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Evidence that the 14 kDa soluble β-galactoside-binding lectin in man is encoded by a single gene

W. Mark ABBOTT and Ten FEIZI*
Section of Glyconjugate Research, MRC Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, U.K.

A full-length cDNA clone for the 14 kDa soluble β-galactoside-binding lectin of man has been isolated from a cDNA library from HepG2 hepatoma cells. The derived amino acid sequence is identical with that of the 14 kDa lectin from human placenta. The results of Northern and Southern blotting of several different human cell lines using a cDNA probe for the 14 kDa lectin suggest the presence of a single gene for this protein. Thus, although there are multiple proteins in the range 14–200 kDa which are antigenically related to this lectin, we would conclude from the present study that there is only one gene for the 14 kDa lectin.

INTRODUCTION

Widely distributed in animal cells is a soluble carbohydrate-binding protein (lectin) of 14 kDa that is readily detected by its affinity for non-reducing terminal β-galactoside residues [1]. The endogenous ligands may additionally include blood-group-related and sialylated oligosaccharides, related to the Galβ1-4Glc or Galβ1-4/3GlcNAc sequences deduced from inhibition-of-binding data [2–4]. The level of this lectin is developmentally regulated in certain cell types [1,5,6]. Immunochemical studies have suggested that the 14 kDa lectins of different animal species are structurally conserved and that they are members of a family of nuclear and cytoplasmic proteins in the range 14 kDa to greater than 200 kDa, whose levels change in transformed and stimulated cells [7–12]. Protein-structure and gene-cloning studies have shown that there are proteins with amino acid sequences related to that of the 14 kDa lectin, and that one of these is a 35 kDa galactose-binding protein which has, in addition to a carbohydrate-binding domain, a second domain homologous with some of the heterogeneous nuclear ribonucleoproteins [13]. In the present paper we describe the isolation of a full-length cDNA clone for the 14 kDa lectin from the human hepatoma cell line HepG2 and demonstrate that the derived sequence is identical with that of the human placental lectin [14]. Together with the results of Northern- and Southern-blotting analyses of RNA and DNA from human cell lines, the data indicate that there is only one gene for the human 14 kDa galactose-binding lectin.

MATERIALS AND METHODS

Cells

The sources and culture conditions for the lymphoblastoid cell lines, CEM and Daudi, and the myeloid cell line, K562, were as described previously [12]. Other cells used were the HepG2 hepatoma cell line (provided by Dr. J. Scott of this Institute, and described in [15]), the monocytic cell line THP-1 [provided by Dr. R. Thorpe, National Institute of Biological Standards and Control (then at Holly Hill, Hampstead, London N.W.3, U.K.)] and the fibroblast cell line, MRC-5 (Flow Laboratories, Irvine, Scotland, U.K.).

Construction and screening of a cDNA library from HepG2 cells

A cDNA library in λgt10 (generously provided by Dr. J. Scott) was prepared from poly(A)$^+$ RNA isolated from HepG2 cells using oligo(dT) as a primer. Approx. 10^6 recombinant phage plaques were screened after transfer to nylon membranes by hybridization to the *Pvu*II fragment of the bovine lectin plasmid pBL-1 [16] labelled by the random priming method [17]. Hybridization was carried out at 65 °C in 3 × SSC, 10 × Denhardt's (0.2% each of bovine serum albumin, Ficoll 400 and polyvinylpyrollidone) and 0.2% SDS. After hybridization filters were washed at 65 °C under conditions of either low or medium stringency (3 × SSC/0.1% SDS or 0.5 × SSC/0.1% SDS respectively) and autoradiographed for 18 h. Positive plaques were purified by rescreening at successively lower plaque densities until all plaques hybridized and then were analysed and sequenced on both strands as described previously [16].

Southern and Northern blotting

High-molecular-mass DNA (10 μg) prepared from HepG2 cells was digested with restriction endonucleases *Eco*RI, *Pst*I, *Bgl*II or *Hin*dIII, electrophoresed through a 0.7%-agarose gel and transferred to nylon membranes. Total cellular RNA was prepared by the lithium chloride/urea method [18]. Poly(A)$^+$ RNA was enriched by one cycle of binding to oligo(dT)–cellulose [19]. RNA was electrophoresed through 1% agarose gels containing 7% formaldehyde and transferred to nylon membranes. Hybridization to a lectin cDNA in both Southern and Northern blots was as described above, under conditions of low stringency. After autoradiography, Northern filters were reprobed with a *Bgl*II restriction fragment of a β-actin gene [20] to assess loading of RNA in each lane.

Abbreviations used: poly(A)$^+$, polyadenylated; SSC, 0.15 M-NaCl/0.015 M-sodium citrate.
* To whom correspondence should be addressed.

