ENDOGENOUS LECTINS OF HUMAN MUSCLE

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1. Introduction

Endogenous carbohydrate binding proteins (lectins) have been detected on the cell surface of a variety of cell types, including cohesive slime moulds [1–3]. Developmental changes in the activities of lectins during the differentiation of slime mould cells has led to the suggestion that they may mediate the cohesion of these cells [4]. In mammalian tissues lectins have been detected by their haemagglutination activities [5–7]; two of which change dramatically during embryogenesis [5,8,9]. One lectin activity is β-D-galactopyranoside specific and the other is most strongly inhibited by certain glycosaminoglycans (GAGS) [9–11]. The biological roles of these lectins are not understood, but some authors have suggested that during myogenesis they may play a role in myoblast fusion [6,7,12,13] although this remains controversial [2,10,14]. We have studied temporal changes in lectins prepared from human muscle grown in vitro and have detected two lectins which appear to be developmentally regulated. We have investigated the saccharide specificities of the lectins, extracted from human foetal and adult post-mortem skeletal muscle. The specificity of human muscle lectins towards β-D-galactopyranosides and GAGS is similar to that of lectins from other species [9–11].

2. Materials and methods

Phosphate-buffered saline (PBS) was 75 mM NaK phosphate, 75 mM NaCl (pH 7.2). Saline was 150 mM NaCl. Extraction buffer contained 2 mM EDTA, 4 mM 2-mercaptoethanol (2ME), 100 mM lactose (BDH, Poole) and 100 mM *N*-acetyl-galactosamine (Sigma, London) in PBS. Keratan sulphate, heparan sulphate and hyaluronic acid were kindly donated by Professor Matthews (Univ. Chicago). Dermatan sulphate and the chondroitin sulphates were purchased from Miles, London.

Soluble lectin extracts were prepared from human muscle as follows: 1–5 g muscle was homogenized in ice-cold extraction buffer (to give 5%, w/v, homogenate) followed by centrifugation at 100 000 × *g* for 1 h at 4°C. The supernatant (containing both lectins) was centrifuged at 70 000 × *g* for 18 h and the resulting precipitate was resuspended in the original volume of PBS (containing 4 mM 2ME and 2 mM EDTA). Both the 70 000 × *g* supernatant (lectin 1) and the suspended precipitate (lectin 2) were dialysed for 18 h against PBS (containing 4 mM 2ME and 2 mM EDTA (3 changes, total vol. 10 litres) at 4°C and stored at –80°C in aliquots.

Human muscle was dissociated enzymatically [15] and secondary monolayer cultures were prepared as in [16]. The cells were seeded at a density of 2–3 × 10^5/10 cm tissue culture plate. Soluble lectin extracts were prepared by washing the cells in ice-cold PBS and then scraping the cells off the surface of the culture dishes into a small volume of ice-cold extraction buffer, sonicating on ice and centrifuging at 100 000 × *g* for 1 h at 4°C. The supernatant which contained both lectins was dialysed and stored as above. Rabbit erythrocytes (type 1 RBC) were collected in Alsever's medium, treated with trypsin and fixed with glutaraldehyde, as in [7]. Type 2 RBC were produced by washing type 1 RBC with absolute alcohol [17]. Haemagglutination tests were performed in Microtiter U-shaped wells. The lectin extract (25 μl) was sequentially diluted 2-fold in each well which contained 25 μl saline. A further 25 μl saline (or saccharide in saline) was added, followed by 25 μl BSA (1%, w/v, in saline) and 25 μl RBC (2%, v/v, in PBS). The plates were rotated for a few minutes and the titre was read after 1 h at room temperature. The titre was defined

as the highest dilution of the extract which produced agglutination. Lectin activity was expressed as titre^{-1} mg^{-1} protein present in 25 μl lectin extract. Protein concentrations were determined by the Lowry method [18] using human serum albumin (Dade) as the standard.

Creatine phosphokinase (EC 2.7.3.2,CK) estimations were carried out on the cell extracts, before centrifugation, using a Boehringer UV activated kit (no. 124 150) as in [19]. CK activities are expressed as μmol creatine produced . min^{-1} . mg protein^{-1} at 30°C.

