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Amino acid sequence of β-galactoside-binding bovine heart lectin

Member of a novel class of vertebrate proteins

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A variety of animal tissues contain β-galactoside-binding lectins with molecular masses in the range 13–17 kDa. There is evidence that these lectins may constitute a new protein family although their function in vivo is not yet clear. In this work the major part of the amino acid sequence of the 13 kDa lectin from bovine heart muscle has been determined. Comparison of this sequence with the cDNA-deduced sequence published for the chick embryo skin lectin showed 58% homology. Comparison of the bovine lectin sequence with partial sequences from two cDNA clones from a human hepatoma library and partial amino acid sequences of human lung lectin showed 70, 40 and 85% homology, respectively. The sequences of these vertebrate lectins are thus clearly related, supporting earlier results of immunological cross-reactivity within this group of proteins. Computer searching of protein sequence databases did not detect significant homologies between the bovine lectin sequence and other known proteins.

Lectin; β-Galactoside binding; Amino acid sequence; Sequence homology; Protein family

1. INTRODUCTION

Bovine muscle tissues contain a soluble protein of subunit molecular mass 13 kDa that binds to β-galactosyl residues [1–3]. Lectins with similar properties have been isolated from a variety of animal tissues (review [4]), and strong antigenic cross-reactions have been observed between the bovine, human, monkey and porcine muscle lectins using both rabbit polyclonal and rat monoclonal antibodies to bovine heart lectin [2,5]. By immunoblotting of bovine tissue homogenates using a monoclonal antibody, 36/8, multiple antigenically cross-reactive components, behaving as proteins in the range 13 kDa to greater than 200 kDa have been revealed in diverse tissues. At least three of these, of 13, 26 and 36 kDa, had β-galactoside-binding activity [5]. Multiple lectins have also been observed in rat lung [6] and 3T3 mouse fibroblasts [7].

Immunoblotting of human lymphocytes with 36/8 antibody has revealed several immunoreactive proteins whose proportions change in transformed and mitogen-stimulated lymphocytes

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[8]. All these findings and the changes in lectin levels during foetal development [4] suggest that the lectins may be a group of 'household' proteins involved in cell growth regulation.

In this report we present approx. 90% of the amino acid sequence of the 13 kDa lectin of bovine heart muscle and compare this with 4 other vertebrate lectin sequences published recently. These comprise a cDNA-derived sequence for the β-galactoside-binding lectin from chicken skin [9], a partial amino acid sequence for human lung lectin, and the deduced sequences from two cDNA clones from a human hepatoma library isolated by immunoscreening [10]. A considerable degree of homology is found between bovine lectin and the other lectin sequences.

2. MATERIALS AND METHODS

Lectin was isolated from a homogenate of bovine heart by affinity chromatography on an asialofetuin-Sepharose 4B adsorbent as described in [1,2]. More than 90% of the protein was detectable as a single band of 13 kDa on SDS-polyacrylamide gel electrophoresis. Reductive carboxymethylation was carried out for the derivatisation of cysteine residues prior to sequence analysis. Protein fragmentation was carried out as described previously [11] using staphylococcal V8 protease (Sigma, Poole, England), TPCK-treated trypsin (Sigma), a lysine-specific protease, from *A. mellea* (a gift from Dr S. Doonan, University of Cork, Ireland) and iodosobenzoic acid cleavage for tryptophan residues [12]. The cleavage products were separated by reverse-phase HPLC using a 25 × 0.46 cm Bakerbond C_4 wide-pore column (HPLC Technology, Macclesfield, England) with gradients based on 0.1% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B). After concentration, the peptides, between 50 and 500 pmol, were sequenced on an Applied Biosystems 470A gas-phase sequencer with 120A on-line PTH analyser (Warrington, England). The determined sequence of bovine heart lectin was compared with all the sequences in the Dayhoff and Doolittle databases updated to August 1985 using the molecular genetic sequencing programmes based at the National Institute for Medical Research, Mill Hill, London. The searching algorithm was that of Korn et al. [13] with the parameters set at homology >60%, a minimum match of 5 amino acids and allowing up to 3 positions to be looped out. An additional homology search was carried out of the National Biomedical Research Foundation Database, updated to August 1986, using the 'Lsearch' programme of the Imperial Cancer Research Fund, London.

3. RESULTS AND DISCUSSION

When 1 nmol bovine heart lectin was applied to the sequencer no clear sequence could be detected, indicating that the protein was N-terminally blocked. However, internal sequences were readily obtained from a lectin protein digest using staphylococcal V8 protease (specific for the C-terminal side of Glu residues) and the resulting peptides were found to have sufficient homology to the reported sequence of chicken skin lectin [9] to allow alignments along the peptide chain. Peptides derived from cleavages with iodosobenzoic acid, trypsin, and lysine-specific protease, were able to provide the requisite overlapping sequences to connect all the V8 protease peptides and allow the unequivocal assignment of a continuous sequence of 125 residues. The alignment with the chicken skin lectin indicates the presence of a blocked N-terminal sequence and work is in progress to identify such a peptide and complete the protein sequence.

