The cDNA cloning of conglutinin and identification of liver as a primary site of synthesis of conglutinin in members of the Bovidae

Jinhua LU,* Steen B. LAURSEN,† Steffen THIEL,† Jens Chr. JENSENIUS† and Kenneth B. M. REID*‡

*MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K., and †Institute for Medical Microbiology, Bartholin Building, Aarhus University, 8000 Aarhus C, Denmark

Bovine conglutinin is a collagen-like, C-type, plasma lectin which belongs to the group of proteins called 'collectins'. Two inosine-containing oligonucleotides were synthesized, based on the published protein sequence for bovine conglutinin [Lee, Leiby, Allar, Paris, Lerch and Okarma (1991) *J. Biol. Chem.* **266**, 2715–2723], and PCR on target DNA from a bovine liver λ gt 11 cDNA library yielded a product of the expected size of 210 bp. Screening of the library with this cDNA fragment identified a single positive clone, with an insert of 0.9 kb, coding for bovine conglutinin from residue 70 to the C-terminus. The 5' cDNA sequence, encompassing 150 bp of the 5' non-translated sequence plus the sequence encoding the leader peptide and the N-terminal residues 1–70, was completed by the use of PCR techniques. The cDNA sequence of bovine conglutinin showed 86% identity with that of bovine lung surfactant protein D (SP-D), and the derived amino acid sequence of bovine conglutinin showed 78% identity with that of bovine SP-D, which included complete

identity of the leader-peptide sequences. The amino acid sequence derived from the cDNA sequence differs from the published protein sequence at four positions. Northern-blot analysis on total RNA, purified from various tissues from cattle, sheep, humans, rats and mice, showed that a strong signal of approx. 1.8 kb is present in bovine liver RNA. A weak signal of similar size was also observed in sheep liver, but not in human, rat and mouse livers. A weak signal, also of 1.8 kb, is present in the lung RNAs of all the species tested. The signals from the lung tissues are likely to be due to the cross-hybridization of the bovine conglutinin cDNA to the SP-D mRNAs of the respective species. The finding of significant signals in only the bovine and sheep liver RNA samples is indicative that serum conglutinin may be present in significant amounts only in members of the Bovidae (the family encompassing cattle, antelopes, sheep and goats) and closely related species.

INTRODUCTION

Bovine conglutinin is a plasma protein which was first described as a factor which agglutinates complement-reacted erythrocytes (Bordet and Gay, 1906). This agglutination is mediated through the Ca^{2+} -dependent affinity of conglutinin for the oligosaccharide on the α -chain of the fixed complement component iC3b (Leon and Yokohari, 1964; Lachmann and Müller-Eberhard, 1968; Hase et al., 1985; Hirani et al., 1985). In the presence of Ca^{2+} , bovine conglutinin binds to terminal non-reducing *N*-acetyl-D-glucosamine, mannose and fucose residues (Loveless et al., 1989). The complete amino acid sequence of bovine conglutinin has been obtained by protein sequencing (Lee et al., 1991), which showed that the molecule has a single type of polypeptide chain composed of an N-terminal cysteine-rich section followed by a collagen-like sequence and a C-terminal non-collagen-like region that contains the C-type carbohydrate recognition domain (CRD) (Drickamer, 1988). A single bovine conglutinin molecule is considered to be composed of four subunits each consisting of three identical chains forming a triple helix via their collagen-like regions and a globular 'head' portion via their C-terminal regions. These subunits are disulphide-linked via their N-terminal portions to form an overall cross-shaped structure (Davis and Lachmann, 1984; Strang et al., 1986). There are a number of recent reports which support the view that bovine conglutinin plays a role in defence against micro-organisms. Bovine conglutinin has been shown to have complement- and phagocyte-dependent bactericidal activity and to enhance the clearance of bacteria from the circulation (Friis-Christiansen et al., 1990).

Also, it has recently been claimed that bovine conglutinin is identical with the serum β -inhibitor which has been described as having both virus-neutralizing and haemagglutination-inhibiting activity with respect to the influenza virus (Hartley et al., 1992). The functions attributed to bovine conglutinin could involve its binding to the C1q receptor (Malhotra et al., 1990) which is found on a wide range of cells and has been designated the 'collectin' receptor (Malhotra et al., 1992).

