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β-Galactoside-Binding Muscle Lectins of Man and Monkey Show Antigenic Cross-Reactions with those of Bovine Origin

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Endogenous β-galactoside-binding lectins were isolated from human heart and from human and rhesus-monkey skeletal muscles. Gel precipitation and radioimmunoassays with rabbit antisera to calf heart lectin revealed antigenic cross-reactions between the primate and bovine muscle lectins.

Considerable information is accumulating on a family of β-galactoside-binding lectins that occur in a variety of animal tissues (Teichberg *et al.*, 1975; Simpson *et al.*, 1978) and differ from the hepatic binding glycoprotein (Hudgin *et al.*, 1974) in being thiol-reducing-group-requiring and bivalent-cation-independent (De Waard *et al.*, 1976). The amount of extractable lectin varies in different tissues and in some organs may be developmentally regulated (Kobiler & Barondes, 1977). β-Galactoside-binding lectins isolated from different tissues of the calf have been shown to be antigenically indistinguishable by gel precipitation and radioimmunoassay with antisera raised in rabbits (Briles *et al.*, 1979). Similarly, lectins of embryonic and young chick tissues could not be distinguished by gel precipitation with rabbit antisera (Kobiler *et al.*, 1978). On the other hand, lectins isolated from calf tissues and chick heart muscle showed little or no antigenic cross-reactions (Briles *et al.*, 1979), and it was suggested that these β-galactoside-binding lectins might be species- rather than tissue-specific.

The present studies describe antigenic cross-reactions between the β-galactoside-binding lectins of bovine heart, human heart and human and rhesus-monkey skeletal muscles as detected by gel precipitation and radioimmunoassays using rabbit antisera against calf heart lectin.

Experimental

Human and animal tissues

A pool of 23 foetal (12–20 weeks gestation) human hearts (wet weight 25g) were obtained from the Foetal Tissue Bank, The Royal Marsden Hospital, Fulham Road, London S.W.3, U.K. Adult human heart (128g) was obtained 4h *post mortem* from a male patient who died of lymphoma. Adult human skeletal muscle (1600g) was a pool of femoral and calf muscles obtained 4h *post mortem* from a patient who died from coronary thrombosis and from two patients who underwent leg amputations for peripheral joint disease. Rhesus-monkey pectoral, femoral and calf muscles (400g) were obtained from the Division of Comparative Medicine at this Institute. Calf and adult cow hearts (360 and 950g respectively) were obtained from a local abattoir. All tissues were stored at −20°C until used.

Abbreviation used: SDS, sodium dodecyl sulphate.

Isolation of soluble β-galactoside-binding lectins

The soluble lectins were isolated from homogenates of the above tissues by using an asialofetuin adsorbent column as described previously (De Waard *et al.*, 1976; Childs *et al.*, 1979). The lectins were eluted from the asialofetuin column with solution A (150mM-NaCl/0.02% NaN_3/20mM-NH_4HCO_3/1mM-dithiothreitol), containing 200mM-lactose (Sigma Chemical Co., Kingston upon Thames, Surrey, U.K.). Lectin protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Assay of lectin haemagglutinating activity

Lectin haemagglutinating activity was assessed by agglutination of trypsin-treated rabbit erythrocytes. Trypsin treatment of erythrocytes was performed as described by Wood *et al.* (1979). Haemagglutination and haemagglutination-inhibition assays were performed by the microtitre technique described previously (Feizi & Monger, 1970), with solution A as diluent.

SDS/polyacrylamide-gel electrophoresis of isolated lectins

SDS/polyacrylamide-gel electrophoresis was performed in 12.5% (w/v) polyacrylamide slab gels containing 0.1% (w/v) SDS by the system of Laemmli (1970). Samples (10μg of protein in 40μl of solution

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A) were made up to 2% (w/v) SDS, 2.5% (v/v) 2-mercaptoethanol, 5% (w/v) sucrose and 0.01% (w/v) Bromophenol Blue and heated at 100°C for 3min before electrophoresis. The run gels were fixed and stained for 18h in methanol/acetic acid/water (9:2:9, by vol.), containing 0.1% Coomassie Blue, and destained in methanol/acetic acid/water (2:1:7, by vol.)

