CALF HEART LECTIN REACTS WITH BLOOD GROUP Ii ANTIGENS AND OTHER PRECURSOR CHAINS OF THE MAJOR BLOOD GROUP ANTIGENS

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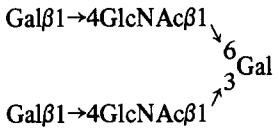
1. Introduction

There is increasing interest in lectins of animal tissues [1]. A lectin isolated from calf hearts reacts with glycoproteins having Galβ1→4GlcNAc sequences at their non-reducing termini [2], for example glycopeptides derived from bovine thymocytes containing Galβ1→4GlcNAcβ1→3 (or 6) structures [3]. It is known that the blood group I antigen is carried on the branched oligosaccharide



[4,5] and the i antigen is expressed predominantly on straight chain oligosaccharides containing the sequence Galβ1→4GlcNAcβ1→3Gal [6]. Both are found as cryptic antigens on the core oligosaccharides of blood group ABH-active secreted glycoproteins [4,7], erythrocyte glycolipids and gangliosides [5,6,8] and as detectable surface antigens on human erythrocytes [9], leucocytes [10] and a variety of animal cell types (R. C., T. F., unpublished observations). They are of special interest because of the developmental changes in their expression on human erythrocytes [9], cell cycle related changes on lymphocytes [11] and their increased expression in certain human adenocarcinoma tissues [12]. Therefore we have investigated the possible role of the blood group I and i antigens as natural receptors for the calf heart lectin. Parallel studies with the β-galactosyl-specific plant lectin, *Ricinus communis* agglutinin 120 [13] have shown that:

(a) The calf heart lectin shows more specificity for the terminal disaccharide units of blood group antigen precursor chains than does the plant lectin;
(b) Ii-active (blood group ABH-inactive) mucins are more potent inhibitors of both lectins than ABH active (Ii inactive) mucins;
(c) Calf heart tissue contains I and i antigens which are potent inhibitors of both lectins.

2. Experimental

The soluble lectin was isolated from an homogenate of calf hearts, as in [2] using an asialofetuin adsorbent column. *R. communis* agglutinin 120 was purchased from Miles Labs Ltd, Slough. The lectins were labelled with carrier-free ^{125}I (The Radiochemical Centre, Amersham) by the chloramine T method [14]. The iodination mixture contained lactose at 20 mM final conc. Specific activities were 1.8×10^2 cpm/ng calf lectin and 3×10^3 cpm/ng *R. communis* agglutinin, as measured by a Nuclear Enterprises 1600 gamma counter.

Inhibition of binding assays with the lectins were a modification of the method in [2]: 10 μl serial 10-fold dilutions of inhibitors were incubated at 4°C with either 10 μl (50 ng) ^{125}I-labelled calf heart lectin or 10 μl (12 ng) ^{125}I-labelled *R. communis* agglutinin in 20 μl either a solution of 0.85% NaCl, 0.01 M NH_4HCO_3, 1 mM dithiothreitol, 1 mg/ml bovine

serum albumin (BSA); or 50 mM phosphate-buffered saline, pH 7.4 (PBS), 1 mg/ml BSA, respectively. After 60 min, 10 μl packed, freshly prepared, trypsinized rabbit erythrocytes (~8 × 10^6 cells) were added and the incubation continued for a further 60 min. The cells were then washed twice with 0.5 ml 0.85% NaCl and their radioactivity measured. All points were performed in duplicate. The counts in the negative control tubes (labelled lectin without erythrocytes and inhibitor) were subtracted from all counts in the assays and inhibitory activity was expressed as either nmol non-reducing galactose termini (oligosaccharides) or μg/ml (mucins) giving 50% inhibition of binding of the lectins relative to the positive controls.

As inhibitors, the following oligosaccharides were used: lactose (Sigma Chemical Co.); Galβ1→4GlcNAc (gift of Dr E. A. Kabat); Galβ1→3GlcNAc and Galβ1→6GlcNAc (gifts of Dr Adeline Gauhé); the straight chain trisaccharides, the branched tri-, tetra- and penta-saccharides (table 1) were synthesized in the laboratory of Dr S. David [15]; the branched octasaccharide isolated from the urine of a patient with GM1 gangliosidosis [16] was a gift from Dr A. Lundblad.