Recently, a new protein, pulmonary surfactant protein D (SP-D), has been characterized in lung lavage (Persson et al., 1989; Lim et al., 1992; Lu et al., 1992). SP-D is also a C-type lectin which contains collagen-like domains (Persson et al., 1990; Rust et al., 1991; Lu et al., 1992; Lim et al., 1992) and it has a high degree of sequence identity with bovine conglutinin (Lu et al., 1992; Shimizu et al., 1992; Lim et al., 1992). In the electron microscope, bovine SP-D has an overall cross-shaped structure which is similar to that of bovine conglutinin (J. Lu, H. Wiedemann, R. Timpl and K. B. M. Reid, unpublished work). Despite their structural similarity, SP-D and bovine conglutinin have quite different carbohydrate specificities, with SP-D having a high affinity for maltose, while bovine conglutinin shows no affinity for maltose but a strong affinity for *N*-acetyl-D-glucosamine (Lu et al., 1992). SP-D associates with surfactant lipids (Persson et al., 1989; Kuroki et al., 1991), and the association is Ca^{2+} -dependent, but no functional role for SP-D has yet been established. However, the other collagen-like lectins, or collectins (bovine conglutinin, mannan-binding protein, lung surfactant protein A) and serum C1q, which have a similar overall structure to SP-D, have been shown to be involved in recognition and

Abbreviations used: CRD, carbohydrate-recognition domain; SP-D, pulmonary surfactant protein D; 1 $\times$ SSC, 0.15 M NaCl/0.015 M sodium citrate.

‡ To whom correspondence should be addressed.

The cDNA sequence of bovine conglutinin will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases.

clearance of micro-organisms; therefore it seems probable that SP-D may also play a similar role. The recent findings, that SP-D can bind to the surface carbohydrates of certain bacteria (*Escherichia coli*, *Salmonella*) and cause agglutination (Kuan et al., 1992) and that SP-D can enhance the production of oxygen radicals by alveolar macrophages (van Iwaarden et al., 1992), is consistent with the SP-D playing a protective role in the lung.

In order to further the understanding of the role of congenitally, its relationship to SP-D and to initiate the search for analogous molecules in other species, it was decided to isolate a cDNA clone for bovine congenitally. The use of the bovine congenitally cDNA probe supports the view that serum congenitally is only synthesized in significant amounts in the livers of members of the Bovidae (cattle, antelopes, sheep and goats) and closely related species.

MATERIALS AND METHODS

The bovine liver λ gt 11 cDNA library was purchased from Clontech (Cambridge Biosciences, Cambridge, U.K.). Cow and sheep tissues were obtained from a local abattoir (Reading, U.K.). Oligonucleotide primers were synthesized using a 381A DNA synthesizer (Applied Biosystems, Warrington, Cheshire, U.K.).

PCR amplification of bovine congenitally cDNA sequence from the liver cDNA library

The PCRs were carried out for 25–35 cycles in reaction mixtures (50 μ l or 100 μ l) containing the appropriate DNA template (approx. 100 ng of DNA or 5×10^6 λ -phages), *Taq* polymerase (2.5 units/100 μ l reaction volume), $1 \times$ *Taq* polymerase buffer (Promega, Southampton, U.K.) and made 0.5 μ M with respect to both primers and 0.2 mM with respect to dNTPs. Two oligonucleotide primers (BC-B and BC-C; Table 1) were synthesized on the basis of the reported protein sequence for bovine congenitally (Lee et al., 1991) over regions where it is significantly different from that of human SP-D (Lu et al., 1992), since the bovine SP-D sequence was not available when the oligonucleotides were synthesized. Inosine was introduced at positions where four different nucleotides would otherwise be required. The two primers were used in a PCR reaction (50 μ l) using approx. 5×10^6 phages (in 1 μ l) from the bovine liver λ gt 11 cDNA library as target DNA, and the reaction was carried out for 35 cycles, with each cycle consisting of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C. To clone PCR products, primers were phosphorylated in reaction mixtures (250 μ l) containing 25 μ M

