to the central hub, is quite stiff, whereas the hub–stem junction is a flexible hinge. From electron micrographs of a pepsin digest of conglutinin, the linkage region is identified as the collagenous portion of the macromolecule. Conglutinin is a multimer of a single polypeptide chain. From sedimentation equilibria of unreduced as compared with reduced and alkylated conglutinin, there are determined to be three disulphide-linked chains. These data, combined with information on the subunit polypeptide of conglutinin, suggest a model for conglutinin in which four disulphide-linked trimers are associated via the *N*-termini to form the intact macromolecule as viewed in the electron microscope. The ultrastructure of conglutinin appears ideally suited to its lectin-like function.

INTRODUCTION

Conglutinin is the component of bovine serum (first described by Bordet & Gay, 1906) that causes the agglutination of erythrocytes that have reacted with antibody and complement. Subsequent studies of the agglutination mechanism showed that complement component C3b is deposited on the erythrocyte surface as a result of complement activation and cleaved by factor I in the presence of factor H to iC3b. This surface iC3b is then bound by conglutinin (Lachmann, 1967; Lachmann & Muller-Eberhard, 1968; Linscott *et al.*, 1978). The conglutinin-binding site on iC3b is located on the carbohydrate-containing portion of the iC3b M_r -68000 α -chain-derived fragment and the binding between iC3b and conglutinin is Ca^{2+} -dependent (Hirani *et al.*, 1985).

The binding properties of conglutinin are similar to those of the CR3 receptor on phagocytes, which also displays Ca^{2+} -dependent binding to iC3b (Ross & Lambris, 1982). Conglutinin shares the property of specificity of carbohydrate binding with a number of lectin-like proteins which participate in the clearance of glycoproteins (Kozutsumi *et al.*, 1980; Mizuno *et al.*, 1980; Stahl *et al.*, 1980; Townsend & Stahl, 1981). The relationship of these binding properties to the physiological role of conglutinin is unknown at present. Although the function of conglutinin has not been defined, experiments in which this macromolecule was

passively administered to mice suggested that it conferred protection from bacterial infection and caused a decrease in the number of invading organisms (Ingram, 1959*a, b*). These results are consistent with the possibility that conglutinin plays a role in intravascular-clearance mechanisms.

Intact conglutinin is a macromolecule of approximately 750 kDa with a frictional-coefficient ratio (f/f_0) of 4 (Lachmann & Richards, 1964). Recently it has been shown that the basic subunit of conglutinin is a single polypeptide chain that is organized into three domains: two non-collagenous domains separated by a collagen-like region (Davis & Lachmann, 1984). Other than the collagens themselves, only conglutinin, Clq (Brodsky-Doyle *et al.*, 1976) and acetylcholinesterase (Rosenberry *et al.*, 1976) are known to contain interspersed collagenous and non-collagenous regions.

In an early electron-microscopic study, conglutinin appeared as a rod-like molecule (Lachmann & Coombs, 1965). On the basis of our current understanding of conglutinin structure, we re-examined this protein by electron microscopy and found the ultrastructure to be quite different than that previously reported.

Here we present electron micrographs of conglutinin and its pepsin fragment. From these electron micrographs and accompanying hydrodynamic data, a model has been deduced for the arrangement of polypeptide chains in conglutinin.

Abbreviations used: C3, complement component C3; C3b, C3 cleaved by C3 convertase; iC3b, C3b cleaved by factor I in the presence of factor H; DTT, dithiothreitol.

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EXPERIMENTAL PROCEDURES

Conglutinin isolation and digestion

Conglutinin was isolated from bovine plasma as previously described (Lachmann, 1967). Pepsin digestion of conglutinin was performed as previously described (Davis & Lachmann, 1984). Fig. 1 shows an SDS/polyacrylamide-gel electrophoresis profile of isolated conglutinin and of its principal pepsin fragment. The electrophoretic mobility of the pepsin fragment is unchanged in the absence of reducing agent (results not shown). SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1971).

