Biochem. J. (1989) 259, 283–290 (Printed in Great Britain)

Soluble bovine galactose-binding lectin

cDNA cloning reveals the complete amino acid sequence and an antigenic relationship with the major encephalitogenic domain of myelin basic protein

W. Mark ABBOTT,* Andrew MELLOR,† Yvonne EDWARDS‡ and Ten FEIZI*§

*Section of Glycoconjugate Research, M.R.C. Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, †Division of Immunology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, and ‡MRC Human Biochemical Genetics Unit, The Galton Laboratory, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, U.K.

A full-length cDNA clone for the 13–14 kDa soluble β-galactoside-binding lectin was isolated from a bovine fibroblast cDNA library. The derived amino acid sequence shows eight differences from a preliminary partial amino acid sequence given previously for the bovine heart lectin. This observation led to a re-examination of the data and correction of the heart lectin protein sequence. Except for a possible polymorphism of the heart lectin at position 57, the fibroblast and heart lectin sequences are considered identical. The epitope recognized by two monoclonal anti-(bovine lectin) antibodies, 36/8 and 9/5, was identified as the tetrapeptide sequence W-G-A/S-E/D by the isolation of several different cDNA clones from a human intestine cDNA library. A similar tetrapeptide is present in all of the soluble β-galactoside-binding animal lectins sequenced thus far. It is also found in myelin basic protein, which we show is antigenically cross-reactive with the lectin. In myelin basic protein the tetrapeptide is a part of the major domain previously shown to be responsible for the induction of experimental allergic encephalomyelitis.

INTRODUCTION

The soluble β-galactoside-binding lectin (subunit molecular mass 13–14 kDa; hereafter referred to as 14 kDa) from bovine muscle (De Waard *et al.*, 1976) is a member of a family of antigenically and structurally related animal lectins (Childs & Feizi, 1979*a*; Carding *et al.*, 1984; Southan *et al.*, 1987; Clerch *et al.*, 1988). It recognizes oligosaccharides with the terminal non-reducing sequence Galβ1-4GlcNAc or Galβ1-3GlcNAc and their analogues with α1-2-linked fucose, α1-3-linked galactose or α2-3-linked sialic acid to the galactose (De Waard *et al.*, 1976; Childs & Feizi, 1979*b*; Abbott *et al.*, 1988). Immunoblotting of various bovine tissues and human lymphocytes with the monoclonal antibody 36/8 to the bovine lectin revealed multiple antigenically cross-reactive proteins in the range 13–200 kDa. The relative proportions of these proteins change in transformed and stimulated lymphocytes (Carding *et al.*, 1985*a*,*b*). The relationship of these cross-reactive proteins to the 14 kDa lectin requires investigation. As a first step, the present studies were undertaken with the aim of obtaining the complete sequence of the bovine lectin and elucidating the structure of the 36/8 epitope. This has been achieved by isolating a full-length lectin cDNA clone and several different human cDNA clones which code for proteins expressing the 36/8 epitope. In addition, these studies have revealed an unsuspected antigenic and structural relationship of the lectin with myelin basic protein.

MATERIALS AND METHODS

Materials

Restriction endonucleases and DNA-modifying enzymes were purchased from Boehringer Mannheim (Lewes, Sussex, U.K.), Pharmacia (Milton Keynes, U.K.) or Gibco–BRL (Uxbridge, Middx. U.K.). The recombinant plasmid pHL14-2 containing a part of the gene for a soluble β-galactoside-binding lectin from a human hepatoma was given by Dr. S. H. Barondes (Gitt & Barondes, 1986). Other chemicals were from Sigma Chemical Co. (Poole, Dorset, U.K.) and were of the highest grade available.

Construction and screening of bovine fibroblast cDNA library

A cDNA library in pBR322 was prepared from polyadenylated RNA isolated from the bovine fibroblast cell line EBTr (Flow Laboratories, Irvine, Scotland, U.K.) by using oligo(dT) as a primer and the dG·dC-tailing procedure. The library (containing approx. 1×10^4 independent recombinants) was screened by hybridization to the *Eco*RI inserts of pHL14-2 labelled by the random priming method (Feinberg & Vogelstein, 1984). Positive colonies were re-screened twice at successively lower densities to ensure their purity.

Analysis of bovine cDNA clones isolated by hybridization to pHL14.2

Plasmid DNA was prepared from clones isolated from the bovine fibroblast cDNA library, digested with *Pst*I and electrophoresed through an agarose gel. The cDNA clone with the largest insert was further analysed. This insert was digested with *Pst*I, *Nsi*I and *Alu*I and the fragments were subcloned into the plasmid vector pSPT18 (Boehringer Mannheim). These fragments were sequenced on both strands by the dideoxy method (Sanger *et al.*, 1977), by using the SP6 and T7 promoter-specific primers (Boehringer Mannheim, and Anglian Biotechnology, Colchester, U.K., respectively). The com-

§ To whom correspondence and reprint requests should be addressed.

These sequence data have been submitted to the EMBL/GenBank Data Libraries.

plete lectin nucleotide sequence was compared with the GenBank DNA sequence database (updated to June 1988) by using the LSEARCH program (Dr. Graham Soundy, Imperial Cancer Research Fund, London).