oligonucleotide, 2.5 mM spermidine, restriction enzyme buffer B (Boehringer Mannheim, Mannheim, Germany), 0.4 mM ATP and 2.5 units of T4 kinase. The mixtures were incubated for 30 min at 37 °C. After heating for 10 min at 100 °C, to inactivate the kinase, the mixtures were used directly in the PCR reactions. Portions of the PCR reactions (1, 2 or 6 μ l) were added to a ligation mixture (10 μ l) containing 10–20 ng of the plasmid pBSKS⁺ (which had been digested with *Hinc*II and dephosphorylated). To amplify the 5' portion of bovine congenitally cDNA, an anti-sense primer (BCR-3; Table 1) was synthesized, based on the 5'-portion sequence of a partial clone for bovine congenitally, and used in PCR reactions (50 μ l) together with either primer F11(sense) or R11(anti-sense), i.e. oligonucleotides based on the λ gt 11 vector sequences upstream (F11), or downstream (R11), of the *Eco*RI cloning site (Table 1). The PCR reactions were carried out for 30 cycles on 1 μ l (approx. 5×10^6 phages) of the bovine liver cDNA library, with each cycle consisting of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 40 s. The PCR products were separated on a 1% (w/v) SeaPlaque low-melting-temperature gel, and the largest product (approx. 0.5 kb) was re-amplified using the same protocol, but with phosphorylated primers (F11 and BCR-3). The reaction mixture was then used for ligation, and the DNA fragment was cloned into pBSKS⁺ as described above. The DNA fragments were sequenced by the double-stranded sequencing technique using the T7 sequencing kit (Pharmacia Biosystems, Milton Keynes, U.K.).

Screening of a bovine liver λ gt 11 cDNA library with the cDNA fragment

Approx. 5×10^5 phages were plated out, and filters were lifted in duplicate. The cDNA fragment (210 bp) amplified from the library, using the primers BC-B and BC-C, was separated from other products of the PCR on a 3% (w/v) NuSieve low-melting-temperature gel. The gel slice containing the 210 bp fragment was cut out and melted, after addition of 8 vol. TE buffer (10 mM Tris/1 mM EDTA, pH 7.4), by heating for 10 min. at 68 °C. The DNA fragment (approx. 20 ng in 10 μ l melted gel solution) was labelled by 30 cycles of PCR reaction with primer BC-B alone, in a 100 μ l reaction volume, after the addition of 5 μ l of [α -³²P]ATP. The labelled PCR product was ethanol-precipitated and dissolved in TE buffer (50 μ l). The filters to be screened were prehybridized for 4–6 h at 42 °C in the prehybridization solution [50% (w/v) formamide/10% (w/v) dextran sulphate/5 $\times$ Denhardt's solution/0.1% (w/v) sodium pyrophosphate/0.1% (w/v) SDS/1 M NaCl/50 mM Tris/HCl/0.1 mg/ml sonicated salmon sperm DNA, pH 7.4]. The labelled mixture was added to the solution, and the hybridization was carried out at 42 °C for 16 h. The filters were washed for 30 min in $2 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl/0.015 M sodium citrate)/0.1% (w/v) SDS at room temperature followed by 30 min in $1 \times$ SSC/0.1% (w/v) SDS at 65 °C, and finally by 30 min in $0.1 \times$ SSC/0.1% (w/v) SDS at 65 °C. The filters were exposed to X-ray film overnight (approx. 16 h) at –70 °C.

Purification of total RNA and Northern-blot analysis

Total RNA was purified from various bovine tissues by a previously described method (Chornazynski and Sacchi, 1987). Briefly, fresh tissues (1.0 g samples), stored in liquid nitrogen, were homogenized in 10 ml of buffer (4 M guanidium thiocyanate/25 mM sodium citrate/0.5% (w/v) Sarkosyl/0.1 M 2-mercaptoethanol, pH 7.4) using an Ultra-Turrax T25 homogenizer (Janke und Kunkel IKA-Labortechnik). Sodium acetate (2 M; pH 4.0; 2 ml), water-saturated phenol (10 ml) and

Table 1 Oligonucleotide primers used in the cloning of bovine congenitally cDNA. (I = inosine)