Fig. 1. SDS/polyacrylamide-gel electrophoresis of isolated conglutinin (lane 2) and the resultant pepsin fragment (lane 1) under reducing conditions

The gel was 10% (w/v) acrylamide; the acrylamide/bis-acrylamide ratio was 37.5, and the gel contained 3% (v/v) 2-mercaptoethanol. M_r values for standard proteins (ovalbumin, trypsinogen and lysozyme) are indicated on the left.

Electron microscopy

Approx. 0.1 mg/ml solutions of conglutinin were dialysed extensively against 0.15 M-ammonium acetate, pH 6.8, mixed with 0.5 vol. of glycerol, sprayed on to freshly cleaved mica, dried *in vacuo*, and rotary-shadowed with tungsten by means of electron-bombardment heating (Slayter, 1976). The estimated average metal-film mass thickness was 100 ng/cm². Samples were examined in the JEM 100cx electron microscope, using a top-entry stage voltage with a 40 μ m objective aperture, and at 100 kV acceleration voltage. Most micrographs were recorded at a magnification of 53 000 $\times$ and enlarged photographically to 160 000 $\times$. High-resolution dark-field images were also obtained, from the same lightly shadowed specimens, by using matched annular condenser and objective apertures.

Alternatively, specimens were negatively contrasted as previously described, with 1% uranyl acetate (Valentine *et al.*, 1968; Slayter, 1983). Calibration was based on micrographs of grating-replica standards and of Indanthrene Olive crystals (Labaw, 1964).

Sedimentation velocity

A model E analytical ultracentrifuge (Spinco Division, Beckman Instruments, Palo Alto, CA, U.S.A.) equipped with a u.v. photoelectrical optical system was used. The temperature was monitored throughout each run and an average of the temperatures was used for calculations.

Conglutinin was dialysed into 10 mM-Tris/HCl/0.15 M-NaCl, pH 8. Both schlieren and u.v. optics were employed to examine a range of protein concentrations from 0.5 to 3 mg/ml. The preparation was centrifuged at 52 000 rev./min in a cell equipped with a 4 $^\circ$ single-sector aluminium centrepiece. The schlieren photographic plates were read on a Gaertner Scientific Corp. (Chicago, IL, U.S.A.) microcomparator. Sedimentation coefficients were corrected for density and temperature to $s_{20,w}$ values.

Sedimentation equilibrium

For these experiments, conglutinin was dialysed into 6 M-guanidinium chloride/50 mM-Tris/HCl, pH 8. A portion of the dialysed conglutinin was then reduced and alkylated by treatment under N₂ with 20 mM-DTT for 1 h at 37 $^\circ$ C, followed by 40 mM-iodoacetamide for 30 min at room temperature. Finally, the reduced and alkylated conglutinin was dialysed against the Tris/guanidinium chloride buffer. Sedimentation-equilibrium centrifugation was performed at two rotor speeds for each conglutinin sample to assess homogeneity. A 12 mm double-sector aluminum centrepiece was used; 0.1 ml of protein solution was injected into the solution side and 0.15 ml of the Tris/guanidinium chloride dialysis diffusate was injected into the solvent side. Concentration gradients were monitored at 280 nm with u.v. optics. M_r values were calculated by a standard procedure (Tanford, 1961).

RESULTS

Electron microscopy

Electron micrographs of native conglutinin were obtained from both negatively contrasted and tungsten-replica preparations. A field of negatively stained conglutinin particles is shown in Fig. 2A.