3. Results

Two types of soluble lectins have been extracted from human muscle. The range of activities in 6 different extracts was 400–900 titre^{-1} mg^{-1} for the first type of lectin (lectin I, assayed with RBC I). The second type (lectin 2) which agglutinated alcohol-washed cells (RBC 2) had a higher activity, ranging from 1000–12 000 titre^{-1} . mg^{-1} . Prolonged centrifugation [11] did not separate these two types of lectins entirely and both were present in the supernatant as well as in the precipitate. The inhibition characteristics of the lectins were investigated and the results are presented in table 1, as the concentration of saccharide or GAG which produced 50% inhibition of haemagglutination activity. Type I lectin was specific for β-D-galatopyranoside residues, as described for lectins from other mammalian species [10,11]. Thiodigalactoside (TDG) and lactose were the most potent inhibitors (fig.1, table 1). Lectin 2 was strongly inhibited by heparin and heparan sulphate (fig.2, table 1).

Monoclonal antibodies to fibronectin [16] did not

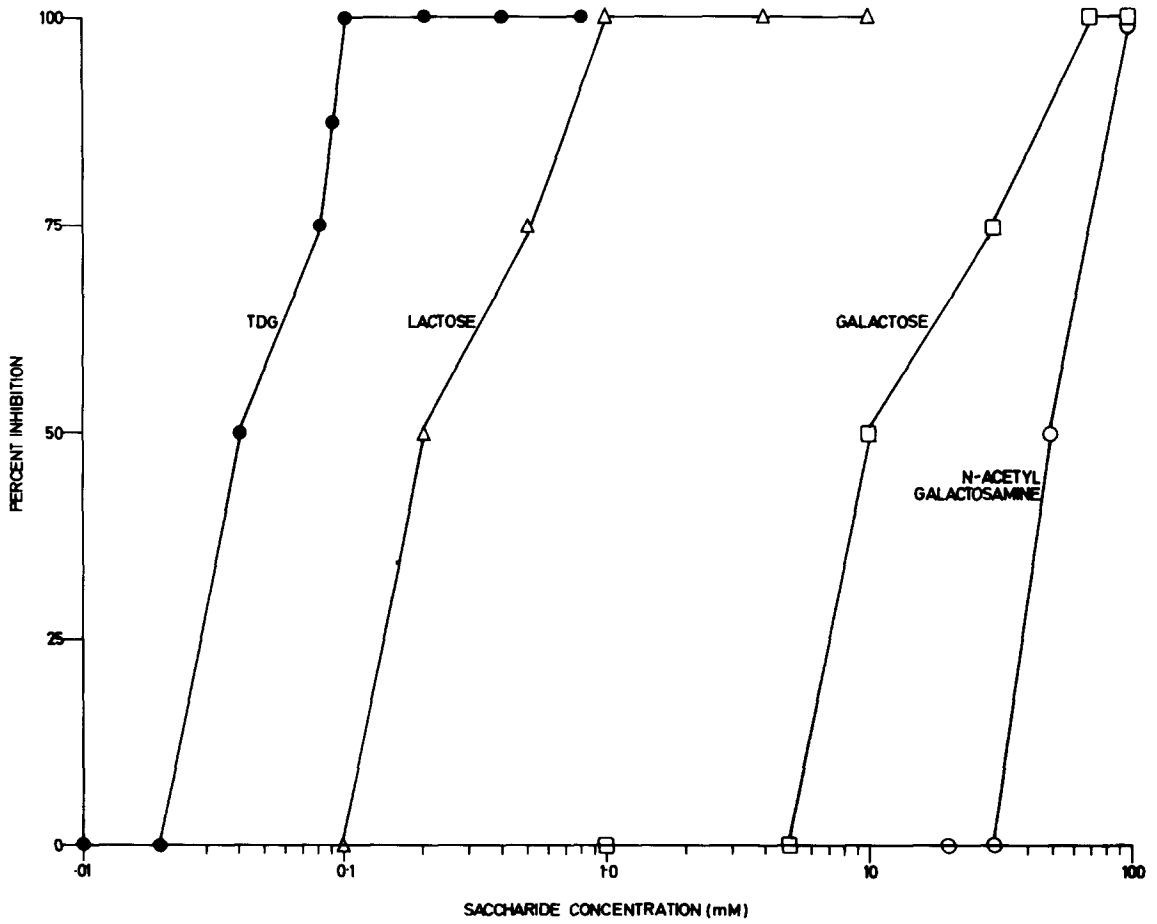

Fig.1. Inhibition of human muscle lectin 1 activity by saccharides.

