Production and characterization of monoclonal antibodies to β -galactoside-binding lectin of bovine heart muscle

Direct evidence that haemagglutinating activity is associated with a 13kDa protein

Simon R. CARDING,* Robin THORPE,† Robert A. CHILDS,* Moises SPITZ† and Ten FEIZI*‡

*Clinical Research Centre, Applied Immunochemistry Research Group, Watford Road, Harrow HA1 3UJ, Middx., U.K., and †National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, U.K.

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With the aim of obtaining monospecific antibodies against the β -galactoside-binding lectin of bovine heart muscle, spleen cells from Lou rats immunized with lectin were fused with the rat myeloma line Y3.Ag1.2.3. Two immunoglobulin M (IgM)-producing clones, designated NIBy 142-36/8 and NIBy 143-9/5, derived from separate fusions, were used to generate ascites containing high-titre binding activity against the 13 kDa component in preparations of lectin. Direct evidence that haemagglutinating activity is associated with the 13 kDa protein was obtained by the specific elution of 13 kDa polypeptides with haemagglutinating activity from an immobilized antibody adsorbent. Solid-phase radiobinding assays and immunoblotting of isolated lectins and/or muscle homogenates confirmed the earlier indications with conventional antisera that the β -galactoside-binding lectins of bovine, human and monkey muscle tissue are antigenically related.

Galactoside-binding lectins have been isolated from a variety of animal tissues (reviewed by Simpson *et al.*, 1978; Barondes, 1981). These proteins have been shown to share the following properties: (a) they recognize non-reducing terminal β -galactoside residues; (b) they are polypeptides in the range 13–15 kDa; and (c) they require thiol reducing groups for haemagglutination. In addition, immunochemical studies with conventional antisera and amino acid analysis have suggested that the β -galactoside-binding lectins among phylogenetically related species are antigenically and structurally related (Childs & Feizi, 1979; Levi & Teichberg, 1982; Roff & Wang, 1983). The precise function of these lectins *in vivo* is not known. However, a number of experiments with purified lectins have suggested that they may have roles, for example, in cellular recognition during myoblast fusion (Gartner & Podleski, 1975; MacBride & Przybylski, 1980), as modifiers of the activity of sialyltransferases

(Scudder *et al.*, 1982), as mediators of cell attachment and spreading (Stojanovic *et al.*, 1983), and even as inducers of immunological tolerance in T-lymphocytes (Levi *et al.*, 1983). These diverse effects, though difficult to interpret at first sight, may well be mediated by the carbohydrate-binding property of these proteins.

Immunocytochemical studies, using conventionally raised rabbit antisera against lectins of this type, have also been difficult to interpret and have suggested that these proteins may be predominantly intracellular in certain cell types and pericellular in others (Nowak *et al.*, 1977; Beyer *et al.*, 1979; Briles *et al.*, 1979; Childs & Feizi, 1980; Beyer & Barondes, 1980). Since the presence of antibodies with additional specificities cannot always be excluded with conventional antisera, it was considered appropriate to raise monoclonal antibodies against the lectin for use in studies of the biosynthesis and subcellular distribution of these proteins.

In the present paper we describe the production and characterization of two hybridoma-derived monoclonal antibodies that recognize the β -galactoside-binding lectin of bovine heart and also react with the lectins of muscle tissues of man and monkey.

Abbreviations used: Ig, immunoglobulin; SDS, sodium dodecyl sulphate; BSA, bovine serum albumin.

‡ To whom correspondence and reprint requests should be addressed.

Experimental

Materials

Na^{125}I and $[^3\text{H}]\text{leucine}$ were purchased from Amersham International, Amersham, Bucks., U.K. Microtitre U-well plates were obtained from Dynatech Laboratories Ltd., Billingshurst, West Sussex, U.K. Tissue-culture plates (24-well) and Petri dishes were from Flow Laboratories Ltd., Irvine, Ayrshire, Scotland, U.K. Nitrocellulose paper was purchased from Schleicher and Schull, Dassel, Germany. Spectrapor dialysis tubing (M_r cut-off 6000–8000) was purchased from Raven Scientific, Haverhill, Suffolk, U.K. Rabbit antisera against Fc fragments of rat immunoglobulin G [IgG(Fc)] and immunoglobulin M [IgM(Fc)] were purchased from Nordic Immunological Laboratories, Maidenhead, Berks., U.K. Sheep antibodies against F(ab')_2 fragments of rat immunoglobulins {anti-[rat F(ab')_2] antibodies} were isolated by affinity chromatography on a column of immobilized rat F(ab')_2 and eluted with 0.1 M glycine/HCl, pH 2.8. For immunization and production of ascites, an inbred subline of Lou rats was used. The myeloma line Y3.Agl.2.3 (Galfré *et al.*, 1979) was kindly provided by Dr. C. Milstein, Laboratory of Molecular Biology, Cambridge, U.K. Adult human skeletal muscle was obtained 29 h *post mortem* from the rectus abdominus muscle of a patient who died from coronary thrombosis.