Construction and screening of human intestine cDNA library

A human intestine cDNA library constructed in the bacteriophage vector λgt11 (Green *et al.*, 1987) and transfected into *Escherichia coli* Y1090 was screened with the monoclonal anti-lectin antibody 36/8 (Carding *et al.*, 1984). Five 140 mm-diam. plates each containing $\sim 1.5 \times 10^5$ plaques were assayed for immunoreactivity by overlaying with 132 mm-diam. nitrocellulose filters (Schleicher and Schuell) impregnated with isopropyl thiogalactoside. The filters were developed as described by Carding *et al.* (1984), except that the second antibody was peroxidase-conjugated sheep anti-rat IgM. The bound conjugate was detected by using the substrate diaminobenzidine [0.2 mg/ml in 22 mM-sodium phosphate buffer (pH 7.0) containing 0.03% (v/v) H_2O_2]. Immunoreactive plaques identified in the initial screen were purified by re-screening at successively lower densities until all plaques obtained were immunoreactive.

Analysis of human cDNA clones producing proteins reactive with antibody 36/8

Recombinant DNA was prepared from immunoreactive plaques (Maniatis *et al.*, 1982), and the cDNA insert size was determined by digestion with *Eco*RI and electrophoresis through an agarose gel. Fusion proteins with β-galactosidase were produced in the *E. coli* host C600 and analysed by SDS/polyacrylamide-gel electrophoresis as described by Price *et al.* (1987).

The cDNA inserts of the nine immunoreactive clones were sub-cloned into the plasmid vector pSPT18 and sequenced on both strands as described above. These sequences and derived amino acid sequences were compared with DNA and protein sequence databases by using the LSEARCH program as described above.

Proteins and peptides

Lectin was isolated from a homogenate of bovine heart by affinity chromatography on an asialofetuin–Sepharose 4B adsorbent as described by Childs & Feizi (1979*a*). Myelin basic protein was purified from human brain as described by Deibler *et al.* (1972). The *N*-terminal fragment 1–118 and the *C*-terminal fragment 119–178 of human myelin basic protein were obtained by BNPS-skatole cleavage (Burnett & Eylar, 1971). The myelin basic protein and peptides were given by Professor R. J. Thompson (University of Southampton).

Antibodies

The rat hybridoma-derived antibodies 36/8 and 9/5, raised against bovine heart-muscle lectin, were those described by Carding *et al.* (1984). An antiserum to human myelin basic protein produced by repeated monthly intradermal injections of the purified protein emulsified in Freund's complete adjuvant into New Zealand White rabbits was given by Professor R. J. Thompson. Peroxidase-conjugated sheep anti-rat IgM and sheep anti-rabbit IgG were purchased from Serotec (Slough, U.K.) and Sigma Chemical Co. respectively.

Solid-phase binding assay

The reactivities of the antibodies and antiserum with proteins and peptides were assayed by a solid-phase binding method described by Carding *et al.* (1984), except that the second antibody used was either a sheep anti-rat IgM–peroxidase conjugate (1:500 dilution) or a sheep anti-rabbit IgG–peroxidase conjugate (1:1000 dilution). Bound conjugate was detected by using the peroxidase substrate 2,2-azinobis-(3-ethylbenzthiazoline-sulphonic acid) (1 mg/ml in 60 mM-citric acid/65 mM-disodium phosphate, pH 4.0, containing 0.03% H_2O_2) and the A_{405} was measured.

RESULTS

Isolation of a full-length cDNA for the bovine fibroblast lectin

Screening of the bovine fibroblast cDNA library with the cDNA insert from the human lectin clone pHL14-2 (containing a part of the lectin gene) detected nine hybridizing colonies. Restriction analysis of the purified recombinant plasmids indicated that only two contained inserts of sufficient length to code for the complete amino acid sequence of the bovine lectin. One of these, designated pBL-1 (insert size approx. 600 bp), was sequenced completely, and the sequence is shown together with the derived amino acid sequence in Fig. 1. Approx. 150 bp of the other full-length clone were sequenced, and were identical with pBL-1. pBL-1 contains 43 bp of 5′ untranslated sequence, the entire coding sequence of 408 bp and 57 bp of 3′ untranslated sequence, excluding the poly(A) tail ($A_n = 40$ bp). A polyadenylation signal, AAUAAA, occurs 29 bp upstream of the poly(A) tail. The open reading frame codes for a polypeptide of 134 amino acids with a predicted molecular mass of 14316 Da. A comparison with the GenBank DNA-sequence database revealed an unexpected homology with an IgE-binding protein from rat basophilic leukaemia cells (Albrandt *et al.*, 1987), in addition to other soluble β-galactose-binding lectins (Ohyama *et al.*, 1986; Gitt & Barondes, 1986).