Gel filtration of cow heart lectin

A portion (7mg) of the lectin eluted from the asialofetuin column was chromatographed on a column (90cm × 1.5cm) of Sephadex G-100 equilibrated in solution A containing 50mM-lactose. The flow rate was 8ml/h and 4ml fractions were collected. Each fraction was monitored for A_{280} and haemagglutinating activity after removal of lactose by dialysis against solution A.

Rabbit antisera against calf heart lectin

Two rabbits (code nos. 171 and 172) were immunized with calf heart lectin isolated from the asialofetuin column. Each rabbit received subcutaneously 0.5ml of *Bordetella pertussis* (whooping-cough) vaccine (The Lister Institute, Elstree, Herts., U.K.), followed 3 days later by 20μg of lectin emulsified in Freund's complete adjuvant injected intradermally into multiple sites on the back. Rabbits were bled after 4 weeks. A third rabbit antiserum against calf heart lectin (De Waard *et al.*, 1976) was kindly given by Dr. S. Kornfeld, Washington University School of Medicine, St. Louis, MO, U.S.A.

Antigenic analysis of isolated lectins

Precipitation tests were performed in 0.6% (w/v) agarose gels (Marine Colloids, Rockland, IL, U.S.A.) in phosphate-buffered saline (150mM-NaCl/6mM-NaH_2PO_4,$2H_2O$/16mM-Na_2HPO_4), pH7.0, containing 0.02% (w/v) NaN_3 and 50mM-lactose.

Calf heart lectin (50μg) was labelled with carrier-free $^{125}I^-$ (The Radiochemical Centre, Amersham, Bucks., U.K.) by the chloramine-T method (Greenwood *et al.*, 1963). The specific radioactivity was 2×10^6c.p.m./μg of protein as measured in a Nuclear Enterprises 1600 Gamma counter. Binding and inhibition of binding assays of the rabbit anti-(calf heart lectin) sera to ^{125}I-labelled calf heart lectin were performed by the double-antibody method using pig anti-(rabbit immunoglobulin G) in the presence of normal rabbit serum, as described previously (Lecomte & Feizi, 1975). Diluent for the assay was phosphate-buffered saline containing 1% (w/v) bovine serum albumin, 1mM-dithiothreitol and 50mM-lactose. In inhibition-of-binding assays, 10μl of serial dilutions of unlabelled lectins were incubated with the rabbit antiserum for 60min before the addition of ^{125}I-labelled calf heart lectin.