Buffers

Phosphate-buffered saline contained 150 mM NaCl/0.02% NaN_3 and 22 mM-sodium phosphate buffer, pH 7.0. Phosphate-buffered saline/BSA also contained 0.1% (w/v) BSA. Solution A contained 150 mM NaCl/10 mM- NH_4HCO_3 /1 mM-dithiothreitol/0.02% (w/v) NaN_3 . Solution B contained phosphate-buffered saline with 200 mM-lactose and 0.1% (w/v) BSA.

β -Galactoside-binding lectins from bovine, human and rhesus-monkey muscle tissues

Adult bovine heart was obtained from a local abattoir and stored at -70°C until used (up to 4 months). The frozen tissue (980 g) was homogenized with four parts (v/w) of 150 mM NaCl/0.02% NaN_3 /10 mM- NH_4HCO_3 /10 mM-2-mercaptoethanol/2 mM-EDTA/200 mM-lactose in an MSE Atomix blender. The homogenate was centrifuged at 2000 g for 60 min at 4°C . The pellet was rehomogenized and to the pooled supernatants $(\text{NH}_4)_2\text{SO}_4$ was added to 50% saturation. After 18 h at 4°C the precipitate was centrifuged at 20000 g at 4°C for 60 min. The pellet was resuspended in 15 mM NaCl/0.02% NaN_3 /10 mM-2-mercaptoethanol/10 mM- NH_4HCO_3 and dialysed against the same solution to remove lactose. The

non-dialysable material was then centrifuged at 62000 g for 60 min before being applied to an asialofetuin–Sephacrose column, prepared as described by de Waard *et al.* (1976). Lectin was eluted with 200 mM-lactose and dialysed against solution A to remove lactose. Lectin activity was assayed by haemagglutination as described below. The yield was 18 mg of protein, determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Lectin was stored at 4°C in 2 mg freeze-dried portions until used.

For affinity chromatography (see below) the lectin was alkylated in order to eliminate the requirement for dithiothreitol, which inactivates IgM antibodies. Alkylation of lectin was performed by incubating the protein (1 mg/ml of a solution containing 150 mM NaCl/0.02% NaN_3 /1 mM-dithiothreitol) at 20°C for 30 min with iodoacetamide (final concentration 10 mM), followed by dialysis against 150 mM NaCl/0.02% NaN_3 .

Homogenates of human and bovine skeletal muscle (approx. 5 g of each tissue) containing lectin were prepared, as described above, and for immunoblotting experiments boiled in the presence of 2% (w/v) SDS for 5 min followed by centrifugation at 2000 g or 15 min.

The lectins of rhesus-monkey and human muscle tissue were those isolated previously (Childs & Feizi, 1979).

Assay of lectin haemagglutinating activity

Lectin haemagglutinating activity was assayed by using trypsin-treated rabbit erythrocytes as described by Childs & Feizi (1979). Each well contained 25 μl of a 1% (v/v) suspension of erythrocytes, 25 μl of lectin solution and 25 μl of solution A. The haemagglutination titre of a lectin solution is expressed as the reciprocal of the highest dilution causing haemagglutination. The number of haemagglutinating units (h.a.u.) is defined as follows:

$$\text{No. of h.a.u.} = \frac{\text{Haemagglutinating titre} \times \text{volume } (\mu\text{l})}{25}$$

Haemagglutination-inhibition assays were carried out by mixing lectin (25 μl containing 4 h.a.u.) with hybridoma supernatant or ascites fluid (25 μl) and erythrocyte suspension (25 μl).

