Comparative study of the structural and functional properties of a bovine plasma C-type lectin, collectin-43, with other collectins

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*Department of Medical Microbiology, Institute of Medical Biology, University of Odense, DK-5000 Odense C, Denmark, †Department of Immunology, Institute of Medical Microbiology, University of Aarhus, DK-8000 Aarhus C, Denmark, ‡MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K., and §Max-Planck-Institut für Biochemie, Martinsried, Federal Republic of Germany

Collectin-43 (CL-43) is a recently described bovine plasma protein containing both collagenous regions and C-type-lectin domains [Holmskov, Teisner, Willis, Reid and Jensenius (1993) *J. Biol. Chem.* **268**, 10120–10125; Lim, Willis, Reid, Lu, Laursen, Jensenius and Holmskov (1994) *J. Biol. Chem.* **269**, 11820–11824]. CL-43 was purified by affinity chromatography on mannan–Sephacrose. On SDS/PAGE under reducing conditions the purified lectin showed a double band at about 43 kDa, with the upper band representing the intact molecule and the lower band a truncated form that lacked the N-terminal nine amino acid residues. Under non-reducing conditions, only one band was seen at 120 kDa. Analytical gel chromatography and sucrose-density-gradient centrifugation of the purified molecule, showed a Stokes radius of 9.1 ± 0.3 nm (91 ± 3 Å) and a sedimentation coefficient ($s_{20,w}$) of 3.6 ± 0.1 S. These values correspond to a molecular mass of 119–138 kDa under non-denaturing condition in solution. The frictional coefficient (f/f_0) was 2.7, indicating

extreme elongation due to the collagenous segment. Only monomer subunits, with 37.4 ± 1.7 -nm-long rods, were seen by electron microscopy. These findings indicate that CL-43, in contrast with the other circulating collectins, is found only as a single subunit composed of three polypeptide chains. Two-dimensional gel electrophoresis showed that CL-43 has two isoforms, with pI values of 4.9 and 5.3, corresponding to the native form and the truncated form of the molecule respectively. CL-43, like conglutinin, lung surfactant protein A and mannan-binding protein (MBP), was shown to bind to the collectin receptor. Bovine MBP caused the activation of the complement system as revealed by the deposition of complement component C4 upon incubation of diluted serum in wells containing MBP bound to solid-phase mannan. CL-43, lung surfactant protein D (SP-D) and conglutinin showed no complement-activating properties under the same conditions. Conglutinin binds fluid- and solid-phase iC3b, while CL-43 and MBP do not show such reactivity.

INTRODUCTION

The collectins are oligomeric lectins with subunits composed of three identical polypeptides, except for surfactant protein A (SP-A), which contains two slightly different polypeptides (Voss et al., 1991). In each collectin polypeptide chain, a short N-terminal sequence, containing two conserved cysteine residues (only one cysteine residue in the case of mature SP-A), is followed by a stretch of repeating Gly-Xaa-Yaa triplets, which allow three polypeptides to twist into the triple-helical structure characteristic of collagenous proteins. The C-terminal region contains a short neck region and the carbohydrate-recognition domain (CRD), which, in the case of mannan-binding protein (MBP), has been found to fold up into an independent globular carbohydrate-binding structure for each polypeptide (Weis et al., 1992). The subunits are linked, covalently through disulphide bonding, and non-covalently, into oligomers of up to six subunits (Holmskov et al., 1994).

Five collectins have now been identified: the pulmonary surfactant proteins SP-A and SP-D, and the plasma lectins conglutinin, MBP (also called ‘mannose-binding protein’) and the recently described collectin-43 (CL-43) (Holmskov et al., 1993a; Lim et al., 1994). The surfactant-associated collectins and MBP have been described in a variety of species, while conglutinin and CL-43, to date, have been described only for members of the Bovidae (hollow-horned ungulates).