Identification of a homologous tetrapeptide sequence W-G-A/S-E/D in the lectin and in antigenically cross-reactive protein products of cDNA clones from human intestine

A screen of the human intestine cDNA library detected 45 plaques producing a fusion protein reactive with the anti-lectin antibody 36/8. Nine of these (designated λ36/8-1 to λ36/8-9) were plaque-purified and further analysed (Table 1). Eight of the clones had short inserts, ranging in size from 125 to 400 bp. Only one clone, λ36/8-5, had a relatively large insert, of 1600 bp. All nine clones expressed fusion proteins that were only slightly larger than β-galactosidase. The nine inserts were sub-cloned into the plasmid vector pSPT18. Insert DNAs from p36/8-6 and p36/8-7 were of similar size, and cross-hybridized, indicating that there were only eight independent clones. The inserts of p36/8-1, p36/8-2 and p36/8-3 were sequenced completely, and the amino acid sequence of both strands of DNA was predicted reading in frame with the β-galactosidase gene (Fig. 2). These sequences were clearly different from the bovine galactose-binding lectin. The only common feature of the derived amino acid sequences is the tetrapeptide W-G-

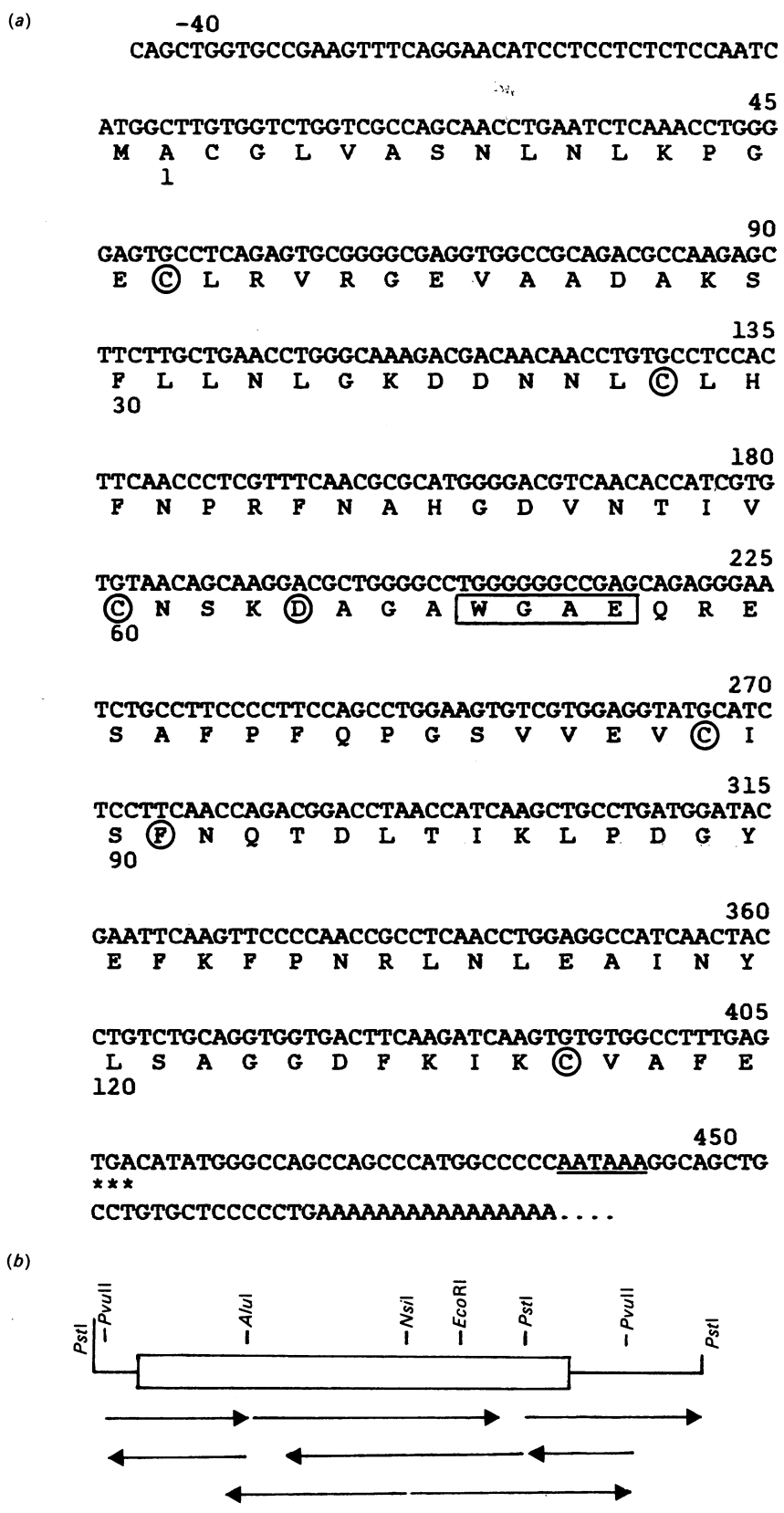

Fig. 1. (*a*) Nucleotide and derived amino acid sequence of the cDNA (pBL-1) of bovine fibroblast β-galactoside-binding lectin and (*b*) strategy for sequencing clone pBL-1

The presumptive poly(A) addition signal is underlined. The stop codon is indicated by three asterisks. The nucleotide sequence is numbered with the adenine of the methionine codon designated as 1. The derived amino acid sequence is numbered with the first alanine designated as 1. The tetrapeptide identified as the epitope recognized by antibody 36/8 is boxed. With the possible exception of the threonine at position 57 (see the Discussion section), the revised sequence for the bovine heart lectin sequence agrees with the derived sequence for the fibroblast lectin. The re-assigned amino acids for the heart lectin are circled.