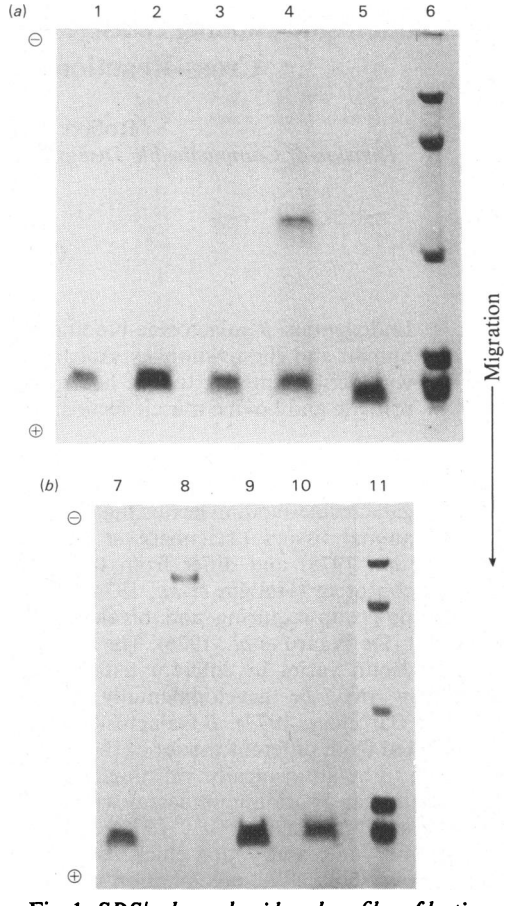

Fig. 1. *SDS/polyacrylamide-gel profiles of lectins*
(*a*) Lectins isolated from: adult human skeletal muscle (lane 1); rhesus-monkey skeletal muscle (2); adult human heart (3); foetal human heart (4); calf heart (5); molecular-weight markers (6) from top to bottom, bovine serum albumin (mol.wt. 68000), ovalbumin (43000), trypsin (23300), myoglobin (17500), cytochrome *c* (13000). (*b*) Cow heart lectin eluted from the asialofetuin adsorbent and fractions obtained after chromatography on Sephadex G-100. Calf heart lectin (lane 10) is shown for comparison. Cow heart lectin eluted from the asialofetuin adsorbent (lane 7), and excluded (lane 8) and included (lane 9) peaks respectively, on a Sephadex G-100 column. Molecular-weight markers (lane 11) were as described in (*a*).

Results

The yields of soluble lectins from the six tissue homogenates (measured as protein/g wet wt.) were: cow heart, 16μg; foetal human heart, 8μg; calf heart, 6μg; adult human skeletal muscle, 4μg; adult human heart and rhesus-monkey skeletal muscle, 2μg. The

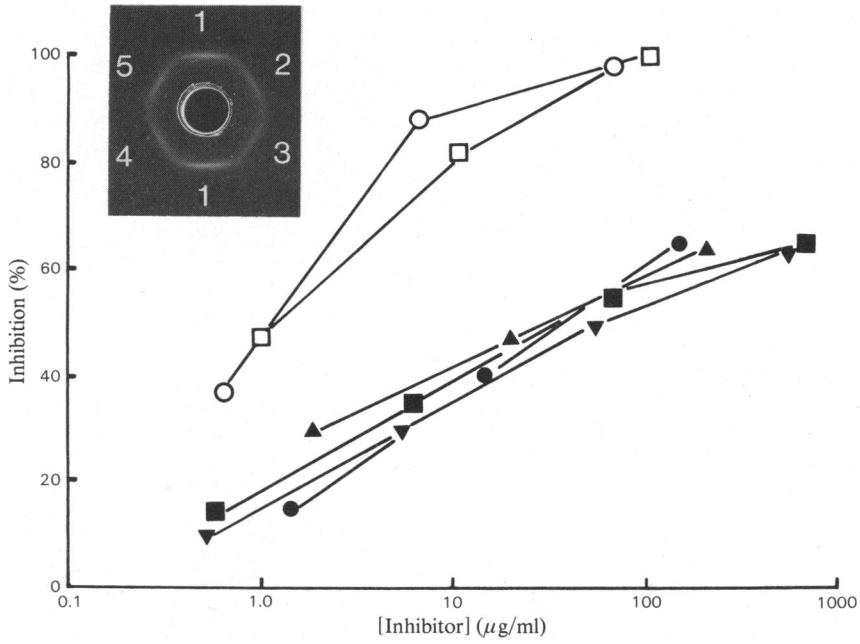

Fig. 2. *Antigenic analysis of bovine, human and rhesus-monkey muscle lectins*
Inhibition of binding of rabbit-172 anti-(calf heart lectin) serum (at a dilution of 1:200) to ^{125}I-labelled calf heart lectin with unlabelled lectins. The inset shows gel-precipitation reactions of the calf heart and four primate lectins with rabbit-172 anti-(calf heart lectin) serum. Symbols: ○, calf heart lectin (inset 1); □, cow heart lectin; ●, foetal human heart lectin (inset 2); ■, adult human heart lectin (inset 3); ▲, rhesus-monkey skeletal-muscle lectin (inset 4); ▼, adult human skeletal-muscle lectin (inset 5).

minimum protein concentrations at which the lectin preparations gave agglutination of trypsin-treated rabbit erythrocytes were in the range 0.4–1.0 μg/ml. This agglutination was inhibited by 2–5 mM-lactose.