Radioiodination of bovine lectin

Alkylated lectin (50 μg) was labelled with 1 mCi of carrier-free ^{125}I by the chloramine-T method (Greenwood & Hunter, 1963) in the presence of 50 mM-lactose. Unbound ^{125}I was removed by chromatography on a column of Sephadex G-25 equilibrated with 50 mM-sodium phosphate buffer, pH 7.0, containing 1% (w/v) BSA and 50 mM-lactose. Lactose was removed by dialysis against

phosphate-buffered saline. Before use, the labelled lectin containing 1.2×10^8 c.p.m. of ^{125}I was absorbed on to an asialofetuin-Sepharose adsorbent and eluted with 200 mM-lactose. Radioactivity recovered in the lactose eluate was 2.4×10^7 c.p.m. (20% of that applied).

Preparation of monoclonal antibodies against β -galactoside-binding lectin from bovine heart

Two female rats were injected subcutaneously at three sites with 60 μg of lectin, in 100 μl of phosphate-buffered saline, emulsified in an equal volume of Freund's complete adjuvant. After 28, 42 and 56 days the animals were given an intraperitoneal boost of 50 μg of lectin in phosphate-buffered saline, and cell fusion was carried out on day 60. Cells from each spleen were fused with cells of the Y3.Ag1.2.3 rat myeloma line (Galfré *et al.*, 1979) as described previously (Galfré *et al.*, 1977). Hybridomas that grew to confluency were screened for antibodies to lectin by solid-phase radiobinding assay. Selected hybridomas designated NIBy 142-36/8 and NIBy 143-9/5 (abbreviated to 36/8 and 9/5 respectively), were cloned twice in soft agar by the method of Galfré & Milstein (1981). Ascites fluid containing monoclonal antibody was obtained 6–10 days after injecting 10^7 cells into the peritoneal cavity of Lou rats. As a control, ascites containing rat hybridoma antibody (IgM class) specific for poliovirus, designated NIBy 25-5/16 (abbreviated to 5/16), was used (M. Spitz, unpublished work).

The heavy-chain class of the cloned monoclonal antibodies was determined by using hybridoma secretion products biosynthetically labelled with [^3H]leucine. Briefly, 5×10^6 cells were incubated in 1 ml of leucine-free Dulbecco's minimal essential medium with added [^3H]leucine (200 μCi), glutamine (1%, w/v) and dialysed 10% (v/v) foetal-calf serum for 6 h at 37°C in an atmosphere containing 5% CO_2 . The supernatants were analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions (see below). [^3H]Leucine-labelled monoclonal IgG and IgM proteins (gifts from Dr. C. Milstein) were included as standards. Labelled polypeptide chains were detected by fluorography as described by Johnstone & Thorpe (1982).

Fractionation of ascites by gel filtration

A portion (3 ml) of a 2-fold-concentrated 36/8 ascites (containing 194 mg of protein) or 5 ml of control ascites (containing 98 mg of protein) were applied to a column (2.6×95 cm: $V_0 = 145$ ml) of Sephadex G-200 equilibrated with phosphate-buffered saline containing 0.02% NaN_3 and 0.1 M-glycine. The excluded IgM fractions were pooled; the protein contents of the 36/8 antibody and the

control antibody pools were 42 mg and 25 mg respectively.

Solid-phase radiobinding assay for anti-lectin antibody activity

A solid-phase radiobinding assay was carried out as described by Cobbold & Waldmann (1981), with slight modification. Microtitre plates (U-well) were incubated at 37°C for 90 min with 50 μl of phosphate-buffered saline containing 0.1% poly-L-lysine. After washing with phosphate-buffered saline, 50 μl of a solution of lectin (30 μg per ml of solution A) was added to each well and incubated at 4°C for 18 h. The plates were washed with phosphate-buffered saline and with distilled water and then incubated at 20°C for 30 min with 50 μl of phosphate-buffered saline containing 3% (w/v) BSA. After washing, dilutions of hybridoma supernatants or ascites were added to the wells and left at 20°C for 2 h. Control wells contained culture medium or control rat ascites, produced by injecting the Y3.Ag1.2.3 myeloma line into the peritoneal cavity of Lou rats. After being washed, the plates were incubated at 20°C for 60 min with ^{125}I -labelled (Greenwood & Hunter, 1963) sheep anti-[rat F(ab') $_2$] containing 10^5 c.p.m. (50 μl /well; sp. radioactivity 10^6 c.p.m./ μg). The plates were washed and dried and the wells counted for radioactivity with a Nuclear Enterprises 1600 or an LKB γ -radiation counter.