The amino acid sequence for the bovine lectin is shown in fig.1, aligned with the deduced sequence from the cDNA of the β-galactoside-binding lectin from chicken skin [9], a partial peptide sequence for the human lung lectin and the deduced sequences for two cDNA clones isolated from a human hepatoma library by screening with an anti-lectin serum [10].

There are considerable homologies between the 5 sequences shown in fig.1. Of the 30 overlapping amino acid positions between all 5 sequences only one position, 77, shows complete variability. Of the 54 overlapping amino acids in the bovine lectin and human lung lectin 46 are identical (85%). Similarly, 70% of the overlapping amino acids are shared between the bovine lectin and the deduced sequence from human clone 2. When the bovine lectin is compared with the chicken skin lectin 58% of the overlapping amino acids are homologous.

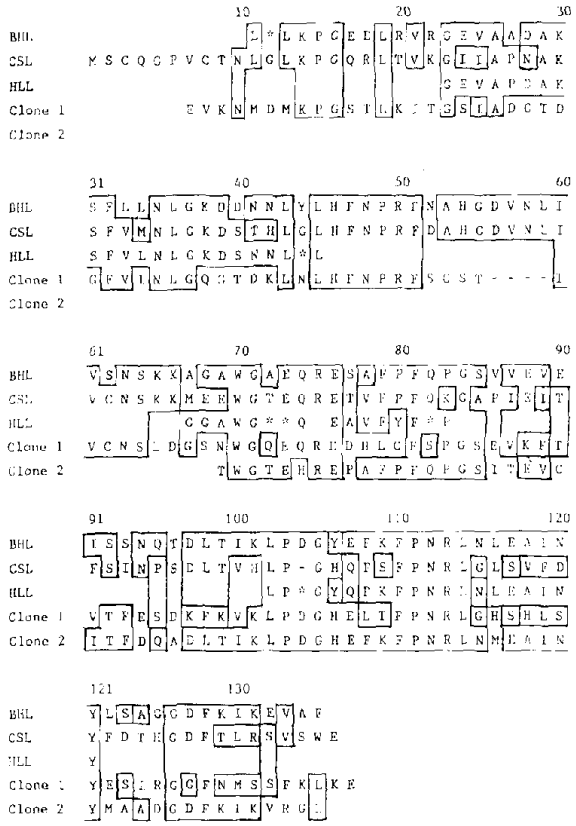

Fig.1. Comparison of amino-acid sequences for 5 vertebrate β-galactoside-binding lectins. BHL, bovine heart lectin determined in this work; CSL, cDNA of chick skin lectin [9]; HLL, peptides from human lung lectin [10]; Clone 1, cDNA from human hepatoma [10]; Clone 2, cDNA from human hepatoma [10]. Dashes indicate gaps introduced for optimal alignment, complete gaps, undetermined sequences, and * uncertain residue assignments. The residue numbering is taken from CSL [9]. Sequence identities between two or more chains are enclosed by boxes.

This value falls to 40% when the bovine sequence is compared to the deduced sequence from human clone 1. With the exception of clone 1 these homologies are in agreement with earlier studies which showed that the bovine lectin is more closely related antigenically to the human lectin [2] than chicken lectin [3]. An alternative alignment of the bovine heart lectin sequence can be produced by inserting 5 spaces after residue 127. Positions 128–132 would then have 4 identities and 1 conserved residue in common with the C-terminal pentapeptide of clone 1. In addition to the region 110–114, the positions 70, 71, 76, 81, 102, 103, 105, and 121 are identical in all 5 sequences. Comparing the bovine heart with the chicken skin lectin also indicates extended regions of highly conserved sequences at positions 45–66 and 102–114. Further investigations, including comparisons between additional vertebrate lectin sequences in relation to their oligosaccharide specificities, will be required to ascertain whether the homologies correspond to the sugar-binding site or some other common property of these proteins.

Using the molecular genetic sequencing program, the bovine sequence was also specifically compared with those of several other galactose-binding proteins, the B chain of *Ricinus communis* agglutinin [14], discoidin I [15], the N-terminal sequence of a galactose-binding protein from an invertebrate [16] and the rat hepatic asialoglycoprotein receptor [17], but no homologies were detected. A search of two data bases with the bovine heart lectin sequence at low stringency gave a large number of hits indicative of random relationships only. Gitt and Barondes [10] were also unable to detect any significant homologies when a database was searched with the sequences of hepatoma clones 1 and 2. This supports the idea that the soluble vertebrate lectins with β-galactoside binding may represent a new protein family.

We have attempted to localise the epitope recognised by the monoclonal antibody 36/8 raised against the bovine lectin which also cross-reacts with human muscle lectin [5] and have recovered antibody binding activity in the V8 protease fragment 24–73. Work is currently underway to define more precisely this epitope and to clarify the possible relationship of the 13 kDa bovine heart lectin to the immunoreactive bands of higher molecular mass detected by Carding et al. [5].

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