Table 1
Inhibition of haemagglutination activity of lectin 1 and lectin 2 by various saccharides and GAGs

Saccharide or GAG	Concentration (mM) inhibiting 50% of lectin 1 activity	Concentration (μg/ml) inhibiting 50% of lectin 2 activity
TDG	0.04	1 mM
Lactose	0.20	–
Melibiose	6.0	–
Galactose	10.0	–
N-Acetylgalactosamine	50.0	5–10 mM
Xylose, glucose, mannose	>100.0	–
Heparin	>100 μg/ml	0.1
Heparan sulphate	–	25.0
Dermatan sulphate	–	250.0
Hyaluronic acid	–	250.0
Chondroitin 4-sulphate	–	1000.0
Chondroitin 6-sulphate	–	2000.0
Keratan sulphate	–	>1000.0

Lectin 1 was tested with RBC1 and lectin 2 with RBC 2 as in section 2. With fresh extracts, wells containing high concentrations of lectin 2 showed no agglutination. This high concentration inhibition has been described in [11]

Table 2
Lectin and CK activities in secondary human muscle cultures (prepared from 20 week foetus)

No. of days in culture	Lectin 1 ($titre^{-1} . mg^{-1}$)	Lectin 2 ($titre^{-1} . mg^{-1}$)	CK at 30°C ($\mu mol . min^{-1} . mg^{-1}$)
1	226	2606	0.30
3	528	8476	0.71
4	500	8000	0.95

Fusion was observed to commence on day 2: lectin 1, activity was inhibited by 1 mM TDG; lectin 2, activity was inhibited by 0.1 mg/ml heparin

inhibit haemagglutination. Temporal changes in lectin activities were followed in extracts of replicate secondary foetal muscle cultures and the results of one experiment are shown in table 1. The levels of lectins increased at least 2-fold during the fusion of myoblasts to myotubes. There was a concomitant increase in the activity of CK, an enzyme marker of muscle cell differentiation [20].

The final degree of differentiation of this culture was relatively high, with a high CK value (1.24 $\mu mol . min^{-1} . ml^{-1}$) and a large proportion of broad, branched myotubes containing numerous nuclei. The levels of lectin 1 and 2 in the dialysed growth medium (60 $titre^{-1} . mg^{-1}$ for both lectins) did not change during the growth and differentiation of this culture [17].

Muscle cultures prepared from an 8 week foetus differentiated poorly, exhibiting a constant low CK level during the growth period (table 3). Only a few thin myotubes containing few nuclei were seen. No changes in either lectin activity were detected during the development of these cultures (table 3).

In all cultures prepared from 6 different muscle specimens, the activities of lectin 2 (2600–17 500 $titre^{-1} . mg^{-1}$) were higher than lectin 1 (200–800 $titre^{-1} . mg^{-1}$).

4. Discussion

The present study indicates that human skeletal muscle contains two soluble lectins which can be