p36/8-1

```
3'GGACCTCGTACCCCTCGACTTTCACAACGTTCCTTTGACGACACA
5'CCTGGAGCATGGGGAGCTGAAAGTGTTGCAAGGAAACTGCTGTGT
   P  G  A [W  G  A  E] S  V  A  R  K  L  L  C

CTTTAAACTAGTCCAGGAACCATAAGTAAAAATACAAAGTTATTGA
GAAATTTGATCAGGTCCTTGGTATTCATTTTTATGTTTCAATAACT
 E  I ***

                                      *** R
CTAAATAAAAAAGGGGGAACATGAATTCTAAATTGC5'
GATTTATTTTTTCCCCCTTGTACTTAAGATTTAACG3'
```

p36/8-2

```
   A  R  W  F  S  G  A  T  A  V  G  L  V  Q  P
3'GCGAGAGGTCTTTGATGGACGCCATCGGTGAGGGTCCTGGACCCC
5'CGCTCTCCAGAAACTACCTGCGGTAGCCACTCCCAGGACCTGGGG
   R  S  P  E  T  T  C  G  S  H  S  Q  D  L  G

 S  T  C  G  P  P  R  L  A  E  L  H  I  Q  S
TCTGCACGTGGGACCCCCAGACTCCCGGAGGTCCACTTAGACTCT
AGACGTGCACCCTGGGGGTCTGAGGGCCTCCAGGTGAATCTGAGA
 R  R  A  P [W  G  S  E] G  L  Q  V  N  L  R

 P  L  G  E  L  A  T  T  T  A  Q  S  L  T  P
CCCTTCTGGGAGGTCACGACAGCAACATCGGACCGACTCCCACCC5'
GGGAAGACCCTCCAGTGCTGTCGTTGTAGCCTGGCTGAGGGTGGG3'
 G  K  T  L  Q  C  C  R  C  S  L  A  E  G  G
```

p36/8-3

```
3'GCGTCTGCCACCTCGCCGCGAAGGAGTCGAGGGCGCCAGTCCGGG
5'CGCAGACGGTGGAGCGGCGCTTCCTCAGCTCCCGCGGTCAGGCCC
   R  R  R  W  S  G  A  S  S  A  P  A  V  R  P

            *** L  G  A  V  P  A  V  R  G  M
GTAGTCCCTACCAGTGTCAGGTCGGTGTCCCCGCTGAGACGGGTA
CATCAGGGATGGTCACAGTCCAGCCACAGGGGCGACTCTGCCCAT
 H  Q  G  W  S  Q  S  S  H  R  G  D  S  A  H

 L  G  G  P  A  A  V  L  D  Q  S  P  C  C  R
GTCAGGGGGACCCCGTCGCTGGTCTAGGACCGACCCCGTCGTGGC
CAGTCCCCCTGGGGCAGCGACCAGATCCTGGCTGGGGCAGCACCG
 Q  S  P [W  G  S  D] Q  I  L  A  G  A  A  P

 S  L  G  E  R
CCTGTCCGGGAGAGC5'
GGACAGGCCCTCTCG3'
 G  Q  A  L  S
```

Fig. 2. Nucleotide and deduced amino acid sequences of both strands of three completely sequenced human cDNA clones designated p36/8-1, p36/8-2 and p36/8-3 that express proteins reactive with antibody 36/8

Peptide sequences that are similar in the three clones and the bovine lectin are indicated by boxing. Stop codons are indicated by three asterisks.

Table 1. cDNA clones expressing fusion proteins reactive with antibody 36/8

The approximate size of insert DNA was estimated by electrophoresis through a 1%-agarose gel, and the approximate size of the fusion protein with β-galactosidase was determined by SDS/polyacrylamide-gel electrophoresis as described in the Materials and methods section.

Clone designation	Insert size (kb) (approx.)	Fusion-protein size (kDa) (approx.)
λ36/8-1	0.127	123
λ36/8-2	0.135	130
λ36/8-3	0.150	133
λ36/8-4	0.350	120
λ36/8-5	1.600	125
λ36/8-6	0.250	123
λ36/8-7	0.300	125
λ36/8-8	0.220	124
λ36/8-9	0.400	123

A/S-E/D. Partial nucleotide sequencing of the five other clones was performed, and possible amino acid sequences were deduced (Fig. 3). Each of these contained nucleotide sequence capable of encoding the tetrapeptide W-G-A/S-E/D in frame with the β-galactosidase gene. A similar tetrapeptide (W-G-A-E) is found in the bovine lectin sequence with the tryptophan at position 68 (Fig. 1).

These results and the previous observation (Southan *et al.*, 1987) that reactivity with monoclonal antibody 36/8 is associated with a peptide fragment containing amino acids 22–71 of the lectin indicate that the tetrapeptide W-G-A/S-E/D is the epitope recognized by 36/8. The second monoclonal antibody, 9/5, raised against the bovine lectin also reacted with fusion proteins encoded by all of the recombinants λ36/8-1 to λ36/8-9, indicating that the epitope for this antibody involves the same tetrapeptide sequence.