The collectins can be divided in two groups according to their quaternary structure. The intersubunit interactions in MBP and SP-A extend through a stretch of the N-terminal section of the collagenous region giving an overall ‘bouquet’-like or certiform shape very similar to that of C1q, the first component of the classical complement pathway (Lu et al., 1990). Conglutinin and SP-D have substantially longer collagen regions, and in the electron microscope (Strang et al., 1986; Lu et al., 1993) they are viewed as cruciform, i.e. a shape like a Greek cross, having four rods of equal length. The rods appear to have some flexibility. The presence of monomers as well as dimers and trimers is also seen to a variable extent (Lu et al., 1993).

The collectins may serve important roles in the innate immune defence against micro-organisms. MBP, conglutinin, SP-A and SP-D have been shown to bind directly to carbohydrates on the surface of various micro-organisms and particles (Kuhlman et al., 1989; Hartley et al., 1992; Kuan et al., 1992; Zimmermann et al., 1992; Haurum et al., 1994), and MBP can, after binding to carbohydrate, activate the classical pathway of complement in a C1q- and antibody-independent way (Ikeda et al., 1989; Kawasaki et al., 1989; Lu et al., 1990; Matsushita and Fujita, 1992). Conglutinin can also cause opsonization by binding to iC3b on the surface of micro-organisms (Friis-Christiansen et al., 1990). The collagenous regions of the collectins are ligands for the collectin receptor on phagocytes and other cells (Malhotra et al., 1990, 1993).

Abbreviations used: SP-A, lung surfactant protein A; SP-D, lung surfactant protein D; CRD, carbohydrate-recognition domain; MBP, mannan-binding protein; CL-43, collectin-43; BK, bovine conglutinin; i.e.f., isoelectric focusing; g.p.c., gel-permeation chromatography.

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We have recently described a new collectin called CL-43 (Holmskov et al., 1993a), which, on the basis of sequence similarity, is closely related to conglutinin and SP-D (Lim et al., 1994). The present paper further describes the structure of CL-43, examines its ability to interact with the complement system and the collectin receptor, and compares the structural and functional properties of CL-43 with those of the other circulating collectins and SP-D.

MATERIALS AND METHODS

Buffers and reagents

Tris-buffered saline (TBS) contained 140 mM NaCl, 10 mM Tris/HCl and 2 mM Na₂N₃, pH 7.2. TBS/Tw was TBS containing 0.05% (v/v) Tween 20 (polyoxyethylene sorbitan monolaurate; Merck-Schuchardt, Darmstadt, Germany). Coating buffer was 0.1 M sodium carbonate/sodium bicarbonate, pH 9.6. Diethanolamine buffer was 10% (v/v) diethanolamine/HCl/7.5 mM Na₂N₃/0.5 mM MgCl₂, pH 9.8. Mannan was prepared from *Saccharomyces cerevisiae* as described by Nakajima and Ballou (1974) and coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) at a concentration of 15 mg of mannan/ml of gel. TSK-maltose gel was prepared by coupling 100 mg of maltose/ml of divinylsulphone-activated Fractogel TSK HW/75(F) (14985; Merck) according to the method of Fornstedt and Porath (1975). Rabbit anti-bovine IgG (heavy and light chains) (code Z247; Dakopatts A/S, Copenhagen, Denmark) was coupled to CNBr-activated Sepharose 4B (Pharmacia) at a concentration of 2.5 mg of IgG/ml of gel in 0.1 M citrate buffer, pH 7.5, for 2 h at 4 °C. Maltose and alkaline-phosphatase-coupled goat anti-rabbit IgG (A-8025) were purchased from Sigma. *p*-Nitrophenyl phosphate disodium salt was from Boehringer-Mannheim, Mannheim, Germany.

SDS/PAGE

Electrophoresis was performed on 4–20% (w/v) polyacrylamide gradient gel with the discontinuous buffers described by Laemmli (1970). Samples were reduced by heating at 100 °C for 3 min in 40 mM dithiothreitol/1.5% (w/v) SDS/5% (v/v) glycerol/0.1 M Tris, pH 8.0, and alkylated by the addition of iodoacetamide to a concentration of 90 mM. Non-reduced samples were heated in sample buffer with 90 mM iodoacetamide. Protein bands were detected by the silver-staining method (Merrill et al., 1982). The molecular-mass markers were those of the low-molecular-mass calibration kit from Pharmacia. The gels were dried between sheets of Cellophane (Walleik and Jensenius, 1982).

