Further studies of oligosaccharide recognition by the soluble 13 kDa lectin of bovine heart muscle

Ability to accommodate the blood-group-H and -B-related sequences

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Oligosaccharide recognition by the 13 kDa soluble lectin from bovine heart muscle has been investigated by inhibition of binding of the ^{125}I -labelled lectin to trypsin-treated rabbit erythrocytes. The results indicate that the Type 1 (Gal β 1-3GlcNAc) and the Type 2 (Gal β 1-4GlcNAc) backbone structures are the basic recognition units, and that the blood-group-H structure, the blood-group-B structure, the 'B-like' structure [afucosyl-(blood group B)] and the α 2-3 sialylated analogues of the backbone structures can also be accommodated and hence are candidate receptor structures for the lectin. A comparison of available inhibition data on six other soluble β -galactoside-binding lectins (three from human lung and three from rat lung) has shown some common features among these and the bovine lectin, e.g. in general a stronger reaction with *N*-acetyl-lactosamine than with lactose, and a lack of reaction with 3-fucosyl-lactose and 6-sialyl-lactose. However, there are distinctive features among the lectins, e.g. differences in relative reactions with the blood-group-A structure, and no two of the lectins appear to be identical in their fine specificities.

INTRODUCTION

Bovine heart muscle contains a soluble lectin with a subunit molecular mass of 13 kDa and a binding specificity for β -galactoside-terminating oligosaccharides (de Waard *et al.*, 1976; Childs & Feizi, 1979*a*). This lectin is a member of a unique family of proteins that are related antigenically (Childs & Feizi, 1979*b*; Carding *et al.*, 1984) and structurally (Ohyama *et al.*, 1986; Gitt & Barondes, 1986; Southan *et al.*, 1987) and which are widely distributed in animal tissues (Barondes, 1984). Previous investigations of oligosaccharide recognition by the bovine heart muscle lectin have shown that Type 1 and Type 2 oligosaccharide backbone sequences (structures 1 and 4 below) are equally potent inhibitors of its binding to trypsin-treated rabbit erythrocytes (Childs & Feizi, 1979*a*). These studies have also shown that linkage of the *N*-acetylglucosamine residue to an internal galactose residue (β 1-3 or β 1-6 linkage as in structures 2, 3, 5 and 6 below) or to mannose (β 1-2 linkage as in structure 7) does not affect reactivity with the lectin.

1. Gal β 1-3GlcNAc (Type 1 backbone)
2. Gal β 1-3GlcNAc β 1-3Gal
3. Gal β 1-3GlcNAc β 1-6Gal

4. Gal β 1-4GlcNAc (Type 2 backbone)
5. Gal β 1-4GlcNAc β 1-3Gal
6. Gal β 1-4GlcNAc β 1-6Gal
7. Gal β 1-4GlcNAc β 1-2Man α 1

8. Gal β 1-4GlcNAc

An additional observation [S. R. Carding & T. Feizi, unpublished work (cited by Scudder *et al.*, 1982)] has been that the Type 2 disaccharide backbone substituted by the blood-group-H-specific α 1-2-linked fucose (structure 8 above) has inhibitory activity towards the bovine lectin. We report here further studies of oligosaccharide recognition by this carbohydrate-binding protein and compare our results with those available on six other soluble mammalian lectins with related specificities, namely three from human lung and three from rat lung.

MATERIALS AND METHODS

Lectin was isolated from a homogenate of bovine heart by affinity chromatography on an adsorbent of asialofetuin-Sepharose 4B as described previously (de Waard *et al.*, 1976; Childs & Feizi, 1979*b*). The purified lectin was carboxymethylated (Carding *et al.*, 1984), and inhibition of binding assays using ^{125}I -labelled lectin (50 ng, containing 10^4 – 10^5 c.p.m.) and trypsin-treated rabbit erythrocytes (8×10^6 cells) in a reaction volume of 50 μ l were performed as described by Childs & Feizi (1979*a*).