In the inhibition-of-binding assays, 25 μl of dilutions of inhibitor were incubated with 25 μl of 36/8 and 9/5 ascites, or purified antibody, at a dilution of 1:10000 at 20°C for 2 h before transferring to microtitre plates coated with bovine heart lectin. The plates were incubated at 20°C for 2 h and, after washing, incubated with ^{125}I -labelled anti-[rat F(ab') $_2$] as described above.

Affinity chromatography of lectin using monoclonal antibodies

Adsorbents consisting of the IgM-rich fraction of 36/8 ascites or control ascites (9 mg of each) coupled to 2 ml of CNBr-activated Sepharose 4B were prepared by the method of Axen *et al.* (1967). The adsorbents were washed with phosphate-buffered saline containing 3 M-KSCN/0.1 M-glycine and equilibrated at 4°C with solution B. ^{125}I -labelled alkylated lectin, 3.7×10^7 c.p.m. in 5 ml of solution B, was applied to both columns at a flow rate of 4 ml/h. After extensive washing with solution B, bound material was eluted sequentially with solution B containing (1) 1 M-NaCl, (2) 1 M-KSCN, and (3) 3 M-KSCN. Eluted fractions were pooled as shown in Fig. 2 (below), dialysed against phosphate-buffered saline containing 5 mM-lactose and 0.1 M- NH_4HCO_3 , and freeze-dried. The radioactivities in the pooled fractions 1, 2 and 3 from the

36/8 antibody column were 3.5×10^3 , 5.6×10^4 and 4.1×10^4 c.p.m. respectively, and, from the control adsorbent, 1.7×10^3 , 6.1×10^3 and 4.2×10^3 c.p.m. respectively. The fractions were reconstituted in 100–400 μ l of SDS/polyacrylamide-gel-electrophoresis sample buffer and the entire fractions subjected to electrophoresis as described below.

The affinity chromatography was repeated with an unlabelled preparation of bovine heart lectin containing a trace amount of ^{125}I -labelled lectin (2×10^4 c.p.m.). Three aliquots of lectin preparation, 200 μ g each, were applied sequentially to the 36/8 antibody column, and radioactivity was detected in the effluent after 600 μ g had been applied. After washing with solution B, the bound material was eluted with solution B containing 3M-KSCN. Fractions containing the eluted radioactive peaks (Fig. 4 below) were dialysed against phosphate-buffered saline containing 0.02% NaN_3 , freeze-dried, and reconstituted to 2ml. When the control antibody column was used as adsorbent, only 200 μ g of lectin preparation was applied, since radioactivity was not retarded (results not shown). The material eluted with 3M-KSCN from the control column was dialysed and proportionately reconstituted to 0.66ml. Lectin activity in the applied and eluted fractions was assayed by haemagglutination of trypsin-treated rabbit erythrocytes.

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was carried out in 4–16% (w/v)-polyacrylamide gradient slab gels and in non-gradient 12.5% (w/v)-polyacrylamide slab or disc gels by the method of Laemmli (1970). Samples in solution A were adjusted to 2% (w/v) SDS/2.5% (v/v) 2-mercaptoethanol/5% (w/v) sucrose/0.01% (w/v) Bromophenol Blue and heated at 100°C for 3 min before electrophoresis. The run gels were fixed and stained for 18 h in methanol/acetic acid/water (5:1:4, by vol.), containing 0.1% Coomassie Blue and destained in methanol/acetic acid/water (2:1:7, by vol.). Disc gels containing radiolabelled lectin were sliced (approx. 2 mm slices) and counted in a Nuclear Enterprises 1600 γ -radiation counter.

Immunoblotting of human and bovine tissue homogenates and lectins

Polyacrylamide-gel electrophoresis and electrotransfer of proteins on to nitrocellulose sheets was carried out as described previously (Childs *et al.*, 1983). After transfer, the nitrocellulose sheets were incubated in phosphate-buffered saline containing 3% (w/v) bovine haemoglobin (Hb/phosphate-buffered saline) at 20°C for 45 min and incubated at 20°C for 16 h in 30 ml of Hb/phosphate-buffered

saline containing either 30 μ l of IgM-rich fraction of ascites fluid or 5 ml of hybridoma supernatant. After washing for 30 min with Hb/phosphate-buffered saline the sheets were incubated at 20°C for 6 h with ^{125}I -labelled sheep anti-[rat F(ab')₂] antibodies (3×10^4 c.p.m./ml; sp. radioactivity 10^6 c.p.m./ μ g). The sheets were then washed with phosphate-buffered saline, air-dried, and exposed to X-ray film with an intensifying screen at –70°C for at least 2 days (unless otherwise stated).