Purification of CL-43, MBP and conglutinin

The mannan-binding lectins present in bovine serum were purified by affinity chromatography on mannan-Sepharose. CL-43 was separated from conglutinin and MBP by selective elution with appropriate sugars, and finally purified using ion-exchange techniques (Holmskov et al., 1993a,b).

Purification of SP-D

Cow lungs were lavaged with approx. 2 l of isotonic NaCl. The lavage was then adjusted to 5 mM CaCl₂/10 mM Tris/5 mM iodoacetamide/5 mM *trans*-4-(aminomethyl)cyclohexanecarboxylic acid (A-6516; Sigma) and 1 µg/ml aprotinin (981 532; Boehringer-Mannheim) (column buffer) and centrifuged at 10000 *g* for 30 min at 4 °C. The supernatant was passed through a 50 ml maltose-TSK column. After washing with column buffer

containing 0.05% (v/v) Emulphogene (BC 720; Sigma), the column was eluted with the same buffer containing 100 mM maltose. Fractions containing SP-D (detected by SDS/PAGE) were pooled and passed through a column of 10 ml of rabbit anti-bovine IgG-Sepharose. The effluent from this column was concentrated by vacuum dialysis and passed through a Superose 6 FPLC column (Pharmacia) equilibrated with TBS containing 10 mM EDTA and 0.05% (v/v) Emulphogene. Fractions containing SP-D (detected by SDS/PAGE) were pooled, and the protein concentration was estimated as described for CL-43 and conglutinin. Approx. 500 µg of SP-D was obtained/litre of lavage.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis was carried out as previous described (O'Farrell, 1975), and the gels were silver-stained. Cells were biosynthetically labelled with [³⁵S]methionine. The major proteins in the cell lysate have previously been compared proteins of known molecular masses and pI values and these were used as internal standards (Madsen et al., 1992). Briefly, the first-dimension run was isoelectric focusing (i.e.f.) with pre-electrophoresis (15 min at 200 V, 30 min at 300 V, then 1 h at 400 V) followed by i.e.f. (18 h at 400 V) in 130 mm × 1.2 mm 4% (w/v) polyacrylamide gels containing 2% ampholines [1.5%, pH 5–7 (Serva); 0.5%, pH 3.5–10 (Pharmacia)]. Thereafter second-dimension SDS/PAGE (15% running gel; 5% stacking gel) was carried out as described by Laemmli (1970). Following preparations for fluorography (Laskey and Mills, 1975), the dried gels were exposed at –70 °C to preflashed Kodak X-Omat films.

Rocket immunoelectrophoresis

Antiserum was raised against CL-43 in rabbits and analysed by crossed immunoelectrophoresis and Western blotting as described by Holmskov et al., (1993a). Rocket immunoelectrophoresis (Laurell, 1966) was used to quantify CL-43 in fractions of serum separated on Superose 6. The electrophoresis was run at 2.5 V/cm for 18 h on 1.5-mm-thick 1% (w/v) agarose gel (Litex HSA; Litex Industry, Copenhagen, Denmark) containing anti-CL-43 antiserum at a level of 15 µl/cm².

Gel-permeation chromatography (g.p.c.)

The Stokes radii (Ackers, 1964) of the collectins were estimated by g.p.c. on a Superose 6 column (HR 10/30, Pharmacia) at a flow rate of 0.5 ml/min. The buffer was TBS containing 10 mM EDTA and 0.05% (v/v) Emulphogene at pH 7.4. Approx. 10 µg of each collectin was applied to the column. Fractions were monitored at 280 nm. CL-43 was also separated in TBS containing 10 mM EDTA without Emulphogene. Calibration of the column was carried out using the following marker proteins: IgM, thyroglobulin, fibronectin, IgG, bovine liver catalase, human serum albumin and myoglobulin.