Primers	Sequences of primers		Corresponding amino acid sequences in congenitally
	5'	3'	
BC-B	GGIAC(A/G)TC(A/G)TTCCA(C/T)TTICC(A/G)TC		333–340
BC-C	AA(C/T)GA(A/G)GCI GTIAC(A/G)ATGGT		271–278
BCR-3	TTCTCTCCAGCTGGTCCAGGCA		90–97
BCR-P	AAACTCGCAGATCACAAGGAG		345–351
BC-16	GGACTAGTGA(A/G)GTIAA(C/T)GCI(T/C)TIAA		197–203
BC-8	ACCAT(C/T)TGNGTACNGC(C/T)TC(A/G)TTYTC		270–278
F11	GACTCTGGAGCCCGTCAGTAT		
R11	GACCAAACTGGTAATGGTAGCGAC

Figure 1 cDNA and derived amino acid sequences of bovine conglutinin (BC) and alignment with those of bovine SP-D (bSP-D) (Lim et al., 1992)

Both the amino acids and the nucleotides are numbered according to the bovine conglutinin sequences. Amino acids are numbered above the line with '+1' taken to represent the N-terminus of mature bovine conglutinin. Nucleotide sequences are numbered at the right-hand end of each line. '|' denotes nucleotides which are identical between the cDNA sequences of bovine conglutinin and bovine SP-D. Amino acid residues differing from those published by Lee et al. (1991) are at positions 153, 190, 198 and 252, where His, Lys, Ala and Val are predicted from the cDNA sequence, while Arg, Ser, Val and Glu are indicated from the protein studies.

chloroform/3-methylbutan-1-ol (49:1, v/v) (2 ml) were added to the homogenate with vortex-mixing after each addition. The mixture was incubated for 15 min on ice and then centrifuged for 20 min at 12000 *g*. Propan-2-ol (10 ml) was then added to the supernatant, and RNA was precipitated by incubation for 10 min on solid CO₂. The RNA was pelleted by centrifugation for 20 min at 12000 *g*, dissolved in 1 ml of water, and stored as precipitate at -20 °C after the addition of 3 vol. of ethanol. Glycogen was separated from the RNA in the liver and muscle

preparations by the addition of 3 vol. of 4 M sodium acetate, pH 7.0, to 1 vol. of RNA in distilled water, followed by incubation for 2 h on ice, and centrifugation for 20 min at 12000 *g*. Samples of purified total RNA (10 µg) were separated on a 1% (w/v) agarose gel run under denaturing conditions and transferred on to Hybond-N membrane using 20 × SSC. The RNA was then cross-linked to the blot using an XL-1500 u.v. cross-linker (Spectronics Corporation, Oxford, U.K.). The blot was incubated for 4–6 h at 42 °C in the prehybridization solution,

and then hybridized for 16 h at 42 °C with the 0.9 kb and the 0.5 kb bovine conglutinin cDNA fragments labelled by 30 cycles of PCR using antisense primers (BCR-P and BCR-3, respectively; Table 1). The blot was washed for 30 min at room temperature in $2 \times \text{SSC}/0.1\%$ (w/v) SDS, followed by washing for 30 min in $1 \times \text{SSC}/0.1\%$ (w/v) SDS and another 30 min in $0.1 \times \text{SSC}/0.1\%$ (w/v) SDS at 65 °C. The washed blot was exposed to X-ray film for 3 days at -70 °C.

PCR amplification of tissue RNA

PCR was carried out using primers BC-16 and BC-8 (Table 1). The PCR protocol was performed with the primers (50 pmol of each primer) and total RNA (100 ng) using the procedure outlined for the Perkin-Elmer RNA-PCR kit (Perkin Elmer, Birkeroed, Denmark). Reverse transcription was carried out at 50 °C for 5 min, then at 72 °C for 5 min. PCR was carried out for 40 cycles with each cycle consisting of 1 min at 95 °C, 1 min at 50 °C and 1 min at 72 °C.

RESULTS

Isolation of a cDNA clone coding for bovine conglutinin

In view of the immunohistochemical data suggesting that liver was one of the primary sites of synthesis of bovine conglutinin (Holmskov et al., 1992), a bovine liver λ gt 11 cDNA library was screened for the presence of bovine conglutinin cDNA clones. In all, 35 cycles of PCR reaction using BC-B and BC-C as primers and approx. 5×10^6 phages from the bovine liver λ gt 11 cDNA library, as template DNA, yielded a product of the expected size of 210 bp. This fragment was re-amplified with phosphorylated BC-B and BC-C, and the resulting PCR reaction mixture was directly used for ligation into *Hinc*II-digested plasmid pBSKS⁺. Sequencing analysis showed that the PCR product coded for the expected region (residues 271–340; Lee et al., 1991) of the bovine conglutinin polypeptide.