Results

Production and isolation of monoclonal antibodies to bovine heart lectin

Production of hybridomas. Two independent fusions were performed with the myeloma cell line Y3.Agl.2.3 and splenocytes from two rats that had been immunized with a preparation of bovine heart lectin. Culture supernatants from wells containing confluent hybridomas were screened for binding to the lectin preparation by using the solid-phase radiobinding assay. Of 221 supernatants screened, 151 showed binding activity at least five times greater than a culture-medium control. Hybridomas from ten positive wells (five from each fusion) that showed antigen-binding activity 5–100 times above the control were cloned in soft agar. All of the clones obtained showed binding activity above the culture-medium control. None of the clones inhibited the haemagglutinating activity of the lectin. One clone from each fusion, designated 36/8 and 9/5, was recloned and used for the production of ascites fluid.

Determination of antibody class. SDS/polyacrylamide-gel electrophoresis of hybridoma secretion products, biosynthetically labelled with [^3H]-leucine, showed that the heavy chain of 36/8 and 9/5 antibodies co-migrated with that of an IgM standard (Fig. 1, inset). Immunoprecipitation studies with antisera against rat IgG and IgM confirmed that the two monoclonal antibodies were of the IgM class (results not shown).

Isolation of IgM antibody from ascites. Sephadex G-200 chromatography of the 36/8 ascites (Fig. 1) showed that the anti-lectin activity was contained in the excluded IgM-rich fraction. No lectin binding activity was detected in the IgM-rich fraction from the control ascites (results not shown).

Affinity chromatography of bovine heart lectin on 36/8 antibody adsorbent

Evidence that the 36/8 antibody reacts with the haemagglutinin rather than a contaminant in the preparation of bovine heart lectin was obtained by affinity-chromatography experiments in the lactose-containing solution B using alkylated lectin.

Fig. 1. Fractionation of 36/8 ascites fluid by gel filtration and SDS/polyacrylamide-gel electrophoresis of the heavy and light chains of [^3H]leucine-labelled antibodies

A 3 ml portion of 2-fold-concentrated 36/8 ascites fluid was applied to a column (2.6 cm $\times$ 95 cm) of Sephadex G-200 as described in the Experimental section. Each fraction (5 ml) was assayed for antibody activity against bovine heart lectin by using the solid-phase radiobinding assay (see the Experimental section). The antibody titre is the reciprocal of the highest dilution at which 10% of maximal binding activity could be detected. The inset is a fluorograph showing electrophoresis in SDS/polyacrylamide gel of [^3H]leucine-labelled and reduced secretion products of hybridomas 36/8 and 9/5 (lanes 2 and 3) and of IgG- and IgM-secreting hybridoma controls (lanes 1 and 4 respectively). The symbols μ , γ and L indicate the positions of migration of IgM and IgG heavy chains and of light chains respectively.

Fig. 2. Micro-scale affinity chromatography of ^{125}I -labelled alkylated bovine heart lectin

An alkylated and iodinated preparation of lectin ($2\mu\text{g}$) containing 3.7×10^7 c.p.m. was applied to adsorbent columns containing 36/8 antibody (—) or control antibody (---); unbound material was washed from the columns (W) and bound material was sequentially eluted with (1) 1M-NaCl, (2) 1M-KSCN and (3) 3M-KSCN as described in the Experimental section. Fraction sizes of washings were 5 ml and of the eluates 1 ml. Fractions were pooled as indicated.