Sucrose-density-gradient centrifugation

Linear sucrose-density gradients (13 ml) of 5–40% (w/v) sucrose in 10 mM sodium phosphate buffer, pH 7.4, containing 0.1% (v/v) Emulphogene were prepared as described by Martin and Ames (1961). Samples of 10–50 µg of purified collectin in 500 µl of 10 mM sodium phosphate buffer, pH 7.4, containing 0.1% (v/v) Emulphogene, were loaded on to the gradients and centrifuged in a Beckman SW40 rotor at 264000 *g* for 24 h at 4 °C. Fractions were collected from the base of the gradient by peristaltic pumping and analysed by SDS/PAGE. The *s*_{20,w} value

Figure 1 SDS/PAGE of reduced (lanes 1–4) and non-reduced (lanes 5–8) bovine lectins on a 4–20% (w/v) gradient gel stained by the silver-staining method

Lanes 1 and 5, CL-43; lanes 2 and 6, SP-D; lanes 3 and 7, conglutinin; lanes 4 and 8, MBP. Abbreviation: *M*, molecular mass.

for each collectin was estimated by comparing their mobilities with those of standard proteins run on the same gradients. The standard proteins used were: bovine liver catalase (11.2 S), human IgG (6.9 S), BSA (4.6 S) and myoglobin (2.3 S). $s_{20,w}$ values used for these proteins were those published by Smith (1970).

Analysis of complement activation

Microtitre plates (Polysorb; Nunc, Kamstrup, Denmark) were coated with mannan (10 µg/ml of coating buffer) by overnight incubation. This incubation, and all the following steps, were carried out in a total volume of 100 µl and at room temperature, and all washes and incubations were carried out with TBS/Tw containing 5 mM CaCl₂ unless otherwise stated. The plates were washed and incubated with 200 µg of human serum albumin (The State Serum Institute, Copenhagen, Denmark) in 200 µl of TBS for 2 h. After they had been washed, the plates were incubated with dilutions of the collectins. As a control the proteins were also applied in buffer containing 10 mM EDTA instead of Ca²⁺. The plates were incubated overnight at 4 °C and washed. Normal human serum depleted of lectins by passage through mannan–Sephadex (and stored at –70 °C) was diluted 90-fold, applied to the wells and incubated for 1 h at 37 °C. The plates were washed and incubated for 2 h with rabbit anti-(human C4) serum (catalogue no. A 065; Dakopatts) diluted 4000-fold. After they had been washed, the plates were incubated for 2 h with alkaline-phosphatase-coupled goat anti-rabbit Ig diluted 2000-fold. After a final wash, the bound enzyme was estimated by adding *p*-nitrophenyl phosphate disodium salt at 1 mg/ml of diethanolamine buffer. The absorbance of the 96 wells was read at 405 nm by means of a multichannel spectrophotometer.

Analysis of iC3b binding

The analysis of binding of purified CL-43 and conglutinin to fluid-phase iC3b was carried out as described by Laursen et al. (1994). Briefly, purified CL-43 or conglutinin were coated on

Figure 2 G.p.c. on Superose 6

(a) A_{280} profile of purified CL-43 (—, left-hand A scale) and of bovine serum (---, right-hand A scale). The fractions from bovine serum were analysed for CL-43 using rocket immunoelectrophoresis (R.i.e., measurements in mm) (—). (b) purified SP-D; (c) purified conglutinin. The positions of the molecular-mass (M) markers indicated were established by chromatography of purified proteins [IgM, 990 kDa; fibronectin (FN), 440 kDa; IgG, 160 kDa; human serum albumin (HSA), 67 kDa].

microtitre plates and, after incubation and washing, the plates were incubated with serum preincubated at 37 °C with Sephadex G-25 to activate the complement system. After incubation and washing, the plates were developed with rabbit anti-(human C3c) serum (catalogue no. A062; Dakopatts).

Binding to the collectin receptor

The collectin receptor was purified from human tonsils and radiolabelled as previously described by Malhotra and Sim (1989). Plates were coated with mannan, and then incubated with dilutions of CL-43 in 10 mM phosphate buffer, pH 7.4, together with radiolabelled collectin receptor. Plates were also coated with conglutinin and incubated with radiolabelled collectin receptor in the presence of CL-43, bovine MBP, BSA or buffer only. After washing, bound radioactivity was eluted with NaOH and counted.