The following three types of complex glycoproteins (mucins) were tested for their reactions with the two lectins:

(a) Substances lacking blood group A, B, H and Lewis activities but showing I and i activities as recognized by all 11 anti-I and anti-i sera tested [4,7,17]; these were, ovarian cyst blood group precursor-like substance F1, a glycoprotein-rich extract of human meconium, Mec and a preparation of sheep gastric mucin;

(b) Human ovarian cyst blood group substances without demonstrable I and i activities [4,7,17]; MSS (blood group A-active), Beach (blood group B-active) and JS (blood group HLe^b-active);

(c) Substances showing activities only with certain of the anti-I and anti-i antisera tested [4,7,17]; these were, ovarian cyst substances 413 (blood group B-active), N-1 phenol insol (blood group Le^a-active), 484 and 502 (both lacking in A, B, H and Lewis activities) and the products of one stage of Smith (periodate) degradation (1st IO_4) [4] of substances MSS, Beach and JS.

I and i antigens were isolated from a clear supernatant of heart homogenate (depleted of lectin by passage over an asialofetuin adsorbent) by affinity chromatography on an anti-I adsorbent [18].

3. Results and discussion

Both lectins were better inhibited by Galβ1→4GlcNAc than by lactose (table 1). The calf heart lectin was equally well inhibited by the disaccharides Galβ1→3GlcNAc and Galβ1→4GlcNAc which occur as the non-reducing termini (type 1 and type 2 chains, respectively) of glycoprotein precursors of the blood group antigens [19]; it was not inhibited by the isomer Galβ1→6GlcNAc (which has not been observed on blood group antigen precursors) at the highest concentration tested. The *R. communis* lectin reacted best with Galβ1→4GlcNAc and less well with the 1→3 and 1→6 linked isomers and did not distinguish between these two latter disaccharides. With the calf lectin the inhibitory activities of straight chain trisaccharides and branched oligosaccharides containing terminal Galβ1→4GlcNAc and/or Galβ1→3GlcNAc units were comparable (per mol/non-reducing galactose) with the disaccharides Galβ1→4 (or 3) GlcNAc, indicating that the linkage of the penultimate GlcNAc to an internal galactose (or mannose) does not affect binding to this lectin. Thus, the calf lectin, while distinguishing 1→4- and 1→3-linked terminal oligosaccharide units from 1→6-linked units, does not distinguish between Galβ1→4GlcNAcβ1→3 (or 6) – sequences associated with I and i specificities [4,6,15,21] and Galβ1→3GlcNAcβ1→3 (or 6) – and Galβ1→4GlcNAcβ1→2 – sequences which have no demonstrable I or i activities [4,15] and weak I activities, respectively (E. Wood, A. Lundblad, T. F., unpublished observations). Neither lectin was inhibited by the branched trisaccharide with two terminal GlcNAc residues linked β1→6 and β1→3 to Gal.

Among the mucins (table 2) the strongly Ii-active substances (a) showed the highest inhibitory activities with both lectins, and the blood group A, B, H, Le^b-active (Ii-inactive) substances (b) which must have a large proportion of substituted precursor chains, were much less inhibitory unless Ii-active oligosaccharide determinants were exposed by periodate degradation. The substances with varying Ii activities (c) showed a range of inhibitory activities with the lectins.