There is clearly a discrete structure composed of four

Fig. 2. Electron micrographs of native conglutinin

A, Negatively contrasted field stained with 1% uranyl acetate (magnification $98\,550\times$); B, gallery of selected negatively stained conglutinin particles (magnification $187\,245\times$); C, gallery of selected tungsten-shadowed conglutinin particles (magnification $126\,000\times$). Arrows indicate selected views where head regions appear to be comprised of several subdomains, frequently three.

relatively stiff strands approx. 2 nm wide by 40 nm in length, each connecting at a small central hub of about 11 nm in diameter. Peripherally the strands emanating from the central hub terminate in a globular head of variable configuration with a diameter somewhat in excess of 10 nm. The maximum extension across a fully spread particle from the outside edge of one head to the outside edge of the opposite head, measured from negatively stained preparations, is about 90 nm. Electron micrographs of conglutinin prepared by the mica-replica technique, using a tungsten-metal coating of approx. 100 ng/cm² mass thickness, are shown as a gallery in Fig. 2B. Details of the molecule can be seen in the enlarged photographs of Fig. 2C. The connecting linkage is a relatively stiff structure, reminiscent of the tail of myosin or of tropollagen. Also, the head region can often be resolved into two, or even three, domains (see arrows in Fig. 2C). The detailed resolution of the molecule is expected to be better in the negatively stained particles than in the metal-replica specimens. Nevertheless, in photographs of conglutinin, it is possible to resolve subdomains in the head regions of the metal-coated particles more easily than in the negatively stained molecules.

Fig. 3 presents histograms of measured arm length, head width, central-lobe width and maximum extension across conglutinin molecules prepared by the negative-contrast method. The sum of twice the peak arm length plus twice the peak head width, plus peak width of the central knot, equals 115 nm. This value should equal the maximum observed extension across the entire molecule, which is, in fact, somewhat less than 95 nm (Fig. 3). The reason that the measured maximum extension is less than the sum of measurements for each component part is that the arms of the molecules lie in a variety of non-linear

combinations. The four arms may be equally spaced around the hub, three may be grouped on a side opposite the fourth, or an intermediate arrangement may occur. The peak values in the distributions in Fig. 3 are considered the values of merit to be used in describing the structure. In most cases in the present study the distributions were approximately Gaussian, so that peak and simple-average values were close.

Fig. 4 shows histograms of similar measurements made from tungsten-shadowed preparations of conglutinin. In these preparations, the sum of two times the peak arm length, plus two times the peak head width, plus the peak width of the central knot, is 130 nm, a value which again is larger than the maximum extension observed in Fig. 4.

The dimensions of most conglutinin parameters as obtained from either negatively stained or metal-replica preparations are similar. The length of the connecting strand obtained from metal-replica measurement is only 4% greater than the peak length, 41 nm, measured from negative-contrast preparations. The size of the central lobe as obtained from the metal replica is slightly smaller than the value obtained from the negatively stained preparation. The maximum extension across the molecule is about 18% greater in the case of the metal-replica measurements.

When measurements of the head region are compared, the diameter of the head is larger in the tungsten-shadowed photographs than in negatively stained electron micrographs (13 as against 11 nm). The difference may be created by the revealment process employed: metal coating tends to exaggerate size, owing to the added metal cap, whereas the negative stain penetrates structures, reducing the apparent dimensions somewhat. A difference in head size of 1 nm must be partially explained by stain penetration, since the amount of metal coating used here

Fig. 3. Histograms of measured size parameters of conglutinin obtained from negatively contrasted particles

The sketch in (a) indicates the parameters measured: a, arm length; b, head width; c, width of central lobe; d, maximum extension across a single particle.

Fig. 4. Histograms of measured size parameters of conglutinin obtained from tungsten-shadowed preparations
Measured parameters are the same as those in Fig. 3.

Fig. 5. Electron micrographs of modified conglutinin

Field of 'collagen-like' segments produced by pepsin degradation, shadow-cast with tungsten (magnification 180000 $\times$).

Table 1. Dimensions from electron microscopy (nm)

Key to abbreviations and symbols: *a*, arm length; *b*, head width; *c*, width of central lobe; *d*, maximum extension (see sketch in Fig. 3(a)). Pk, peak; Av, average; W shadow/DF, tungsten shadowing/ dark-field micrographs. For entire distribution of data, see Fig. 3 (negative stain), Fig. 4 (W shadow/DF) and Fig. 6 (W shadow/DF of pepsin digest).