Electron microscopy

Purified CL-43, conglutinin and SP-D at a concentration of 50 µg/ml were dialysed against 0.2 M NH₄HCO₃ and, after

addition of an equal volume of glycerol, sprayed on to freshly cleaved mica discs. Rotary shadowing with carbon/platinum and electron microscopy was carried out as previously described (Engel et al., 1981).

RESULTS

Purification of bovine CL-43, MBP, concglutinin and SP-D

The three bovine plasma collectins were purified by affinity chromatography on mannan-Sepharose as described previously (Holmskov et al., 1993a,b). SP-D was purified by maltose-TSK affinity chromatography, followed by depletion affinity chromatography on an anti-(bovine IgG)-Sepharose 4B column. As a final step the four lectins were subjected to g.p.c. The purified lectins were compared by SDS/PAGE (Figure 1). Lanes 1–4 show CL-43, SP-D, concglutinin and MBP under reducing conditions, and lanes 5–8 show the same lectins under non-reducing conditions. CL-43 shows a major band at 43 kDa, with a minor band 1 kDa lower, corresponding to the truncated form of CL-43. Only one band at 120 kDa is seen under non-reducing conditions. Under reducing conditions SP-D shows a major band at 45 kDa and a minor band at 86 kDa. The minor band is likely to be a dimer, because it was found to have the same pI as the 45 kDa band (see below). Concglutinin also sometimes shows a non-reducible dimer which might be caused by interchain ester bonds (Andersen et al., 1992). One major SP-D band at 160 kDa and three minor bands above 200 kDa are seen under non-reducing conditions. These minor bands probably represent disulphide-linked higher oligomers of SP-D. Reduced concglutinin shows a major band at 44 kDa and a minor band at 40 kDa corresponding to the intact and the truncated forms. Under non-

reducing conditions concglutinin shows a ladder pattern with bands ranging from 37 kDa to 260 kDa. MBP shows a single band at 28 kDa under reducing conditions and three closely spaced bands at 160–180 kDa and two closely spaced bands at 200 kDa under non-reducing conditions.

G.p.c.

Both purified CL-43 and CL-43 in serum (analysed by rocket immunoelectrophoresis) were eluted from the Superose 6 column at the same position corresponding to a Stokes radius of 9.1 ± 0.3 nm (91 ± 3 Å). The presence or absence of non-ionic detergent made no difference to the behaviour of CL-43 in g.p.c. SP-D was, as had been shown previously (Lu et al., 1993) eluted in two main peaks: one corresponding to the void volume, V_0 , and another just before CL-43 at a position corresponding to a Stokes radius of 9.3 ± 0.3 nm. Concglutinin was eluted at a major peak at V_0 (Figure 2).

Sucrose-density-gradient centrifugation

Sucrose-density gradient centrifugation was carried out for CL-43, SP-D and concglutinin. An $s_{20,w}$ of 10.5 ± 1.0 S was found for concglutinin, while values of 3.6 ± 0.15 S and 3.5 ± 0.15 S were found for CL-43 and for the form of SP-D eluted at the position on the column corresponding to a Stokes radius of 9.3 nm. The void-volume SP-D was not detected by the sucrose-density-gradient centrifugation.

Electron microscopy

CL-43 was eluted from g.p.c. in a single peak and was examined by electron microscopy. Two different purifications gave similar

Figure 3 Electron microscopy of rotary-shadowed CL-43

Arrows indicated where three CRDs can be seen attached to a single rod. The insets are: a, SP-D; b, concglutinin.

Figure 4 Two-dimensional gel electrophoresis of (a) CL-43, (b) SP-D, (c) conglutinin and (d) MBP

The gels were stained by the silver-staining method. Abbreviation: *M*, molecular mass.

results. A total of 40 particles were examined, and all were monomers with rod length of 37.4 ± 1.7 nm (Figure 3). As has been described previously (Lu et al., 1993), conglutinin was seen as oligomers, up to tetramers, of subunits having a rod length of 38 nm (inset b to Figure 3), and SP-D was, like CL-43, seen mainly as monomer subunits ($\sim 85\%$), with rod length of 46 nm, but oligomers up to the tetramer, as shown in Figure 3 (inset a) were also seen (Lu et al., 1993).