Oligosaccharides

Lactose (structure 9, Table 1), and the α 2-3 and α 2-6 sialylated analogues (structures 12 and 13) were from Sigma Chemical Co. (Poole, Dorset, U.K.). The blood-group-H-active oligosaccharides (structures 10 and 20), lacto-*N*-neotetraose (structure 14), lacto-*N*-tetraose (structure 19) and their fucosylated analogues (structures 18, 21 and 22) were isolated from human milk and were gifts of Dr. W. M. Watkins and Dr. A. S. R. Donald. The blood-group-A tetrasaccharide (structure 11) isolated from neonatal faeces was a gift of Dr. A. Lundbland

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Table 1. Relative activities of oligosaccharides as inhibitors of the binding of bovine heart lectin to trypsin-treated rabbit erythrocytes

Oligosaccharide designation	Structure	Inhibitory activity*
Lactose-based oligosaccharides		
9	Gal β 1-4Glc	1
10	Gal β 1-4Glc 1,2 Fuca	0.8
11	GalNAc α 1-3Gal β 1-4Glc 1,2 Fuca	–†
12	NeuAc α 2-3Gal β 1-4Glc	0.4
13	NeuAc α 2-6Gal β 1-4Glc	–
Type 2-based oligosaccharides		
4	Gal β 1-4GlcNAc	3.7
14	Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc	2.0
15	Gal α 1-3Gal β 1-4GlcNAc-R ₁ ‡	1.9
16	Gal α 1-3Gal β 1-4GlcNAc-R ₂ ‡ 1,2 Fuca	0.7
17	Gal β 1-4GlcNAc 1,3 Fuca	–
18	Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc 1,3 Fuca	0.2§
Type 1-based oligosaccharides		
1	Gal β 1-3GlcNAc	4.4
19	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc	2.2
20	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc 1,2 Fuca	1.2
21	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc 1,4 Fuca	–
22	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc 1,2 1,4 Fuca Fuca	0.2§

* Inhibitory activity is expressed relative to that of lactose; the results are compiled from seven separate experiments in which 50% inhibition of lectin binding was observed with 9.5–20 nmol of added lactose.

† – Indicates no inhibition at the highest levels tested; these were 43, 32, 56 and 37 nmol for oligosaccharide structures 11, 13, 17 and 21 respectively.

‡ R₁ is O(CH₂)₈CO₂CH₃; R₂ is OCH₂–CHOH–CH=CH–CHOH–CH₂OH.

§ Less than 50% inhibition observed at the highest levels tested; these were 46 and 50 nmol for oligosaccharides 18 and 22. Inhibitory activities were calculated by extrapolation of inhibition curves from approx. 30% inhibition.

|| Results taken from Childs & Feizi (1979b).

and Dr. H. Sabharwal. The blood-group-B tetrasaccharide BR_L 0.44 (structure 16) derived from an ovarian-cyst substance was a gift of Dr. E. A. Kabat. The blood-group-‘B-like’ trisaccharide (structure 15) was chemically synthesized and was from Chembiomed, Edmonton, Alberta, Canada. *N*-Acetyl-lactosamine (structure 4) and the type-1-based analogue (structure 1) were chemically synthesized and were gifts from Dr. S. David and Dr. A. Veyrières. The chemically synthesized oligosaccharide structure 17 (3-fucosyl-*N*-acetyl-lactosamine) was a gift from Dr. P. Sinay.

RESULTS AND DISCUSSION

The inhibition of binding experiments (summarized in Table 1) confirm previous observations (Childs & Feizi, 1979a) that the bovine heart lectin does not discriminate between the unsubstituted Type 1 and Type 2 disac-

charide backbones (cf. structure 1 with 4, and structure 14 with 19 in Table 1) and that both sequences have superior inhibitory activities compared with the unsubstituted disaccharide lactose (structure 9). Recognition by the lectin does not appear to extend beyond the non-reducing disaccharide backbone sequence, since extension of the oligosaccharide backbone sequences, as in structures 14 and 19, did not result in increased inhibitory activities relative to the corresponding disaccharide structures. Substitutions of the backbone sequences with either blood-group-H or blood-group-B-related monosaccharides, as in structures 10, 20, 15 and 16, only slightly impaired reactivity with the lectin, whereas blood group A substitution, as in structure 11, abolished reactivity (Table 1). Substitution of the backbones with α 1-4- and α 1-3-linked fucose residues, as in structures 17, 18 and 21, markedly impaired or abolished inhibitory activity. The effect of terminal sialic acid substitution