Technique	<i>a</i>		<i>b</i>		<i>c</i>		<i>d</i>	
	Pk	Av	Pk	Av	Pk	Av	Pk	Av
Negative staining	41.0	37.9	11.0	10.6	11.0	11.4	92.0	95.2
W shadow/DF	40.8	39.3	13.3	12.9	10.4	11.0	108.3	105.2
W shadow/DF of pepsin digest*	35.2	36.4	—	—	—	—	—	—

* Segment length as measured from micrographs of pepsin digest is about 12% less than dimension *a*, the connecting-arm length.

is estimated to add less than 1 nm of thickness (Slayter, 1976, 1983).

When made visible by metal coating, the high- M_r residual fragment of conglutinin isolated after limited pepsin proteolysis is revealed as rather homogenous and rod-like (Fig. 5). The peak of the distribution of rod lengths was found to be $35 \text{ nm} \pm 8$. It is noteworthy that this length is only slightly shorter than that of the connecting segment found in intact conglutinin, suggesting that these uniform rods correspond to that portion of the native molecule.

The measured dimensions for intact conglutinin and the pepsin fragment of conglutinin are summarized in Table 1.

Sedimentation velocity

In a sedimentation-velocity study of conglutinin, sedimentation coefficients for this molecule were determined for the range of concentrations described in the Experimental procedures section. At the protein concentrations studied, sedimentation values displayed a high concentration-dependence; values ranged from 7.5 S at 0.6 mg/ml to 4.8 S at 3.1 mg/ml. Each sedimentation coefficient was corrected to an $s_{20,w}$ value and plotted against concentration (Fig. 6a).

The extrapolated y-intercept of this plot, the value for $s_{20,w}^0$, was 8.0S. At physiological ionic strength and pH, conglutinin sedimented as a single sharp peak, and thus appeared not to be a complex of associating and dissociating subunits. This is suggested also by the negative slope in the plot of sedimentation coefficient versus concentration. A very rapidly sedimenting peak, often present in the earliest scans during a run, was most likely composed of very large conglutinin aggregates.

Sedimentation equilibrium

The conglutinin polypeptide has an apparent M_r 48 000 on SDS/polyacrylamide-gel electrophoresis. Sedimentation equilibrium was performed on the molecule to determine the absolute molecular mass (M) of the subunit polypeptide. Also, the number of covalently linked peptide chains within the macromolecule was determined from the ratio of the M values of unreduced and reduced and alkylated conglutinin in 6 M-guanidinium chloride. Representative equilibrium data are displayed in Fig. 6(b). The M of each conglutinin sample was determined at two rotor speeds to ensure M homogeneity within each sample. A summary of the results is shown in

Fig. 6. Sedimentation of conglutinin

(a) Sedimentation coefficient of intact conglutinin as a function of protein concentration (*c*). (b). Sedimentation equilibrium of conglutinin in 6 M-guanidinium chloride. The sedimentation equilibrium of conglutinin under dissociating conditions before and after reduction and alkylation. Representative data are shown for conglutinin, unreduced or reduced and alkylated in 50 mM-Tris/HCl (pH 8)/6 M-guanidinium chloride. r^2 is the square of the distance from the centre of rotation. ●, Reduced and alkylated conglutinin at 12000 rev./min; ○, unreduced conglutinin at 10000 rev./min.

Table 2. Sedimentation-equilibrium-derived molecular masses of conglutinin in 6 M-guanidinium chloride

10 ⁻³ × Rotor speed (rev./min)	Molecular mass (kDa)	
	Unreduced (U)	Reduced and alkylated (R + A)
10	102.3	
12	126	
14		35.3
14		32.95
16		31
Average ± s.d.	114.15 ± 11.9	33.1 ± 1.8
Ratio U/R + A . . .	3.4 ± 0.7	

Table 2. If a sample were heterogenous with respect to M , one would expect a higher M estimate at the lower rotor speed; for both conglutinin samples, this was not the case. The ratio of these two values (unreduced over reduced and alkylated) should yield the number of covalently linked polypeptide chains in conglutinin: the value of this ratio is 3.4 ± 0.7 .