Nucleotide sequences from the eight recombinants were submitted to the GenBank DNA sequences database (updated to June 1988) to search for sequence similarities. The cDNA insert of p36/8-1 is identical with part of the human aldolase β gene from nucleotide positions 1342 to 1466 (Sakakibara *et al.*, 1985). This homology is in the non-coding part of the aldolase β gene, and furthermore the immunoreactive tetrapeptide is expressed from the anti-sense strand. Thus it seems unlikely that the protein product of p36/8-1 would exist in Nature, and it is expressed merely as a result of cloning and expression in λgt11. The insert of p36/8-8 contains an *Alu* repetitive sequence, which is similar to many sequences in the database, although none showed an exact match. No significant similarities were detected with the other six independent clones.

The tetrapeptide sequence confirmed as the cross-reactive epitope through studies with myelin basic protein

A search was made of the National Biomedical Research Foundation database for protein sequences containing any of the four possible permutations of the tetrapeptide sequence W-G-A/S-E/D. Only two such proteins were found; myelin basic protein from several different mammalian species (Table 2) has the sequence W-G-A-E at position 116–119, and cytochrome *b* from the mitochondrion of *Trypanosoma brucei* contains the sequence W-G-S-E, at positions 167–170. When purified human myelin basic protein was tested for antigenic cross-reactivity with the purified bovine heart-muscle lectin the results were unequivocal. Myelin basic protein reacted with both anti-lectin monoclonal antibodies 36/8 and 9/5, and the lectin clearly reacted with the polyclonal rabbit antiserum to myelin basic protein (Figs. 4*a* and 4*b*). The two fragments of myelin basic protein produced by chemical cleavage at the *C*-terminal

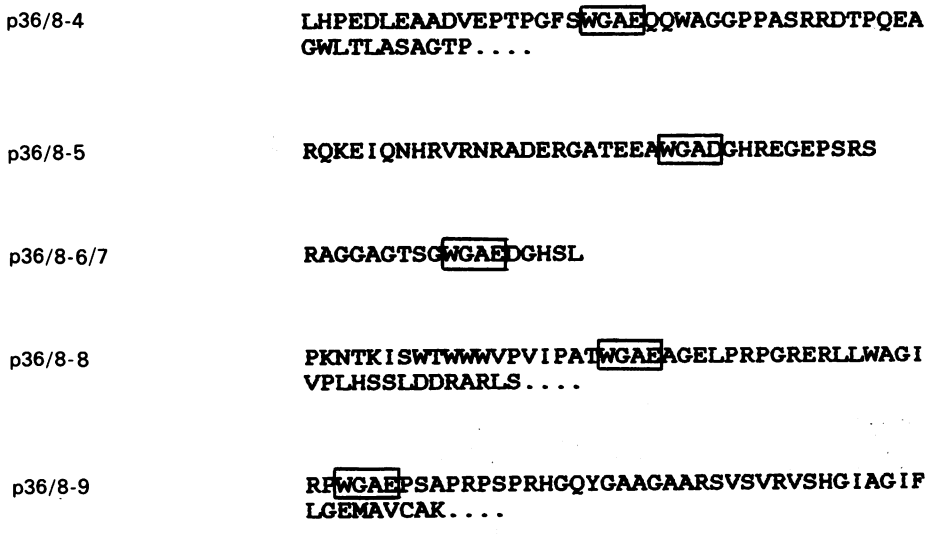

Fig. 3. Possible deduced amino acid sequences of the six partially sequenced human cDNA clones that express proteins reactive with antibody 36/8

A stop codon in frame with the β-galactosidase gene of λgt11 was found with clones p36/8-5 and p36/8-6/7. The common tetrapeptide sequence is indicated by boxing.

Table 2. Comparison of the conserved tetrapeptide sequence found in several β-galactoside-binding lectins, myelin basic protein and cytochrome *b* of *Trypanasoma brucei*

The myelin-basic-protein sequences are from human, chimpanzee, monkey, bovine, rabbit and guinea-pig tissues.

Source	Tetrapeptide	Reference
Bovine 14 kDa lectin	WGAE	Southan *et al.* (1987); the present work
Human hepatoma clone 1	WGQE	Gitt & Barondes (1986)
Human hepatoma clone 2	WGTE	Gitt & Barondes (1986)
Chicken skin lectin	WGTE	Ohyama *et al.* (1986)
Human placenta lectin	WGTE	Paroutaud *et al.* (1987)
Electric eel lectin	WGTE	Paroutaud *et al.* (1987)
Rat lung lectin	WGTE	Clerch *et al.* (1988)
Mouse 14 kDa lectin	WGTE	Raz *et al.* (1988)
Mouse 34 kDa lectin	WGKE	Raz *et al.* (1988); Jia & Wang, (1988)
Myelin basic protein	WGAE	Carnegie (1971); Eylar *et al.* (1971*b*)
Cytochrome *b* (*T. brucei*)	WGSE	Benne *et al.* (1983)