Fig. 1. Comparison of oligosaccharide recognition by seven β -galactoside-binding lectins

The inhibitory activities (relative to lactose) of the eight oligosaccharides are taken from reports by Leffler & Barondes (1986) and Sparrow *et al.* (1987) and from the present study. The numbers refer to the oligosaccharide designations shown in Table 1. Symbols for lectins: ■, bovine heart lectin; ▲, △, and □, human lung lectins HL14, HL22 and HL29 respectively; ●, ○, ▽, rat lung lectins RL14.5, RL18 and RL29 respectively. The lectins are divided into two groups, (a) and (b), according to their ability to react with the blood-group-A oligosaccharide (structure 11).

was also investigated: the NeuAc α 2-3-substituted lactose (structure 12) retained inhibitory activity, whereas the α 2-6 substituted analogue (structure 13) did not.

Taken together, these and the previous observations with the bovine heart lectin suggest that Gal β 1-3GlcNAc and Gal β 1-4GlcNAc backbone sequences are the basic recognition structures, and that the combining site of this lectin can accommodate several naturally occurring analogues based on these backbones. Thus the blood-group-H, -B and -'B-like' substituents as well as the α 2-3-sialylated analogues are accommodated, whereas the blood-group-A substituent and α 2-6-sialylated analogue are not. It will be interesting to investigate how this range of '*in vitro*' reactivities relates to the oligosaccharide recognition by the bovine lectin *in vivo*. From previous immunochemical and structural studies it is known that the bovine species possesses the biosynthetic machinery for the several oligosaccharide sequences with which the lectin reacts *in vitro*, such as the Type 1 and Type 2 backbones, 2-3-linked sialic acid and the blood-group-H and -'B-like' structures (Beiser & Kabat, 1952; Mizuochi *et al.*, 1979; Hanfland, 1975).

Oligosaccharide recognition by six other soluble lectins, three from rat lung (Leffler & Barondes, 1986) and three from human lung (Sparrow *et al.*, 1987), has also been investigated recently by using asialofetuin as the reference immobilized glycan. In Fig. 2 we have compared the relative inhibitory activities of eight oligosaccharides, which were tested with the six lectins in the previous studies and with the bovine lectin in the present study. The seven lectins have some common features: none reacts with 3-fucosyl-*N*-acetyl-lactosamine (structure 17) or 6-sialyl-lactose (structure 13), and with one exception (rat lung lectin, RL18) they react more strongly with *N*-acetyl-lactosamine (structure 4) than with lactose (structure 9).

The common feature of the non-reactive (or poorly reactive) oligosaccharides, such as those with Type 1 or Type 2 backbones having fucose linked at C-4 or C-3 of *N*-acetylglucosamine/glucose and sialic acid linked at C-6 of galactose (Figs. 2b, 2d and 3a), is that these substitutions result in modification or masking of the

Fig. 2. Proposed conformations for oligosaccharides based on Gal β 1-3GlcNAc (a and b) and Gal β 1-4GlcNAc (c and d)

The preferred conformations of fucose linked α 1-4 or α 1-3 to *N*-acetylglucosamine of Type 1 and Type 2 backbones respectively [deduced from n.m.r. analysis and potential-energy calculations (Spohr *et al.*, 1985; Hounsell, 1987)], are such that these residues would lie along the 'top' of the molecule, as indicated in the Figure, thus interfering with interactions with the hydroxy groups shown in bold. R = -H or -3Gal β 1-4Glc.

Fig. 3. Proposed conformations for oligosaccharides based on Galβ1-4Glc

(a) One possible conformation for α2-6 sialyl-lactose (structure 13) has been drawn from space-filling molecular models. For all rotomers around the α2-6 glycosidic bond the sialic acid would block recognition of the 'top' face of the molecule having hydroxy groups shown in **bold**. (b) The proposed solution conformation for α2-3 sialyl-lactose is shown, based on conformational information given by Sabesan *et al.* (1984) for the ganglioside G_{M1}. The *N*-acetamido group and glycerol side chain of the sialic acid are orientated away from the C-4 hydroxy and CH₂OH group of galactose, allowing unrestricted access of the lectin to the hydroxy groups shown in **bold**. (c) The proposed solution conformation for the blood-group-A oligosaccharide (structure 11) is shown, based on conformational information given by Lemieux *et al.* (1985) for a blood-group-B oligosaccharide. From space-filling molecular models of this conformer it can be seen that the *N*-acetamido group is in close proximity to the C-4 hydroxy group of galactose.