DISCUSSION

Ultrastructure of conglutinin

In the present study the $s_{20,w}^0$ for conglutinin was found to be 8.0 S, similar to the value of 7.8 S reported previously (Lachmann & Richards, 1964). However, the ultrastructure of this protein is strikingly different than previously reported (Lachmann & Coombs, 1965). Conglutinin, as shown in electron micrographs, is clearly a tetramer composed of four 'lollipop'-shaped structures radiating from a central hub. The overall configuration of the molecule was the same in electron micrographs prepared by negative staining and by the tungsten-replica method. Dimensions of the linkage region and central hub domains of conglutinin were approximately the same for both preparations, but the diameter of the head region was smaller in the negatively stained specimens. Unexpectedly, that region is less well-resolved in the photographs of negatively stained conglutinin. These observations may be explained by stain penetration into peripheral regions, obscuring structure at the fringe of the head region and thus yielding the apparently smaller diameter.

The pepsin fragment of conglutinin is predominantly collagen-like, as suggested by the amino acid composition (Davis & Lachmann, 1984) and substantiated by sequence analysis (A. E. Davis III, unpublished work). Electron microscopy of the collagen-like domain isolated from the pepsin digest of conglutinin yielded dimensions of 35 nm × 2 nm. Similar values for the connecting arm regions of conglutinin (41 nm × 2 nm) suggest that the high- apparent- M_r pepsin fragment is derived from that portion of the molecule. The width of this rod also agrees with reported electron-micrographic dimensions for the collagen triple helix (Traub & Piez, 1971), suggesting that this region is composed of a single collagen triple helix.

Intact conglutinin exhibits a strongly negative c.d. spectrum at 202 nm, suggesting that a portion of the protein exists in the conformation characteristic of collagenous triple helices (C. J. Strang & P. V. Hauschka,

unpublished work). The magnitude of the observed minimum (-6200 degrees·cm²·dmol⁻¹ at 202 nm) is strikingly similar to the magnitude of the corresponding trough in the c.d. spectrum reported for C1q (-5800 degrees·cm²·dmol⁻¹ at 200 nm) (Brodsky-Doyle *et al.*, 1976), suggesting that these two proteins may contain similar percentages of triple helix.

Although the arms of the conglutinin are quite stiff, there appears to be a flexible region at the hub-arm junction, since the relative two-dimensional angle between a pair of arms varies from 20° to 180°. This flexibility provides conglutinin with an obvious advantage in binding multiple groups spaced at variable intervals on a cell surface or a complex.

Subunit composition of conglutinin

The intact conglutinin molecule seen in the electron micrographs is a multimer of the polypeptide chain characterized previously (Davis & Lachmann, 1984). The number of disulphide-linked polypeptide chains in conglutinin was estimated by sedimentation equilibrium, in 6 M-guanidinium chloride, of unreduced conglutinin as compared with reduced and alkylated conglutinin. It was necessary to use denaturing conditions to disrupt any non-covalent interactions and to prevent partial association of the individual reduced and alkylated polypeptide chains. The M of unreduced conglutinin in 6 M-guanidinium chloride was found to be 114150 ± 11900 . The M of the conglutinin polypeptide as determined by sedimentation equilibrium of reduced and alkylated conglutinin in 6 M-guanidinium chloride was found to be 33100 ± 1800 . This is similar to the results from SDS/polyacrylamide-gel electrophoresis (which gives only an estimate of M_r), when allowance is made for the collagen-like nature of a significant portion of the polypeptide. Owing to their large glycine content, collagenous proteins have a lower average molecular mass per residue than globular proteins (Furthmayer & Timpl, 1971). The ratio of M_r of unreduced conglutinin to that of reduced and alkylated conglutinin is then 3.4 ± 0.7 , suggesting that three polypeptides are disulphide-linked to form a subunit of conglutinin.