Complete cDNA and derived amino acid sequences of bovine conglutinin

The 210 cDNA fragment was radiolabelled by PCR with BC-B (an antisense oligonucleotide), which allowed the production of single-stranded probes with high specific radioactivity. A single positive clone containing an insert of 0.9 kb was identified by the screening of approx. 5×10^6 phages. The DNA sequence of this clone showed that it coded for bovine conglutinin from residue 74 (Gly) to the C-terminal residue, and also contained 42 bp of the 3'-non-translated sequence, but not the polyadenylation signal (Figure 1). An anti-sense primer was synthesized based on a portion of the 5'-region of this cDNA clone (BCR-3; Table 1) and used to amplify the 5'-sequence which was absent in this bovine conglutinin cDNA by PCR with primer F11 or R11, using the cDNA library as target DNA. Four products were obtained in the PCR reaction using primers F11 and BCR-3, and the largest, of approx. 0.5 kb, was cloned by blunt-ended ligation and sequenced. The sequence of this cDNA fragment showed that it coded for the N-terminal portion of bovine conglutinin which is absent in the isolated cDNA clone, a leader sequence of 20 amino acid residues and approx. 150 bp of the 5'-non-translated sequence. Apart from four residues, the derived sequence is identical with the reported protein sequence for bovine conglutinin (Lee et al., 1991; Figure 1). The presence of a leader peptide is consistent with bovine conglutinin being a serum protein. The cDNA sequence also confirmed the presence of a cysteine residue where a glycine residue would be expected

in the collagen-like region (at position 38). This residue is considered to form inter-chain and/or inter-subunit disulphide linkages (J. Lu, unpublished work).

Northern-blot analysis and analysis of tissue RNA by PCR

Total RNAs purified from bovine, sheep, human, rat and mouse tissues were separated on a 1% (w/v) agarose gel under denaturing conditions and blotted on to a Hybond-N membrane. The blot was hybridized with both the 0.5 kb and the 0.9 kb bovine conglutinin cDNA fragments, which had been radio-labelled by PCR using anti-sense primers (BCR-3 and BC-P respectively). At a final washing stringency of $0.1 \times \text{SSC}/0.1\%$ (w/v) SDS at 65 °C for 30 min, a strong signal of 1.8 kb was observed in bovine liver RNA (Figure 2, lane 7) which is consistent with the bovine conglutinin cDNA clones being

Figure 2 Northern-blot analysis of total RNAs (10 µg) isolated from bovine, sheep, human, rat and mouse tissues

The 18 S and 28 S RNAs are taken as RNA size markers. Tissues analysed were: bovine muscle (1), tonsil (2), parotid (3), kidney (4), lung (5), spleen (6) and liver (7); human liver (8); sheep lung (9), spleen (10) and liver (11); rat lung (12) and liver (13); and mouse lung (14) and liver (15).

Figure 3 PCR products obtained

The PCR products obtained, using primers BC-16 and BC-8, and RNA-PCR kit on bovine RNA from (1) liver, (2) lung, (3) intestine lymph node, (4) spleen, (5) tonsil, (7) cortex of the adrenal gland, (8) hip lymph node, (9) kidney, (10) unfractionated blood cells and (11) thymus were loaded on an agarose gel, and, after electrophoresis, the gel was stained with ethidium bromide. The M_r markers are shown in lane 6.

isolated from the liver cDNA library. Weaker signals of the same size were also observed in all the lung RNA tracks on the Northern blot (Figure 2; lanes 5, 9, 12 and 14). These are likely to be the mRNAs coding for SP-Ds, since SP-D and conglutinin have a high degree of sequence identity (86 %; bovine conglutinin versus bovine SP-D) and the cross-hybridization of bovine SP-D cDNA with bovine conglutinin mRNA had been reported (Lim et al., 1992). No signal was detected in the RNAs isolated from bovine muscle, tonsil, parotid, kidney and spleen (Figure 2, lanes 1, 2, 3, 4 and 6). A weak signal of similar size was also observed in sheep liver RNA, but not in human, rat and mouse liver RNAs (Figure 2, lanes 8, 11, 13 and 15), even when washing the blot at a lower stringency ($1 \times \text{SSC}/0.1\%$ (w/v) SDS; results not shown). RNAs isolated from a range of bovine tissues were also analysed by PCR (Figure 3). The PCR results also indicated that liver is a primary site of conglutinin synthesis, since a fragment of the expected size was only seen in the liver track (Figure 3).