hydroxy groups shown in **bold** in these Figures i.e. the axial C-4 hydroxy group of galactose, the CH₂OH group at C-6 of galactose and the equatorial C-3 or C-4 hydroxy group of *N*-acetylglucosamine/glucose. These groups have been proposed as the major determining factors in recognition of β-galactoside-binding lectins of rat and human lung (Leffler & Barondes, 1986; Sparrow

et al., 1987), and our results with the bovine heart lectin are in agreement with this hypothesis.

The relative importance of the C-4 hydroxy group of galactose for recognition by each of the lectins can be deduced from their reactivities with the blood-group-A-active oligosaccharide and a consideration of oligosaccharide conformation. As shown in Fig. 3(c), the blood-group-A oligosaccharide structure (11) is likely to adopt a conformation where the *N*-acetamido group of *N*-acetylglucosamine α1-3-linked to galactose is in close proximity to the C-4 hydroxy group of the latter residue. For some of the lectins (BHL, HL14 and RL14.5), substitution with *N*-acetylglucosamine greatly decreased reactivity, showing the importance of an accessible C-4 hydroxy group. In the present study with the bovine heart lectin, where blood-group-B and 'B-like' oligosaccharides were included as inhibitors, the results show that substitution by Galα1-3 rather than GalNAcα1-3 does not result in a major decrease in reactivity. This indicates that it is the *N*-acetamido group alone of *N*-acetylglucosamine that is involved in the masking of lectin recognition. For other lectins (HL22 and RL18) this substitution with *N*-acetylglucosamine had little effect on reactivity, suggesting that the C-4 hydroxy group of galactose may not be required or that the acetamido group may be accommodated in the lectin-combining site. For two of the lectins, namely HL29 and RL29, blood-group A-active oligosaccharides are the preferred ligands (Fig. 1). The molecular models for α2-3-sialyl-lactose (structure 12, Fig. 3b) show that, unlike the GalNAcα1-3 substituent, the sialic acid is orientated such that the *N*-acetamido group is away from the C-4 hydroxy group and CH₂OH group of galactose. This explains why, despite the bulky nature of this substituent, the inhibitory activity of α2-3-sialyl-lactose is retained with all the lectins.

The importance of the *N*-acetamido group of *N*-acetylglucosamine in binding is more difficult to understand, as both Galβ1-3GlcNAc and Galβ1-4GlcNAc have, in most cases, increased reactivity compared with lactose, although the *N*-acetamido group of the Type 1 sequence, but not the Type 2 sequence, is removed spatially from the C-4 hydroxy group of galactose, the C-6 CH₂OH group of galactose and the C-3/4 hydroxy group of *N*-acetylglucosamine (see Figs. 2a and 2c). This raises the possibility that the *N*-acetamido group is not directly involved in binding, but somehow serves to stabilize the ligand-receptor interaction.

As discussed above, lectins of this family are widely distributed in animal tissues (Barondes, 1984). Immunochemical (Childs & Feizi, 1979b; Carding *et al.*, 1985), biochemical (Roff & Wang, 1983; Sparrow *et al.*, 1987) and molecular-genetic (Gitt & Barondes, 1986) studies suggest that multiple forms of these proteins may occur in individual tissues. From the present studies and those of Leffler & Barondes (1986) and Sparrow *et al.* (1987) it appears that no two of the lectins are identical in their carbohydrate-binding specificities. It will be an exciting development if it can be shown that the differing oligosaccharide specificities of these lectins relate to the polymorphisms of carbohydrate structures in the cells, or the individuals, from which they are derived.

We are grateful to Dr. W. M. Watkins, Dr. A. S. R. Donald, Dr. E. A. Kabat, Dr. A. Lundblad and Dr. H. Sabharwal for oligosaccharides from natural sources, to Dr. S. David,

Dr. A. Veyrières, Dr. P. Sinay and Chembiomed for the chemically synthesized oligosaccharides and to Mrs. Maureen Moriarty for the preparation of the manuscript.

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Received 17 February 1988; accepted 7 March 1988