These results are in contrast with the results from SDS/polyacrylamide-gel electrophoresis, in which the unreduced conglutinin migrated with a mobility similar to that of a cross-linked hexamer of the conglutinin polypeptide, suggesting that six chains are disulphide-linked (Davis & Lachmann, 1984). To compare the behaviour of conglutinin in these two solutions, gel filtration of conglutinin was performed in 50 mM-Tris/HCl, pH 8, with either 6 M-guanidinium chloride or 0.2% SDS. For comparison, standard proteins in the appropriate denaturant were employed. In the 6 M-guanidinium chloride buffer, conglutinin had an M_r of 108000, when compared with IgG, bovine serum albumin, C3 and chymotrypsinogen standards, whereas, in the SDS solution, conglutinin had an M_r of more than 1 million when compared with the same standards and with IgM and thyroglobulin. It appears then that conglutinin forms non-covalently associated aggregates in SDS, and that results obtained in SDS do not give a true value for the smallest covalently linked multimer of the conglutinin polypeptide.

The molecular-mass values for the domains of the conglutinin polypeptide taken from a previous work add

up to an apparent M_r 43 000 (Davis & Lachmann, 1984). The collagenous domain is, in fact, somewhat smaller, owing to the high mass percentage of glycine (Furthmayer & Timpl, 1971) in this fragment. Also, it is likely that the molecular mass of a collagen 'tail' is added into the SDS/polyacrylamide-gel electrophoresis molecular mass of the C-terminal domain, since hydroxyproline and hydroxylysine are still present in hydrolysates of this fragment. The small N-terminal domain is 2.7 kDa as determined from amino-acid-sequence analysis. We believe the subunit polypeptide to be 3.3 kDa as determined from sedimentation-equilibrium data, and from this value we estimate the collagen domain to be approx. 12 kDa and the C-terminal domain to be approx. 18 kDa in size.

Assembly of conglutinin

The covalently linked subunit of conglutinin must then be assembled into three domains, as described in Fig. 7: an N-terminal domain of 3×3 kDa, a collagenous domain of 3×12 kDa and a C-terminal globular domain of 3×18 kDa. Four of these subunits are then associated via the N-terminal regions to form the intact conglutinin molecule, as shown in the electron micrograph and diagrammatically depicted in Fig. 7. The cysteine residues that participate in interchain disulphide bonds are most likely located within, or near, the C-terminal globular portion of the polypeptide. Although amino-acid-composition data from hydrolysis of the pepsin fragment of conglutinin suggest that one or two cysteine residues are located within the collagen-like region (Davis & Lachmann, 1984), this fragment exhibited the same mobility on SDS/polyacrylamide-gel electrophoresis in the unreduced or reduced state. Therefore it must be concluded that cysteine residues within the pepsin fragment do not participate in intermolecular cross-linking. There are no cysteine residues in the N-terminal region. We previously reported that residue no. 5 from the N-terminus was cysteine (Davis & Lachmann, 1984), whereas this residue is in fact threonine, as has been determined in four subsequent sequencing runs. On our h.p.l.c. program, a phenylthiohydantoin-threonine degradation product is eluted coincidentally with the phenylthiohydantoin derivative of carboxymethylcysteine O-methyl ester. In the later sequencing runs, particular care was taken in handling this residue. The derivatization reaction was done immediately after peptide-bond cleavage and only phenylthiohydantoin-threonine was identified. In addition, threonine was identified by amino acid analysis after backhydrolysis, and no carboxymethylcysteine was present. Thus the C-terminal globular portion of the polypeptide chain must be the locus of the interchain disulphide linkages. For this to be the case, all three chains of the conglutinin polypeptide trimer must be aligned in a parallel N-C-arrangement, a condition that is consistent with the relative arrangement of polypeptides in all known collagenous triple helices (Prockop *et al.*, 1979).