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                                            GGGC
  -40
TGACAGCTGGTGCGCCTGCCCGGGAACATCCTCCTGGACTCAATC
                                              45
ATGGCTTGTGGTCTGGTCGCCAGCAACCTGAATCTCAAACCTGGA
 M  A  C  G  L  V  A  S  N  L  N  L  K  P  G
    1
                                              90
GAGTGCCTTCGAGTGCGAGGCGAGGTGGCTCCTGACGCTAAGAGC
 E  C  L  R  V  R  G  E  V  A  P  D  A  K  S

                                             135
TTCGTGCTGAACCTGGGCAAAGACAGCAACAACCTGTGCCTGCAC
 F  V  L  N  L  G  K  D  S  N  N  L  C  L  H
 30
                                             180
TTCAACCCTCGCTTCAACGCCCACGGCGACGCCAACACCATCGTG
 F  N  P  R  F  N  A  H  G  D  A  N  T  I  V

                                             225
TGCAACAGCAAGGACGGCGGGGCCTGGGGGACCGAGCAGCGGGAG
 C  N  S  K  D  G  G  A  W  G  T  E  Q  R  E
 60
                                             270
GCTGTCTTTCCCTTCCAGCCTGGAAGTGTTGCAGAGGTGTGCATC
 A  V  F  P  F  Q  P  G  S  V  A  E  V  C  I

                                             315
ACCTTCGACCAGGCCAACCTGACCGTCAAGCTGCCAGATGGATAC
 T  F  D  Q  A  N  L  T  V  K  L  P  D  G  Y
 90
                                             360
GAATTCAAGTTCCCCAACCGCCTCAACCTGGAGGCCATCAACTAC
 E  F  K  F  P  N  R  L  N  L  E  A  I  N  Y

                                             405
ATGGCAGCTGACGGTGACTTCAAGATCAAATGTGTGGCCTTTGAC
 M  A  A  D  G  D  F  K  I  K  C  V  A  F  D
 120
TGAAATCAGCCAGCCCATGGCCCCCAATAAAGGCAGCTGCCTCTG
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CCCCG
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Fig. 1. Nucleotide and derived amino acid sequence of the cDNA of human β-galactoside-binding lectin from HepG2 cells

The nucleotide sequence is numbered with the adenine of the initiating methionine designated as '1'. The derived amino acid sequence is numbered with the first alanine residue designated '1'.

RESULTS

Screening of the HepG2 cDNA library with a bovine lectin cDNA probe under the conditions of low stringency revealed 20 hybridizing plaques. All 20 plaques still hybridized when washed more stringently. Eight of the plaques were purified and restriction analysis showed that all eight had an internal *Eco*RI site. The clone with the longest insert was sequenced completely (Fig. 1) and the derived sequence found to be identical with the complete amino acid sequence of a 14 kDa β-galactoside-binding lectin from human placenta [14].

In order to resolve the question of the multiplicity of genes for 14 kDa β-galactoside-binding lectins in man, we examined the lectin gene and its expression by Southern- and Northern-blot analyses. A Southern blot of DNA isolated from the HepG2 cell line is shown in Fig. 2(*a*). Even under conditions of low stringency, the pattern of hybridizing bands is consistent with the presence of only one gene with an internal *Eco*RI site (nucleotide positions 316–321 in Fig. 1) and probably an internal *Pst*I site in an intron.

The expression of the human lectin gene in the lymphoblastoid, myeloid, fibroblast, monocytic and hepatoma cell lines was analysed by Northern blotting under conditions of low stringency. All the cell lines tested had a transcript of approx. 750 bp (Fig. 2*b*). In addition to this small transcript there was a faintly hybridizing band at 4–5 kb in some of the cell lines (Fig. 2*a*; lanes M, D, H and K). Although this is approximately the right position for the 28 S ribosomal RNA, it is not ribosomal RNA as it is greatly enhanced in poly(A)$^+$-enriched RNA (Fig. 2*c*). Although it could represent a related gene, it seems more likely that it is unprocessed precursor RNA as there are additional faint bands between the 750 bp and 4–5 kb transcripts (e.g. Fig. 2*b*, lane M, and Fig. 2*c*) and the strength of hybridization of these larger transcripts was the same as the 750 bp message (results not shown).

DISCUSSION

The aim of the present study was to investigate whether there are multiple genes coding for the 14 kDa soluble β-galactoside-binding lectin in man. We have isolated a full-length cDNA clone for this lectin from the human hepatoma cell line HepG2 and shown that the derived sequence is identical with that for the human placental lectin [14]. This result, together with results of the Northern and Southern blots of several cell lines, indicates that there is only one gene coding for this protein. Since the completion of this work we have learnt that Couraud *et al.* [21] have revised the amino acid sequence for a 14 kDa lectin they had isolated from human placenta (cf. [22]) and that, with the exception of a leucine/tyrosine polymorphism at positions 104 and 119, the revised sequence is identical with that obtained by Kasai and his associates [14] and with the derived sequence for the 14 kDa lectin in the HepG2 cells reported here and in HL60 cells [21].