DISCUSSION

Northern-blot and PCR analysis showed the presence of bovine conglutinin mRNA in the liver, which is consistent with the detection of bovine conglutinin, at the immunohistochemical level, in the liver (Holmskov et al., 1992). The hybridization of bovine conglutinin cDNA to mRNAs isolated from bovine, sheep, rat and mouse lungs is likely to be due to those mRNAs coding for SP-D, since bovine conglutinin shows a high sequence identity with SP-D (Lim et al., 1992; Lu et al., 1992; Shimizu et al., 1992) and it is known that bovine SP-D cDNA hybridizes to bovine conglutinin mRNA (Lim et al., 1992). This view, that the conglutinin cDNA probe cross-reacts with SP-D mRNA present in lung tissue, is further supported by the finding that PCR carried out on bovine lung and liver RNA, using conglutinin specific primers, only amplified the correct product from the liver sample (Figure 3). No signal was observed in the Northern blotting of human, rat and mouse liver RNAs with the bovine conglutinin cDNA probe (Figure 2). This is in agreement with the finding that the bovine SP-D cDNA probe hybridizes with bovine liver RNA, whereas the rat and human SP-D cDNA probes do not detect any signal in rat and human liver RNAs, respectively (Lim et al., 1992; Rust et al., 1991; Shimizu et al., 1992). Conglutinin has not been fully characterized in any other species except the cow, although a conglutinin-like molecule has been reported to be present in human plasma (Thiel et al., 1987). However, the behaviour of this protein on SDS/PAGE, as a band of 60 kDa under reducing conditions, suggests that it is structurally distinct from bovine conglutinin. The presence of a weak signal in the Northern blot of sheep liver RNA, using the bovine conglutinin cDNA probe, is suggestive that conglutinin may be a protein which is only present in the cow, and closely related species, and may be a product of a recent duplication of the SP-D gene. This is also suggested by the fact that bovine SP-D shows a higher sequence identity with bovine conglutinin (78 %; Lim et al., 1992) than with that of human and rat SP-D (67 and 65 % respectively). Bovine SP-D and bovine conglutinin also both have a depletion of two Gly-Xaa-Yaa triplets in the collagen-like regions when compared with human and rat SP-D sequences (see Figure 3 in Lim et al., 1992). The Northern-blot and PCR-analysis data, however, does not rule out the possibility that, if conglutinin is present in other species, it may be of non-hepatic origin. It is also possible that conglutinin may be expressed at a much lower level in other species than in members of the Bovidae and, therefore, cannot be observed by cross-

hybridization and can also not be detected in the sera of those species using an assay which easily detects the relatively abundant bovine conglutinin (Lachmann, 1967). The fact that the signal in the Northern blot of sheep liver RNA is even lower than that obtained with sheep lung RNA supports the latter suggestion (Figure 2).

The 1.3 kb bovine conglutinin cDNA sequence shows an 86 % sequence identity with that of bovine SP-D (Figure 1). Within the translated region, the sequence identity is higher in the 5' portion (91 % identity) which codes for the N-terminal region, including the collagen-like sequence, than that in the 3' region coding for the C-terminal, non-collagen-like, sequence (78 % identity). This is consistent with the finding that, although these two proteins show a strong structural similarity, they differ in their carbohydrate specificities (Lu et al., 1992). The complete identity of the 98 bp between nucleotides 120 and 217 in both bovine conglutinin and bovine SP-D means that the proteins are identical over their leader peptides. The significance of the complete conservation of the two leader sequences at both the protein and gene levels is not clear. A similar degree of sequence identity is also found over a stretch of 133 bp in the collagen-like region (between nucleotides 461 and 593), where there are only mismatches at two points, and only one of these results in a change in the amino acid sequence.