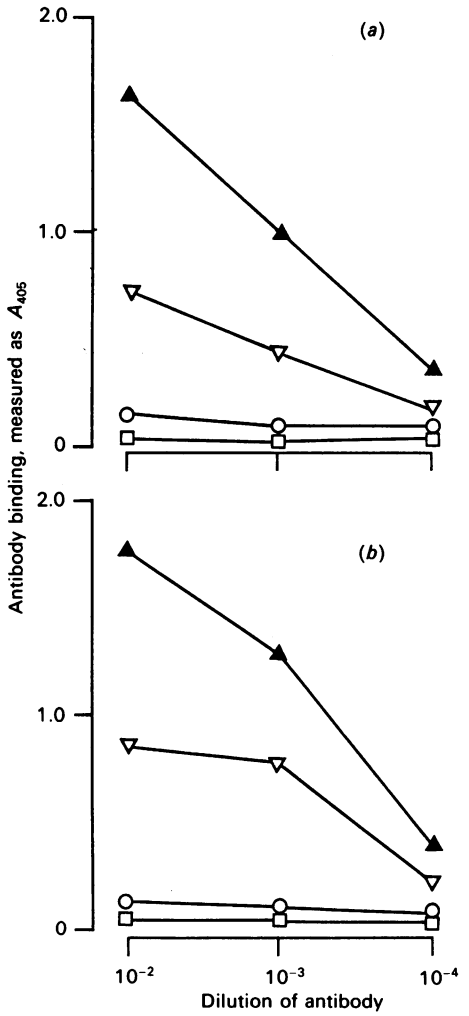

Fig. 4. Antigenic cross-reactions between bovine heart lectin (▲) and myelin basic protein (▽), but not the *N*-terminal peptide 1–118 (○) or *C*-terminal peptide 119–178 (□) of myelin basic protein, by using anti-lectin antibodies 36/8 (*a*) and 9/5 (*b*)

Binding of the two antibodies to the proteins or peptides immobilized on microtitre plates was measured as described in the Materials and methods section.

side of tryptophan-116, which reacted with the rabbit antiserum (Fig. 5*a*), did not react with the anti-lectin antibodies (Figs. 4*a* and 4*b*), thus emphasizing the importance of the intact tetrapeptide sequence in the epitope recognized by the anti-lectin antibodies. Antibody against the *Trypanosoma*, mitochondrion cytochrome *b* was not available for testing.

DISCUSSION

The salient features of these studies are: (1) the prediction of the complete amino acid sequence of the 14 kDa bovine fibroblast lectin from a full-length cDNA; (2) the identification of the tetrapeptide sequence W-G-A/S-E/D as an integral part of the epitope recognized by the monoclonal anti-lectin antibodies 36/8 and 9/5; and (3) the demonstration that myelin basic protein, which shares the tetrapeptide sequence, is immunologically cross-reactive with the bovine lectin.

The primary protein structure derived from the DNA sequence of the bovine fibroblast lectin differs in eight positions from the preliminary partial amino acid sequence given previously for the bovine heart lectin (Southan *et al.*, 1987). The data for the heart lectin have been re-examined and seven of the residues re-assigned, as indicated in Fig. 1. These now agree with the derived fibroblast lectin sequence. At position 57 the derived sequence indicated threonine. However, there was evidence of either threonine or leucine at this position in the bovine heart protein sequence, suggesting a possible polymorphism. With this latter exception, it would appear that the β-galactoside-binding proteins from bovine heart and the fibroblast cell line are identical.

As with the chicken skin lectin (Ohyama *et al.*, 1986) and the rat lung lectin (Clerch *et al.*, 1988), there was no evidence in the bovine lectin sequence of a signal peptide characteristic of many membrane-associated and secreted proteins. This is in agreement with immunofluorescence studies of human lymphoblastoid cells (Carding *et al.*, 1985*b*) and various bovine tissues (S. J. Thorpe, S. Carding, P. Fryer & T. Feizi, unpublished work), using the anti-lectin antibody 36/8, which show predominant intracellular staining, rather than at the cell surface.

There is a considerable similarity between the three

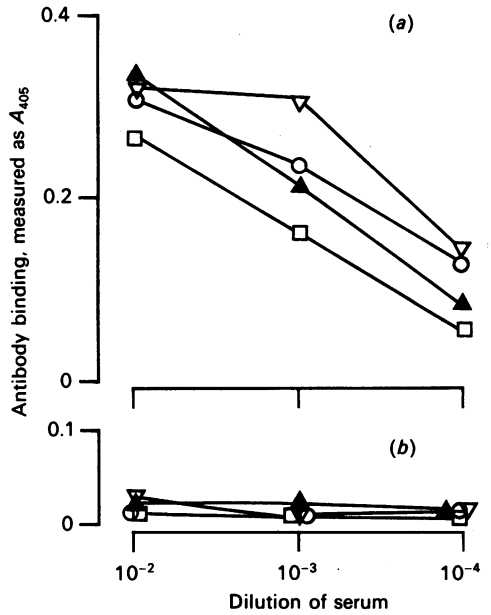

Fig. 5. Binding of rabbit anti-(myelin basic protein) serum (*a*) to bovine heart lectin (▲), myelin basic protein (▽) and to *N*-terminal peptide 1-118 (○) and *C*-terminal peptide 119-178 (□) of myelin basic protein