Calculation of molecular masses from Stokes radii and $s_{20,w}$ values

The collectins are very elongated proteins, and realistic estimates of molecular mass cannot be made by g.p.c. alone, as standards of known molecular mass and similar elongation are not available. Instead, the molecular mass under non-denaturing conditions was calculated from Stokes radius and $s_{20,w}$ using the relationship:

$$M = (6\pi\eta N a s) / (1 - v\rho)$$

where M is the molecular mass, η is the viscosity of water at 20 °C, N is Avogadro's number, a is the Stokes radius, s is $s_{20,w}$, v is the partial specific volume of the protein and ρ is the density of water at 20 °C (Siegel and Monty, 1966).

v was calculated as 0.712 for bovine SP-D, using the known amino acid sequence of the secreted form, and the same value was used for the other collectins (Schachman, 1957; Perkins, 1986). The calculation does not take the carbohydrate content into account. CL-43 and conglutinin have no N-linked carbo-

hydrate, but SP-D may have a single N-linked oligosaccharide molecule per polypeptide. All three proteins contain hydroxylysine residues in the collagenous region that are potential O-glycosylation sites, but the contribution of O-glycosylation is small (0.36 kDa/site).

The calculation yielded a molecular mass between 119.2 and 137.6 kDa for CL-43. The range reflects the error limits noted above in the determination of a and s . This is consistent with the disulphide-linked subunit of 120 kDa seen on SDS/PAGE under non-reducing conditions. It was not possible to calculate an accurate Stokes radius for the major peaks of SP-D or conglutinin seen in Figure 2, as these are eluted too close to V_0 , and must have Stokes radius greater than 11 nm. These peaks are likely to correspond to the trimers and tetramers of subunits seen in electron microscopy. The secondary peak of SP-D (Figure 2b) has Stokes radius of 9.3 ± 0.3 nm and an $s_{20,w}$ of 3.5 ± 0.15 S, indicating a molecular mass between 118.4 and 137.6 kDa.

The frictional coefficient, f/f_0 , for CL-43 and the lower-molecular-mass form of SP-D was calculated (Siegel and Monty, 1966) as 2.7, reflecting the very elongated structures seen by electron microscopy.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis revealed that CL-43 has two isoforms with pI values of 4.9 and 5.3 corresponding to the native form and to a truncated form of the molecule respectively (Figure 4a). Like rat SP-D (Perrson et al., 1989), bovine SP-D

Figure 5 Deposition of C4 on collectin-reacted solid-phase mannan

Mannan-coated ELISA wells were incubated with the purified collectins in the presence of Ca^{2+} . The wells were then incubated at 37 °C with lectin-depleted human serum diluted 90-fold in Ca^{2+} -containing buffer. Deposition of C4 fragments on to the coated plastic surface as an indicator of activation of the classical complement pathway was measured by alkaline phosphatase-labelled anti-C4 antibody. ○, MBP in the presence of Ca^{2+} ; ●, MBP in the presence of EDTA; △, CL-43 in the presence of Ca^{2+} ; ▲, CL-43 in the presence of EDTA.

Figure 6 Binding of CL-43 to the collectin receptor

(a) Radiolabelled collectin receptor (250 ng; 10^6 c.p.m.) was incubated in the presence of 2.5 µg of CL-43, MBP, BSA or buffer only (10 mM phosphate, pH 7.4) on conglutinin-coated microtitre plates. (b) Radiolabelled collectin receptor was incubated in the presence of dilutions of CL-43 (○) or BSA (□) on a mannan-coated microtitre plate.

showed a charged train of molecules with pI values from 5.2 to 7.5 (Figure 4b). The molecule at 86 kDa shows the same isoforms as the 45 kDa molecule. Both the intact and the truncated form of conglutinin show several isoforms with pI values from 5.0 to 6.0 and from 5.2 to 7.4 respectively (Figure 4c). MBP has two isoforms with pI values of 4.6 and 5.2 (Figure 4d).