It is possible that an intermolecular disulphide-bonded segment from the N-terminal or C-terminal portion of the collagenous region is missing in the pepsin fragment. However, such a linkage would not affect the proposed architecture of the intact macromolecule. It would be predicted that the macromolecule of 12 chains we have described has M_r of 397 000. This value is substantially different than the 750 000 reported previously (Lachmann

Fig. 7. Structural composition of bovine conglutinin

A plausible model for the number and organization of polypeptide chains in intact conglutinin is shown. We suggest that three chains are disulphide-linked to one another in the C-terminal, non-collagenous regions of the chains, and that this trimer is folded as described to form a monomer subunit. Four of these subunits then associate in a non-covalent fashion via the N-termini to form the intact conglutinin seen in electron micrographs.

& Coombs, 1965) and probably reflects the different methods of measurement. In the previous study, the sedimentation and diffusion coefficients for conglutinin were determined under separate experimental conditions, whereas in the present study, where sedimentation equilibrium was used, the two measurements were determined under identical conditions, thereby increasing the precision of the measurement.

The structure and composition of conglutinin most closely resembles the complement sub-component C1q, which, like conglutinin, is a collagen-like serum protein. Electron micrographs of C1q give the appearance of a bunch of tulips; this macromolecule is composed of six peripheral globular portions connected to a central fibril-connecting strand (Shelton *et al.*, 1972; Knobel *et al.*, 1975). The connecting strands and the central fibril comprise the collagen-like region of C1q, whereas the peripheral globular portions contains the C-terminal non-collagen-like regions (Brodsky-Doyle *et al.*, 1976;

Reid *et al.*, 1972). Thus C1q and conglutinin are somewhat similar in overall appearance and composition, except for the manner in which the strands are associated, since in conglutinin there is no association of multiple collagenous helices.

The location of the iC3b-binding site on conglutinin is not known, but multiple iC3b-binding sites must exist, since iC3b-coated erythrocyte are agglutinated by conglutinin. A valence of four, or multiple of four, would be predicted on the basis of the ultrastructure of conglutinin. An analogous numerical relationship has been demonstrated for C1q binding to IgG. IgG binds to C1q through the peripheral globular domains (Reid *et al.*, 1977) and 12 or 18 molecules of IgG are bound per C1q molecule (Schumaker *et al.*, 1976). The ultrastructure of conglutinin is also reminiscent of several other binding proteins; for example, thrombospondin (Coligan & Slayter, 1984), clathrin (Ungewickell & Branton, 1981; Crowther & Pearse, 1981; Harrison & Kirchhausen, 1983; Kirchhausen & Harrison, 1984), C4b-binding protein (Dahlback *et al.*, 1983) and laminin (Timpl & Martin, 1982; Odermatt *et al.*, 1981). Although each of these proteins is made up of multiple arm-like extensions from a central area, none are known to contain collagen-like regions.

The ultrastructure of conglutinin appears ideally suited for the function of this macromolecule as a binding protein. Recent binding studies with conglutinin and C3 fragments (Hirani *et al.*, 1985) show that, when immobilized on a plastic surface, conglutinin binds in a Ca^{2+} -dependent manner to the carbohydrate portion of C3b, iC3b and C3c. When C3 fragments are immobilized on the erythrocyte surface, conglutinin only binds iC3b, possibly because the carbohydrate on other bound C3 fragments is not accessible. The binding properties of conglutinin are similar to those of other lectin-like proteins that participate in the clearance of plasma glycoproteins. Conglutinin links C3 fragments, perhaps acting as a soluble receptor by also bridging C3 fragments to a second macromolecule in a manner analogous to the linkage made by C1q between the antibody Fc domain and the C1r and C1s zymogen components of the C1 complex (Strang *et al.*, 1982; Poon *et al.*, 1983). Further investigations will undoubtedly be directed at the nature of the binding of conglutinin to cells and to other plasma proteins.

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