Panel (*b*) shows the lack of binding of a non-immune rabbit serum to these proteins. The binding assays with the proteins and peptides immobilized on microtitre plates were performed as described in the Materials and methods section.

complete 14 kDa lectin sequences published so far. The revised bovine lectin sequence shares 85% of its amino acids with the rat lung lectin (Clerch *et al.*, 1988) and 56% with the chicken skin lectin (Ohyama *et al.*, 1986). The polypeptide regions at positions 44–49, 58–62 and 68–74 (the last includes the tetrapeptide W-G-A/S-E/D) are very conserved not only among the 14 kDa lectins but also among related galactose-binding proteins (Gitt & Barondes, 1986; Raz *et al.*, 1988; Jia & Wang, 1988), suggesting that these protein regions may be important functionally.

The IgE-binding protein detected in a search of the GenBank database may be predicted to be a carbohydrate-binding protein. A close examination of its sequence showed that it contains the W-G-K-E sequence and has more than 95% identity with a galactose-binding protein (CBP35) from mouse 3T3 fibroblasts (Jia & Wang, 1988), suggesting that it is probably the rat homologue of CBP35. The finding that an IgE-binding protein is a member of this family of proteins is intriguing, as it is suggestive that lectin–carbohydrate interactions may mediate some of the effects of IgE.

The tetrapeptide sequence W-G-A/S-E/D as part of a larger peptide is clearly sufficient for recognition by the two antibodies 36/8 and 9/5. The tetrapeptide (W-G-X-E) is conserved among soluble β-galactoside-binding lectins that have been partially or fully sequenced (Table 2). It will be interesting to investigate whether they all react with the two monoclonal antibodies.

The occurrence of the tetrapeptide sequence in myelin basic protein and the antigenic cross-reaction with the lectin is of considerable immunological interest. The tetrapeptide in myelin basic protein is a part of the main domain involved in the induction of experimental allergic encephalomyelitis (Eylar *et al.*, 1971*a*), Trp^{116}-Gly-Ala-Glu-Gly-Gln-Lys^{122}. It would be of interest to investigate if the tetrapeptide epitope shared between the lectins and myelin basic protein is an autoantigen in autoimmune diseases of man.

The conserved nature of the tetrapeptide among lectins from different species (Table 2) suggests that it may be important functionally, for example in carbohydrate binding. There is a precedent for short function-associated peptides, i.e. the R-G-D sequence on the cell-binding domains of the integrin family of proteins (Ruoslahti & Pierschbacher, 1986). It has been suggested previously (Levi & Teichberg, 1981; Paroutaud *et al.*, 1987) that the conserved tetrapeptide in question is involved in β-galactoside binding, as lactose was found to enhance the fluorescence of the lectin and to protect the tryptophan from oxidation. However, neither 36/8 or 9/5 antibodies interfered with lectin haemagglutination, nor did lactose at a concentration of 0.2 M prevent the lectin binding to a 36/8-antibody adsorbent (Carding *et al.*, 1984); this lactose concentration is 500 times that required to give 50% inhibition of lectin binding to rabbit erythrocytes (Abbott *et al.*, 1988). These results cannot be taken as conclusive, however, as the two antibodies react preferentially with the denatured lectin (Carding *et al.*, 1984) and only precipitate the native protein poorly (S. R. Carding & R. A. Childs, unpublished work).

Myelin basic protein has been shown to bind to certain gangliosides (Yohe *et al.*, 1983; Ong & Yu, 1984). In addition the phosphorylation of myelin basic protein (at Ser^{115}) is inhibited by added gangliosides (Jesse-Chan, 1987; Kreutter *et al.*, 1987). These observations raise the intriguing possibility that (*a*) there may be carbohydrate-binding activity in this part of myelin basic protein, which includes the tetrapeptide epitope

$$\overset{115}{\text{FSWGAEGQK}}$$

and (*b*) that interaction with carbohydrate may regulate phosphorylation.

At present it is difficult to be certain that the W-G-A/S-E/D sequence is involved in carbohydrate binding, and indeed this sequence may confer an additional function on the galactose-binding lectin. The concept that lectins may have more than one function is not new. For example, discoidin I has a carbohydrate-binding site and a distinct cell-recognition tripeptide signal, R-G-D (Gabius *et al.*, 1985), and receptors for insulin-like growth factor II and elastin each have distinct sites for protein–protein and protein–carbohydrate interactions (Tong *et al.*, 1988; Hinek *et al.*, 1988). The 35 kDa galactose-binding lectin may also be bifunctional, since it contains two distinct domains, one of which is characteristic of soluble β-galactoside-binding proteins, whereas the other shows homology with proteins of the heteronuclear ribonucleoprotein complex (Jia & Wang, 1988).

The 36/8 monoclonal antibody used in the present studies detects several other proteins after electrophoresis and immunoblotting (Carding *et al.*, 1985*a*,*b*) and has identified a number of unrelated cDNA clones. Some of these gene products may represent a family of proteins related to the 14 kDa lectin (Roff & Wang, 1983; Gitt &

Barondes, 1986; Raz *et al.*, 1988; Hinek *et al.*, 1988; Jia & Wang, 1988), but others may be unrelated, except for the W-G-A/S-E/D sequence. From our present studies it seems likely that immunoreactive proteins of 17–18 kDa seen after electrophoresis of brain and spinal cord (Carding *et al.*, 1985*a*) represent myelin basic protein.