The derived amino acid sequence for bovine conglutinin (Figure 1) differs from the reported protein sequence (Lee et al., 1991) at four positions, each involving one residue (at positions 153, 190, 198 and 252; full details are given in the legend to Figure 1). The cause of those differences is not clear, but errors in protein sequencing and cDNA cloning are possible. Also it is possible that bovine conglutinin may consist of two slightly different polypeptide chains, as in the case of human surfactant protein A (Floros et al., 1986). The derived sequence confirmed the unexpected presence of a cysteine residue in the position of a triplet glycine residue of the collagen-like region of bovine conglutinin (Figure 1), which is considered to form interchain, and possibly inter-triple-helix, disulphide bonds (J. Lu, unpublished work).

The publication of the complete amino acid sequence for bovine conglutinin by protein sequencing (Lee et al., 1991) allowed many choices in the design of oligonucleotide primers for use in the PCR. Primers BC-B and BC-C were designed to be specific for bovine conglutinin and to yield a product of only 210 bp, which allows a reaction time as short as 15 s at 72 °C. A short chain-reaction time should reduce the range of non-specific products obtained and therefore enhance the proportion of the target sequence. This was the case in the PCR reaction, using the BC-B and BC-C oligonucleotide primers, where only one major band was observed on the gel (results not shown). The use of inosine in the synthesis of primers BC-B and BC-C reduced the complexity and therefore also contributed to the specific amplification of the target sequence. The 210 bp cDNA fragment isolated from the library by PCR was a very effective probe in the subsequent screening of the same library. This strategy is especially useful when the target sequence is scarce in the library. The same library had, without success, been screened three times, using oligonucleotide probes based on peptide sequences of bovine conglutinin, and screening with anti-(bovine conglutinin) antibodies was also negative. Screening of approx. 5×10^5 phages of the library with the 210 bp PCR product only identified a single positive clone, which indicated that the number of clones for bovine conglutinin is low in this library. By means of PCR, a significantly larger number of phages can be easily screened and, in the present study, approx. 5×10^6 phages were used, which is equivalent to 10 times the number which could be conveniently screened by conventional methods using single

oligonucleotide probes. The sequence over the 5' portion of bovine conglutinin cDNA was readily obtained by use of PCR with an antisense, conglutinin specific, primer (BCR-3) and primers (F11 or R11) based on the vector (λ gt 11) sequences around the *Eco*RI cloning site.

Recently, the three-dimensional structure of the C-type CRD of rat MBP-A was characterized by X-ray crystallography and was shown to contain two Ca^{2+} -binding sites (Weis et al., 1991). Mutagenesis and expression studies have also provided information on the importance of certain residues within the C-type CRD with respect to carbohydrate binding (Queensberry and Drickamer, 1992; Tiemeyer et al., 1992). Results from the two groups have led to similar conclusions, i.e. that mutations on both conserved and some less- or non-conserved residues could reduce the affinity of the domain for the carbohydrate without changing its ligand specificity and, in some cases, abolish binding. SP-D and bovine conglutinin show an exceptionally high degree of amino acid sequence identity, but show different carbohydrate specificities, and therefore the comparison of the amino acid sequences of the two lectins over the C-type CRD should provide some indication of the potential residues responsible for the differences in carbohydrate specificity. The characterization of cDNA sequence for bovine SP-D (Lim et al., 1992) and its alignment with sequences of human and rat SP-D and the reported protein sequence for bovine conglutinin has allowed the identification of 16 positions within the C-type CRD which are conserved in all the three SP-Ds, but not in bovine conglutinin, and therefore these residues appear suitable sites for mutagenesis studies designed to attempt to change the carbohydrate specificity of the conglutinin CRD so that it becomes similar to that of SP-D.