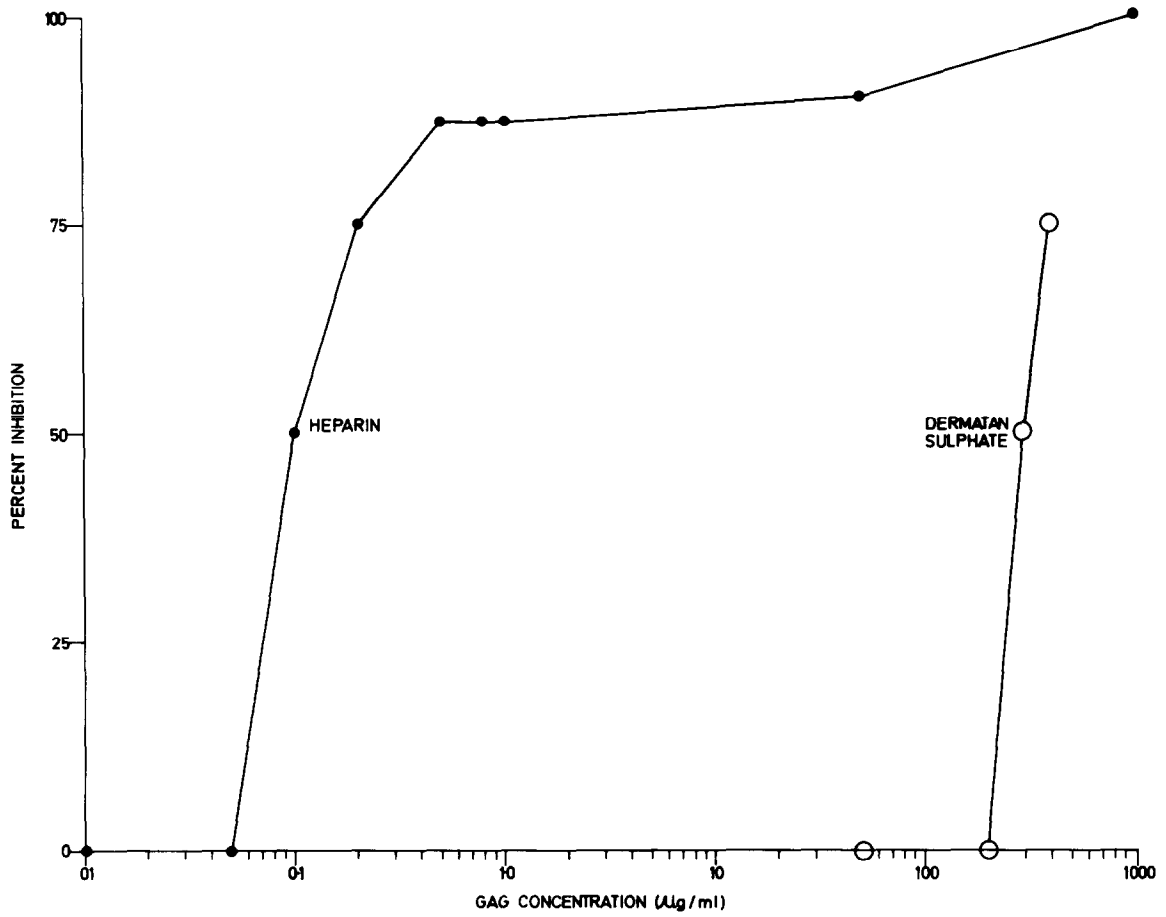

Fig.2. Inhibition of human muscle lectin 2 activity by glycosaminoglycans.

Table 3
Lectin and CK activities in human muscle cultures prepared from 28 week foetus

No. of days in culture	Lectin 1 titre^{-1} . mg^{-1})	Lectin 2 titre^{-1} . mg^{-1})	CK at 30°C (μmol . min^{-1}. mg^{-1})
3	800	6400	0.28
4	760	6094	0.25
5	888	6110	0.27
6	850	6808	0.14

The first myotubes were observed on day 4

detected by their haemagglutination activities. The haemagglutination activities of human muscle lectin 1 is inhibited by galactose-containing saccharides and is more sensitive to TDG, lactose, melibiose, galactose and *N*-acetyl–galactosamine than the crude chick embryo muscle lectin [7]. The GAGS, heparin and heparan sulphate are the most potent inhibitors of lectin 2; heparin being 20-fold more potent as an inhibitor of human muscle lectin compared to chick muscle lectin [7]. However, dermatan sulphate is only a weak inhibitor of the human muscle lectin. Heparin and heparan sulphate contain galactose, as well as xylose and the saccharide repeating units consist of glucuronic and iduronic acids and glucosamine [21].

Lectin 2 does not appear to be identical to fibronectin, an extracellular protein with haemagglutination activity and the capacity to interact with heparin, as antibodies to fibronectin do not inhibit agglutination [17]. Increases in both lectin 1 and lectin 2 were found during the fusion of human myoblasts, resembling the changes in lectin 1 activity reported in extracts of cultures of the L6 muscle cell line [1,6,7 12]. In contrast, there were no changes in lectin 1 and 2 in primary chick embryo muscle cultures, although lectin 2 activity was detected in the growth medium after fusion [17]. We have found no evidence for increases in either lectin activity in dialysed growth medium from human muscle cultures even after extensive myoblast fusion and myotube differentiation was observed, in contrast to the reports of others [17]. The involvement of a galactosyl-specific lectin in myoblast fusion has been investigated by the inclusion of TDG in the cell growth medium. This produced inhibition of fusion in the L6 muscle line [6] but not in primary cultures of chick embryonic muscle [14], rat muscle [2] or human skeletal muscle (Dr Rose Yasin, personal communication). Therefore the role of endogenous lectins in muscle remains unclear.

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