Analysis of complement activation and binding

We studied deposition of C4 components from human serum on to mannan preincubated with bovine MBP, CL-43, SP-D or conglutinin in the presence of Ca^{2+} , or, as a control, in the presence of EDTA. A dose-dependent deposition of C4 was observed after incubation with MBP in the presence of Ca^{2+} (Figure 5). By contrast, CL-43, SP-D and conglutinin did not increase C4 deposition over the level seen after incubation with MBP in the presence of EDTA.

Conglutinin bound, as previously shown, to fluid-phase iC3b.

CL-43 and MBP in parallel assays exhibited no binding of iC3b (results not shown).

Binding to the collectin receptor

Clq, conglutinin, MBP and SP-A all bind to the collectin receptor (Malhotra et al., 1990, 1992). CL-43 as well as bovine MBP could inhibit the binding between conglutinin and radio-labelled collectin receptor (Figure 6a), and the binding of radio-labelled collectin receptor was dependent on the concentration of solid-phase CL-43 (Figure 6b).

DISCUSSION

We have recently characterized CL-43 as a plasma C-type lectin containing collagenous regions (Holmskov et al., 1993a). The determination of the sequence of CL-43 confirmed the presence of collagenous structure and showed a 70 % identity with bovine SP-D and 74 % identity with conglutinin (Lim et al., 1994). The present study extends these findings, comparing the three collectins at the quaternary-structural level and also with respect to their interaction with complement and the collectin receptor.

Electron microscopy of two different preparations of CL-43 showed the presence of only monomers of the structural units all with a rod length of 37 nm (Figure 3). Both preparations were eluted as one symmetrical peak at a position corresponding to a Stokes radius of 9.1 ± 0.3 nm. When serum was separated on same column and fractions were analysed for CL-43 by immunochemical means, CL-43 was found to be eluted at exactly the same position as purified CL-43 (Figure 2), indicating that the absence of oligomers is not an artefact induced by the purification procedure.

The cDNA of CL-43 encodes a polypeptide of 301 amino acid residues, which is significantly shorter than the 349 amino acid residues of bovine SP-D and the 351 amino acid residues of conglutinin (Lim et al., 1994). The difference in length is caused by two deletions in the collagen region of CL-43, which has only 38 Gly-Xaa-Yaa triplets compared with the 57 Gly-Xaa-Yaa triplets of bovine SP-D and conglutinin. These deletions account for the shorter rod length of CL-43 (37 nm) as compared with bovine SP-D (46 nm) found by electron microscopy. Assuming a triple-helical rod has a length of 0.286 nm/amino acid (Taub and Piez, 1971), the predicted length of the triple-helical rod in CL-43 is 33 nm, which is in fair agreement with the observed value of 37 nm. The predicted length of the triple-helical rods in both SP-D and conglutinin is 49 nm, and there is presently no explanation for the discrepancy between the predicted and the observed 38 nm rod length of conglutinin. Conglutinin has a cysteine residue at position 38 which might be involved in interchain disulphide bonding (Figure 11 of Lee et al., 1991). This might possibly prevent the first five Gly-Xaa-Yaa of each chain from participating in a triple helix, making the rod shorter than predicted.

The estimates of molecular masses of the collectins by SDS/PAGE under denaturing conditions and in non-denaturing conditions by analytical g.p.c. and sucrose-density-gradient centrifugation are consistent with each other, but still show minor anomalies. The apparent size of the CL-43 polypeptide chain from SDS/PAGE is 43 kDa, and that of the covalent oligomer seen under non-reducing conditions is 120 kDa. This is in good agreement with the estimate of 119–138 kDa seen under non-denaturing conditions. However, the molecular mass of the single polypeptide as estimated from sequence is only 31.5 kDa,

and the reason for the discrepancy between this value and the other estimates is not clear. O-linked carbohydrate is unlikely to make a contribution of more than 2 kDa per polypeptide. No higher oligomers of CL-43 were seen by g.p.c. or sucrose-density-gradient centrifugation, a finding consistent with our not seeing any oligomers under the electron microscope.