Table 1
Comparison of the potency of oligosaccharides in inhibiting the binding of radioiodinated calf heart lectin and *R. communis* 120 agglutinin to trypsinized rabbit erythrocytes

Oligosaccharides	nmol non-reducing galactose termini giving 50% inhibition of binding	
	Calf heart lectin	*R. communis* agglutinin
Galβ1→4Glc (lactose)	18	28
Galβ1→4GlcNAc[a]	4.9	9.5
Galβ1→3GlcNAc	4.1	27
Galβ1→6GlcNAc	> 54	24
Galβ1→4GlcNAcβ1→3Gal[a]	5.7	19
Galβ1→4GlcNAcβ1→6Gal[a]	5.7	12
Galβ1→3GlcNAcβ1→3Gal	5.7	> 38
Galβ1→3GlcNAcβ1→6Gal	5.7	57
GlcNAcβ1↘6 Gal 3↗ Galβ1→3GlcNAcβ1 Galβ1→4GlcNAcβ1↘6 Gal 3↗ Galβ1→3GlcNAcβ1	8.4	194
Galβ1→4GlcNAcβ1→2Manα1↘3 Manβ1→4GlcNAc[a] 6↗ Galβ1→4GlcNAcβ1→2Manα1	4.2	12
GlcNAcβ1↘6 Gal 3↗ GlcNAcβ1	> 364	> 364

[a] Exhibit inhibitory activity toward certain anti-I or anti-i antibodies (4,15,21], E. Wood, A. Lundblad, T. F., unpublished observations)

Since the I and i antigens contain recognition sites for the calf lectin, we examined the calf heart homogenate for the presence of I and i antigens. The eluate from the anti-I adsorbent column, 0.85 mg dry wt, containing 2% hexose (as determined by phenol sulphuric acid assay) [20] was tested for inhibitory activity with two anti-I sera (Ma and Step) and one anti-i serum (Tho), by radioimmunoassy [8,15]. It had potent inhibitory activity (relative to its hexose content) with all three antisera, giving 50% inhibition of binding of the anti-I and -i sera to a radioiodinated Ii-active glycoprotein at concentrations of 0.3, 1 and 6 μg hexose/ml, respectively. The eluate also had inhibitory activity toward the calf lectin and the *R. communis* lectin at hexose concentrations of 60 μg/ml and 1 μg/ml, respectively (table 2).

These studies show that among blood group substances, those with I and i activities carry receptors for the calf and the *R. communis* lectins and raise the possibility that I and i antigens may be among natural receptors for the former. Immunocytochemical studies should identify the cellular location of Ii antigens in heart tissues and their relationship to the distribution of the lectin.

Calf heart lectin may be only one among several which has been selected for and enriched from heart

Table 2
Comparison of the potency of blood group substances with and without Ii activities in inhibiting the binding of radioiodinated calf heart lectin and *R. communis* 120 agglutinin to trypsinized rabbit erythrocytes

Inhibitors	No. anti-I or -i sera inhibited	Concentration (μg/ml) giving 50% inhibition of binding	
		Calf heart lectin	*R. communis* agglutinin
(a) Reactive with all anti-I and -i sera			
Fl	11[a]	100	0.45
Mec	11	45	1
Sheep gastric mucin	11	90	0.6
(b) Not reactive with anti-I and -i sera			
MSS	0	> 1000	20
Beach	0	1500	170
JS	0	450	40
(c) Reactive with certain anti-I and -i sera			
413	7	320	20
N-1 phenol insoluble	2	650	6
484	5	250	30
502	4	300	30
MSS 1st IO_4	3	100	7
Beach 1st IO_4	4	90	1
JS 1st IO_4	2	500	> 1200
Ii active substance from calf heart	3[b]	60	1

[a] The figures indicate the number of anti-I or anti-i sera inhibited by each mucin
[b] The Ii-active substance eluted from the supernatant of the calf heart homogenate was tested with only two anti-I and one anti-i sera. The inhibitory activity of this substance with the two lectins was expressed as μg hexose/ml giving 50% inhibition. The hexose content of this substance was 2% compared with 20–30% for the mucins

The blood group I and i activities of the mucins have been evaluated with 11 antisera (6 anti-I and 5 anti-i) by radioimmunoassays [17]

homogenate by adsorption to asialofetuin and elution with lactose. Future studies with other carbohydrate adsorbents and elution with more complex oligosaccharide chains may reveal more discriminatory lectins. Such an approach would open the way for the systematic investigation of the biological functions of defined cell surface carbohydrate structures, such as the blood group antigens and their specific membrane-associated lectins.

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