In SDS/polyacrylamide gels (Fig. 1), the calf and cow heart lectins migrated identically, with an approx. monomer mol.wt. of 13000. The three human and the rhesus-monkey lectins were consistently slightly slower in their migration than the two bovine lectins (approx. monomer mol.wts. 13500). The lectin preparations contained minor components with approx. mol.wts. of 27000, 54000 and 68000, probably corresponding to dimer, tetramer and pentamer forms of the lectins (De Waard *et al.*, 1976). The 27000-mol.wt. component was most evident in the foetal human heart lectin preparation.

When the cow heart lectin preparation was chromatographed on a column of Sephadex G-100, 60% of the protein was in the excluded volume of the column and the remaining 40% eluted as a single included peak. The components under both peaks were found to have haemagglutinating activity at 4 μg/ml and 1 μg/ml respectively. In SDS/polyacrylamide gels (Fig. 1*b*) the excluded-peak material gave bands corresponding to mol.wts. of approx. 26000 and 54000, and the included-peak material gave a single band corresponding to an approx. mol.wt. of 13000.

Antigenic analysis of the lectins

The three rabbit antisera against calf heart lectin showed similar reactivities in gel-precipitation tests and radioimmunoassays; their reactivities were the same both in the presence and absence of lactose. No reactivities against calf heart lectin were detected in preimmune rabbit serum by gel-precipitation tests or radioimmunoassays. The six lectins of bovine, human and rhesus-monkey origins gave single precipitin lines without spur formations (Fig. 2, inset). However, antigenic differences among the lectins were revealed in competitive-binding assays in which the unlabelled lectins were used as inhibitors of the binding of rabbit anti-(calf heart lectin) sera to ^{125}I-labelled calf heart lectin (Fig. 2). The calf and cow heart lectins had similar inhibition curves, giving 50% inhibition of binding at 1 μg/ml. The three human and the rhesus-monkey lectins as a group were approx. 40-fold less active, giving 50% inhibition in the range 30–60 μg/ml.

The specificity of rabbit-172 anti-(calf heart lectin)

serum towards the lactose-binding lectin rather than possible muscle-tissue contaminants was investigated by immunofluorescence (results not shown). Briefly, when calf heart lectin was added to cryostat sections of cow heart tissue, followed by rabbit-172 antiserum and fluorescein-conjugated goat anti-(rabbit immunoglobulin G), strong fluorescent staining was observed surrounding the muscle cells. This staining was abolished if lectin was added in the presence of 50mM-lactose.

Discussion

The present studies indicate that the β-galactoside-binding lectins are relatively stable proteins that can be successfully extracted from fresh and post-mortem tissues. As the soluble and not the particle-associated form of these lectins was extracted in the present studies, the yields reported represent only a fraction of the total lectin content of the tissues. The bovine and primate lectins were indistinguishable in gel-precipitation tests with rabbit anti-(calf heart lectin) sera. However, in competitive-binding radioimmunoassays with the calf heart lectin as labelled reference antigen, the bovine lectins were 30–60 times more active than the primate lectins. The human foetal and adult heart and human and rhesus-monkey skeletal-muscle lectins gave similar inhibition curves with shallow slopes characteristic of cross-reactive antigens.

These observations on the cross-reactivities between the bovine and primate lectins and the apparent lack of any such cross-reactivity between the bovine and chick heart lectins (Briles *et al.*, 1979) suggest a taxonomic variation in structure of these lectins analogous to that observed with cytochrome *c* (Dickerson & Geis, 1969).

The biological functions of these widely distributed animal lectins are not yet known. Their differing subcellular locations in various tissues (Beyer *et al.*, 1979) have raised the possibility that these lectins may play different roles in different organs. It seems more likely that they play a common role in different cell types, for their recognition structures, which include the blood-group-Ii antigens (Childs & Feizi, 1979; Childs *et al.*, 1979), and other carbohydrate structures with terminal β-galactosyl residues (De Waard *et al.*, 1976), are commonly occurring cellular components. Some speculations on possible modes of action of endogenous lectins of animal tissues have recently been presented by Bowles (1979). They seem ideally suited for regulatory roles such as those involving receptor-mediated internalization (Goldstein *et al.*, 1979) and transport of biologically important molecules.

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