Alkylation did not affect the haemagglutinating activity of the lectin, and the alkylated lectin was found to be stable in the absence of reducing agent over a period of 3 months. When $2\mu\text{g}$ of ^{125}I -labelled alkylated lectin was chromatographed on the 36/8-antibody or control-antibody adsorbent and eluted with $1\text{ M-NaCl}/1\text{ M-}$ and 3 M-KSCN (Fig. 2), only the fractions eluted with KSCN from the 36/8-antibody adsorbent were found to contain a radioactive component with a subunit molecular mass of 13 kDa (Fig. 3), corresponding to the major protein in the preparation of the lectin. Secondly, when $600\mu\text{g}$ of unlabelled lectin preparation (115200 h.a.u.) containing 1 ng of ^{125}I -labelled lectin ($2 \times 10^4\text{ c.p.m.}$), was chromatographed on the 36/8-antibody adsorbent, 16000 h.a.u. (14% of

that applied) was recovered in the fraction eluted with 3 M-KSCN , and the radioactivity and haemagglutinating activity that was not retained on this adsorbent were substantially retarded (results not shown). When the control-antibody adsorbent was used, there was no retardation of radioactivity, and no haemagglutinating activity could be detected in the 3 M-KSCN eluate when $200\mu\text{g}$ of lectin was chromatographed.

Immunoblotting of isolated bovine lectin and muscle homogenates

Immunoblotting of purified bovine heart lectin with the IgM-rich fraction of antibody 36/8 (Fig. 4, lane 1) showed staining of 13 kDa and a 26 kDa components. With the homogenates of bovine heart and skeletal muscle (lanes 2 and 3 respectively

Fig. 3. SDS/polyacrylamide-gel-electrophoretic profiles of a preparation of bovine heart lectin labelled with ^{125}I and of fractions eluted from the antibody adsorbents

Electrophoresis was performed as described in the Experimental section; migration was from left to right. (a) Sample of ^{125}I -labelled bovine heart lectin as applied to the adsorbent columns. (b), (c) and (d), fractions eluted from the adsorbent columns with 1 M-NaCl , 1 M-KSCN and 3 M-KSCN respectively as described in the text; ● and ○ designate eluates from 36/8- and the control-antibody columns respectively. Arrows indicate positions of migration of molecular-mass markers: phosphorylase a , 94 kDa ; bovine serum albumin, 68 kDa ; albumin, 43 kDa ; trypsin, 23.3 kDa ; cytochrome c , 12.5 kDa .

Fig. 4. Autoradiography showing immunostaining of purified lectins and homogenates of bovine and human muscle tissue

Isolated lectins ($1\mu\text{g}$ each) from bovine heart, human adult and foetal heart and rhesus-monkey skeletal muscle (lanes 1, 5, 6 and 7 respectively) and homogenates ($100\mu\text{g}$ protein) of bovine heart and of bovine and human skeletal muscles (lanes 2, 3 and 4 respectively) were electrophoresed on SDS/polyacrylamide gels, electrophoretically transferred on to nitrocellulose sheets, and immunostained with the IgM-rich fraction of 36/8 antibody followed by ^{125}I -labelled anti-[rat F(ab')_2] (as described in the Experimental section), except that autoradiography was prolonged to 5 days in the case of human skeletal-muscle homogenate. Lane CB contained $15\mu\text{g}$ of purified bovine heart lectin, which was stained with Coomassie Blue.

Fig. 5. Antigenic cross-reactivity of the bovine, human and monkey muscle lectins in the solid-phase radiobinding assay with the 36/8 monoclonal antibody

The binding of the IgM-rich fraction of 36/8 ascites to purified bovine, human and monkey lectins (a) and the inhibition of binding of 36/8 antibody to bovine lectin by the bovine, human and monkey lectins (b) were assayed as described in the Experimental section. Symbols: ●, bovine heart lectin; □, human-adult heart lectin; ■, human-foetal heart lectin; ○, rhesus-monkey skeletal-muscle lectin.

ively), only the 13kDa component was revealed. Similar results were obtained with the 9/5-antibody ascites (results not shown).

Cross-reactivity of the lectins of human and rhesus-monkey muscle tissues

Solid-phase radiobinding assays showed that both the 36/8 antibody, assayed as an IgM-rich fraction (Fig. 5a) or ascites, and the 9/5 antibody, assayed as ascites (results not shown), reacted with preparations of lectin isolated from bovine heart muscle and to a lesser extent with those from adult and foetal human heart and from rhesus-monkey skeletal muscle. However, in inhibition-of-binding assays, using bovine lectin as the immobilized antigen, the human and rhesus-monkey lectins showed little inhibitory activity (Fig. 5b).