REFERENCES

- Bordet, J. and Gay, F. P. (1906) *Ann. Inst. Pasteur* **20**, 467–498
- Chornazynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- Davis, A. E. and Lachmann, P. J. (1984) *Biochemistry* **23**, 2139–2144
- Drickamer, K. (1988) *J. Biol. Chem.* **263**, 9557–9560
- Floros, J., Steinbrink, R., Jacobs, K., Phelps, D., Kriz, R., Reeny, M., Sultzman, L., Jones, S., Tausch, H. W., Frank, H. A. and Fritsch, E. F. (1986) *J. Biol. Chem.* **261**, 9029–9033
- Friis-Christiansen, P., Thiel, S., Svehag, S. E., Dessau, R., Andersen, O. and Jensenius, J. C. (1990) *Scand. J. Immunol.* **31**, 453–460
- Hartley, C. A., Jackson, D. C. and Anders, E. M. (1992) *J. Virol.* **66**, 4358–4363
- Hase, S., Kikuchi, N., Ikenaka, T. and Inoue, K. (1985) *J. Biochem. (Tokyo)* **98**, 863–874
- Hirani, S., Lambris, J. D. and Muller-Eberhard, H. J. (1985) *J. Immunol.* **134**, 1105–1109
- Holmskov, U., Teisner, B., Pedersen, N. T., Laursen, S. B., Rasmussen, H. B. and Jensenius, J. C. (1992) *Immunology* **76**, 169–173
- Kuan, S.-F., Rust, K. and Crouch, E. (1992) *J. Clin. Invest.* **90**, 97–106
- Kuroki, Y., Shiratori, M., Ogasawara, Y., Akihiro, T. and Akino, T. (1991) *Biochim. Biophys. Acta* **1086**, 185–190
- Lachmann, P. J. (1967) *Adv. Immunol.* **6**, 479–527
- Lachmann, P. J. and Müller-Eberhard, H. J. (1968) *J. Immunol.* **100**, 691–698
- Lee, Y. M., Leiby, K. R., Allar, J., Paris, K., Lerch, B. and Okarma, T. B. (1991) *J. Biol. Chem.* **266**, 2715–2723
- Leon, M. A. and Yokohari, R. (1964) *Science* **143**, 1327–1328
- Lim, B. L., Lu, J. and Reid, K. B. M. (1993) *Immunol.* **78**, 159–165
- Loveless, R. W., Feizi, T., Childs, R. A., Mizuochi, T., Stoll, M. S., Oldroyd, R. G. and Lachmann, P. J. (1989) *Biochem. J.* **258**, 109–113
- Lu, J., Willis, A. C. and Reid, K. B. M. (1992) *Biochem. J.* **284**, 795–802
- Malhotra, R., Thiel, S., Reid, K. B. M. and Sim, R. B. (1990) *J. Exp. Med.* **172**, 955–959
- Malhotra, R., Harum, J., Thiel, S. and Sun, R. B. (1992) *Eur. J. Immunol.* **22**, 1437–1445
- Persson, A., Chang, D., Rust, K., Moxley, M., Longmore, W. and Crouch, E. (1989) *Biochemistry* **27**, 6361–6367
- Persson, A., Chang, D. and Crouch, E. (1990) *J. Biol. Chem.* **265**, 5755–5760
- Queensberry, M. S. and Drickamer, K. (1992) *J. Biol. Chem.* **267**, 1831–1841
- Rust, K., Grosso, L., Zhang, V., Chang, D., Persson, A., Longmore, W., Cai, G.-Z. and Crouch, E. (1991) *Arch. Biochem. Biophys.* **290**, 116–126
- Shimizu, H., Fisher, J. H., Papst, P., Benson, B., Lau, K., Mason, R. J. and Voelker, D. R. (1992) *J. Biol. Chem.* **267**, 1853–1857
- Strang, C. J., Slayter, H. S., Lachmann, P. J. and Davis, A. E. (1986) *Biochem. J.* **234**, 381–389
- Thiel, S., Baatrup, G., Friis-Christiansen, P., Svehag, S. E. and Jensenius, J. C. (1987) *Scand. J. Immunol.* **26**, 461–468
- Tiemeyer, M., Brandley, B. K., Zshihara, M., Swiedler, S. J., Greene, J., Hoyle, G. W. and Hill, R. L. (1992) *J. Biol. Chem.* **267**, 12252–12257
- van Iwaarden, J. F., Shimizu, H., van Golde, P. H. M., Voelker, D. R. and van Golde, L. M. G. (1992) *Biochem. J.* **286**, 5–11
- Weis, W. I., Kahn, R., Fourme, R., Drickamer, K. and Hendrickson, W. A. (1991) *Science* **254**, 1608–1615