For SP-D, the molecular mass estimated from sequence is 35.7 kDa per polypeptide. Again, O-linked carbohydrate, and possibly one N-linked carbohydrate molecule, may add up to 3–4 kDa per polypeptide. The value from SDS/PAGE of 45 kDa is reasonably consistent with this. G.p.c. and electron microscopy indicate that SP-D exists in two sizes: the larger oligomers were beyond the size range of g.p.c. analysis. The smaller molecules seen on g.p.c. and in sucrose-density-gradient centrifugation was calculated to have a molecular mass of 118–138 kDa under non-denaturing conditions, consistent with a single unit containing three polypeptide chains, as for CL-43.

Conglutinin was also too large for analysis in this g.p.c. system, but an $s_{20,w}$ of 10.6 S was derived.

I.e.f. of the four collectins examined showed very different patterns (Figure 4). No N-glycosylation sites are found in the CL-43 sequence, and i.e.f. of CL-43 revealed only one isoform of the intact molecule with a pI of 4.9 (Figure 4a). The truncated form of the molecule, lacking the first nine amino acids, showed a pI of 5.3, and this increase in pI is explained by the loss of a net charge corresponding to three acidic groups within the fragment that is removed to yield the truncated molecule.

Conglutinin is less acidic than CL-43, showing four isoforms of the intact molecule with pI values ranging from 5.3 to 6.1 (Figure 4c). Conglutinin has one potential glycosylation site involving Asn³¹⁷-Asn³¹⁸-Ser³¹⁹, but Asn³¹⁷ is one of the residues involved in sugar binding (Drickamer, 1992), and therefore it seems unlikely that conglutinin is N-glycosylated. The different isoforms of the molecule could only in part be due to allelic variation in the molecule, since the serum used for the purification came from a single cow.

Reduced bovine SP-D, like rat SP-D (Perrson et al., 1989), appears as a charge train with pI values ranging from 5.2 to 7.2 (Figure 4b). Bovine SP-D has one potential glycosylation site at residue 70, within the fifteenth Gly-Xaa-Yaa repeat. Variation in glycosylation could account for some of the variability seen, e.g., through sialylation of O-linked carbohydrates, but the possibility that there are different allelic forms of the molecule should also be considered, since the lung lavage used to prepare SP-D was from a pool from seven cows. The two isoforms of bovine MBP could be glycosylation variants or could be allelic forms.

It is well established that conglutinin binds to a high-mannose group on the α -chain of the complement degradation product iC3b (Lachmann and Müller-Eberhard, 1968; Hirani et al., 1986; Laursen et al., 1994). Although both MBP and CL-43 bind to mannose, which should allow the binding to the sugar residues on both the α - and β -chain of iC3b, we were not able to detect binding between these collectins and iC3b. This observation highlights the exceptional selectivity of conglutinin for iC3b, likely caused by secondary- or tertiary-structural features of iC3b, but an explanation for the lack of binding to iC3b of the other collectins is still missing.

MPB has an overall bouquet-like (or sertiform) structure very similar to that of C1q, the first component in the classical complement pathway, and it has been shown previously that human and mouse MBP can activate the classical pathway of the complement system after binding to sugar ligands without the involvement of antibody and C1q (Ikeda et al., 1987; Lu et al., 1990; Holt et al., 1994). Bovine MBP is here shown to activate the classical complement system of human serum in a manner

similar to that shown by human MBP, while the cruciform collectins (conglutinin and SP-D) and CL-43 have no complement-activating capacity (Figure 5). Comparison of SP-A and MBP in the same type of assay, failed to reveal any significant activity of SP-A purified from normal human lung lavage (A. Harum, S. Thiel and J. C. Jensenius, unpublished work).

Conglutinin, MBP and SP-A bind to the collectin receptor (Malhotra et al., 1990). CL-43 was also found to bind to the collectin receptor, and the binding could be inhibited by conglutinin or C1q (Figure 6).

Although no biological role has yet been established for CL-43, the sugar-binding profile of the molecule indicates that CL-43, like the other collectins, binds to carbohydrate moieties on the surface of micro-organisms, after which the binding to the collectin receptor mediates the binding and internalization of the CL-43-opsonized micro-organisms.

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