When the 36/8 antibody was used, the antigenic

cross-reactivities of the bovine, human and rhesus-monkey lectins were confirmed by immunoblotting with the purified lectins and the homogenate of human skeletal muscle (Fig. 4, lanes 4–7). In each case, autoradiography revealed immunostaining of a 13kDa component. In addition, there was immunostaining of a 26kDa component in the human skeletal-muscle homogenate (lane 4) seen after 5 days of autoradiography.

Discussion

These studies show that the two rat hybridoma antibodies, NIBy 142-36/8 and NIBy 143-9/5, are directed against the β -galactoside-binding lectin of bovine muscles. First, both antibodies react in immunoblotting experiments with a component of bovine muscle homogenates having the same apparent molecular mass, 13kDa, as the major component in preparations of lectin. In some preparations a 26kDa component was also stained. The ability of the two antibodies to react with the lectin treated with SDS indicates that the peptide antigenic determinants they recognize are stable to denaturation. Secondly, the material eluted from the 36/8-antibody adsorbent consists of a 13kDa protein with haemagglutinating activity. Since the affinity-chromatography experiments were performed in the presence of lactose, they provide direct evidence that binding to the antibody adsorbent was via a determinant on the lectin rather than a trace contaminant associated with this protein. These observations corroborate the evidence from gel-filtration experiments of Roff & Wang (1983) that the haemagglutinating activity is associated with the 13kDa protein.

Alkylation of lectin not only enabled the affinity-chromatography experiments to be performed in the absence of reducing agent (the latter would inactivate IgM antibodies), but also protected the lectin against oxidation during repeated chromatographies, dialysis and storage. The preservation of the lectin activity after carboxymethylation with iodo[^{14}C]acetamide was noted previously (Sanford *et al.*, 1982). It can be envisaged that this protective effect will be much exploited for the long-term storage of the lectin at 4°C.

Both in solid-phase radioimmunoassays and in immunoblotting experiments the two antibodies reacted to a considerable extent with the purified β -galactoside-binding lectins of human heart (foetal and adult) and rhesus-monkey skeletal muscle. However, these mammalian lectins are not antigenically identical, for, in comparison with the bovine lectin, the human and monkey lectins were poor inhibitors of the binding of the two hybridoma antibodies to immobilized bovine heart

lectin. This indicates that only a part of the antigenic determinant on the bovine lectin is expressed on the human and monkey lectins. These observations are in agreement with the results obtained with conventionally raised rabbit antisera against bovine heart lectin (Childs & Feizi, 1979). Also, with a rabbit antiserum against bovine heart lectin, Briles *et al.* (1979) found only a weak reaction with the chicken heart lectin. Neither the rabbit antibodies in a previous study (Childs & Feizi, 1979) nor the rat hybridoma antibodies in the present study inhibited the haemagglutinating activity of the bovine lectin. On the other hand, a rabbit antiserum to chick muscle lectin has been reported to inhibit haemagglutinating activity of this lectin (Nowak *et al.*, 1977). Taken together, these results suggest that: (a) the rabbit polyclonal and the rat monoclonal antibodies against bovine lectin recognize determinants in the 'framework' rather than the sugar-binding site of the bovine lectin; (b) the 'framework' regions of these lectins have structural homologies and the homologies may be greatest between species that are phylogenetically related; and (c) the rabbit antiserum against chick lectin reacts with determinants associated with, or in close proximity to, the sugar-binding site of this lectin. It will be interesting to determine whether the lack of antibody responses by the rabbit and rat to the sugar-binding site of the bovine lectin is due to this site being structurally conserved (invariant) in mammals.

In the affinity-chromatography experiments, only a subpopulation (14%) of the lectin applied was bound to the antibody adsorbent, whereas a substantial proportion was retarded. This raises the possibility that the lectin that had been isolated by absorption on to asialofetuin-Sepharose and elution with lactose contains more than one population of galactose-binding protein(s), which have different binding affinities with the monoclonal 36/8 antibody. Further investigations are required to determine whether these differences represent structural variants of the lectin (isolectins) analogous to those described for chicken tissues (Beyer *et al.*, 1979) and in cultured 3T3 fibroblasts (Roff & Wang, 1983) or whether the retarded material represents lectin that has been partially degraded during the isolation procedures.

The monoclonal antibodies 36/8 and 9/5 will be of value in immunocytochemical studies of the lectin and in monitoring the biosynthesis and metabolism of this widely distributed but poorly understood family of carbohydrate-binding proteins.

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