We are grateful to Professor S. H. Barondes for providing the recombinant plasmid pHL14-2, to Professor R. J. Thompson for providing preparations of myelin basic protein, peptides and antiserum, and to Mrs. Sheila Kingsley for the preparation of the manuscript.

REFERENCES

Abbott, W. M., Hounsell, E. F. & Feizi, T. (1988) Biochem. J. **252**, 283–287
Albrandt, K., Orida, N. K. & Liu, F.-T. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 6859–6863
Benne, R., De Vries, F., Vanden Berg, J. & Klover, B (1983) Nucleic Acids Res. **11**, 6925–6941
Burnett, P. R. & Eylar, H. (1971) J. Biol. Chem. **246**, 3425–3430
Carding, S. R., Thorpe, R., Childs, R. A., Spitz, M. & Feizi, T. (1984) Biochem. J. **220**, 253–260
Carding, S. R., Childs, R. A., Thorpe, R., Spitz, M. & Feizi, T. (1985*a*) Biochem. J. **228**, 147–153
Carding, S. R., Thorpe, S. J., Thorpe, R. & Feizi, T. (1985*b*) Biochem. Biophys. Res. Commun. **127**, 680–686
Carnegie, P. R. (1971) Biochem. J. **123**, 57–67
Childs, R. A. & Feizi, T. (1979*a*) Biochem. J. **183**, 755–758
Childs, R. A. & Feizi, T. (1979*b*) FEBS Lett. **99**, 175–179
Clerch, L. B., Whitney, P., Hass, M., Brew, K., Miller, T., Werner, R. & Massaro, D. (1988) Biochemistry **27**, 692–699
Deibler, G. E., Martenson, R. E. & Kies, M. W. (1972) Prep. Biochem. **2**, 139–165
De Waard, A., Hickman, S. & Kornfeld, S. (1976) J. Biol. Chem. **251**, 7581–7587
Eylar, E. H., Westall, F. C. & Brostoff, S. (1971*a*) J. Biol. Chem. **246**, 3418–3424
Eylar, E. H., Brostoff, S., Hashim, G., Coccam, J. & Burnett, P. (1971*b*) J. Biol. Chem. **246**, 5770–5784
Feinberg, A. P. & Vogelstein, B. (1984) Anal. Biochem. **137**, 266–267
Gabius, H.-J., Springer, W. R. & Barondes, S. H. (1985) Cell **42**, 449–456
Gitt, M. A. & Barondes, S. H. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7603–7607
Green, F., Edwards, Y., Houri, H.-P., Povey, S., Ho, M. W., Pinto, M. & Swallow, D. (1987) Gene **57**, 101–110
Hinek, A., Wrenn, D. S., Mecham, R. P. & Barondes, S. H. (1988) Science **239**, 1539–1514
Jesse-Chan, K.-F. (1987) J. Biol. Chem. **262**, 2415–2422
Jia, S. & Wang, J. L. (1988) J. Biol. Chem. **263**, 6009–6011
Kreutter, D., Kim, J. Y. H., Goldenring, J. R., Rasmussen, H., Ukomada, C., Delorenzo, R. J. & Yu, R. K. (1987) J. Biol. Chem. **262**, 1633–1637
Levi, G. & Teichberg, V. I. (1981) J. Biol. Chem. **256**, 5735–5740
Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, New York
Ohyama, Y., Hirabayashi, J., Oda, Y., Ohno, S., Kawasaki, H., Suzuki, K. & Kasai, K. I. (1986) Biochem. Biophys. Res. Commun. **134**, 51–56
Ong, R. L. & Yu, R. K. (1984) J. Neurosci. Res. **12**, 377–393
Paroutaud, P., Levi, G., Teichberg, V. I. & Strosberg, A. D. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 6345–6348
Price, G. J., Jones, P., Davison, M. D., Patel, B., Eperon, I. C. & Critchley, D. R. (1987) Biochem. J. **245**, 595–603
Raz, A., Carmi, P. & Pazerini, G. (1988) Cancer Res. **48**, 645–649
Roff, C. F. & Wang, J. L. (1983) J. Biol. Chem. **258**, 10657–10663
Ruoslahti, E. & Pierschbacher, M. D. (1986) Cell **44**, 517–518
Sakakibara, M., Mukai, T., Yatsuki, H. & Hori, K. (1985) Nucleic Acids Res. **13**, 5055–5069
Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 5463–5467
Southan, C., Aitken, A., Childs, R. A., Abbott, W. M. & Feizi, T. (1987) FEBS Lett **214**, 301–304
Tong, P. Y., Tollefsen, S. E. & Kornfeld, S. (1988) J. Biol. Chem. **263**, 2585–2588
Yohe, H. C., Jacobson, R I. & Yu, R. K. (1983) J. Neurosci. Res. **9**, 401–412

Received 22 August 1988/10 November 1988; accepted 16 November 1988