Gitt & Barondes [23] identified three different lectin sequences by partial amino acid sequencing of a 14 kDa human lung lectin and isolating by immunoscreening two cDNA clones (clones 1 and 2) from a library prepared from a human hepatoma xenograft grown in mice. Their lung lectin sequence is almost identical with the HepG2 sequence we report here. Clone 1 is clearly different, although related to the HepG2 lectin sequence. It may be that it is a gene distinct from that for the 14 kDa lectin and codes for a protein cross-reacting antigenically with this lectin. Its exact relationship to the 14 kDa lectin would be clarified by Southern blotting and/or identification of the size of its transcript or protein product. Clone 2 is also different from the HepG2 sequence, but is identical with a cDNA sequence for 14 kDa lectin of mouse reported by Harrison and co-workers [24] and is now considered to be of murine origin.

Thus there is remarkable agreement between the sequences now available for the 14 kDa lectin in different cell types and tissues of man. These results are mirrored

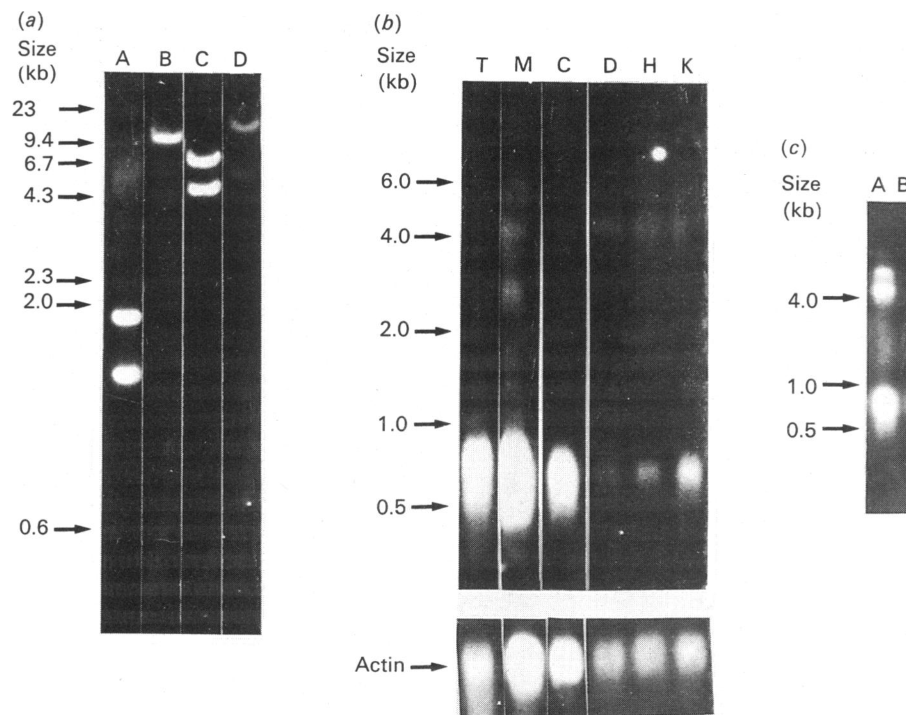

Fig. 2. (a) Southern-blot analysis of human genomic DNA isolated from HepG2 cells, (b) Northern-blot analysis of RNA from human cell lines and (c) Northern-blot analysis of RNA from Daudi cells

(*a*) DNA was digested with either *Pst*I (lane A), *Hin*dIII(B), *Eco*RI (C) or *Bgl*II (D) and probed with a bovine lectin cDNA as described in the Materials and methods section. The autoradiograph is shown in the negative form. Values at the left indicate sizes in kb as estimated by using λDNA digested with *Hin*dIII. (*b*) A 20 μg portion of total cellular RNA was electrophoresed, blotted and probed as described in the Materials and methods section. Abbreviations for cell lines: T, THP-1; M, MRC-5; C, CEM; D, Daudi; H, HepG2; K, K562. Numbers at left indicate sizes in kb estimated by a 1 kb 'ladder' (Gibco-BRL, Paisley, Renfrewshire, Scotland, U.K.). Hybridization of an actin probe to the same blot is shown below. (*c*) A 10 μg portion of poly(A)$^+$-enriched RNA (lane A) or 10 μg of total cellular RNA (lane B) were electrophoresed, blotted and probed as described in the Materials and methods section. Sizes were estimated as in (*b*).

in bovine tissues, where the sequences found for the lectin in the heart and a fibroblast cell line are almost identical [16], and in the rat, where sequences reported for the lung and uterus protein are identical [25]. We therefore conclude that there is likely to be only one gene for the soluble 14 kDa β-galactoside-binding lectin in each species. However, there is clearly a family of genes related to that of the 14 kDa lectin, which would include as distinct members the gene for CBP35 [13] and Clone 1 [23] and possibly those for other proteins of higher molecular masses that are antigenically cross-reactive with this protein.

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