THE MONOCLONAL ANTIBODY ANTI-SSEA-1 DISCRIMINATES BETWEEN FUCOSYLATED TYPE 1 AND TYPE 2 BLOOD GROUP CHAINS

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1. Introduction

The mouse hybridoma antibody termed anti-SSEA-1 recognises a differentiation antigen which appears at the 8-cell stage of mouse embryos and becomes restricted to only certain cells in differentiated tissues [1]. In [2] anti-SSEA-1 bound to a glycoprotein-rich extract of human meconium. This binding formed the basis of a radioimmunoassay which was used to investigate the antigenic determinant recognised by the antibody. Inhibition assays with glycoproteins of known blood group activities and with a variety of natural and chemically synthesized oligosaccharides showed that this antigenic determinant involves a fucosylated structure distinct from blood group H.

Among the oligosaccharides tested as inhibitors of anti-SSEA-1 the most potent was one derived from a partially degraded ovarian cyst glycoprotein [3] and designated N-1 R_L 0.71a:

$$\begin{array}{l} \text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 6\ (?)\text{-3hexenetetrol} \\ \qquad\qquad\ \uparrow 1,3 \\ \qquad\qquad\ \text{Fuc}\alpha \end{array}$$

However, preparations of the milk oligosaccharides lacto-*N*-fucopentaose II (LNFP II) and lacto-*N*-difucohexaose II which have the Le^a-active, terminal non-reducing sequence:

$$\begin{array}{l} \text{Gal}\beta 1 \rightarrow 3\text{GlcNAc}\beta 1 \rightarrow \qquad \text{(from [4])} \\ \qquad\qquad\ \uparrow 1,4 \\ \qquad\qquad\ \text{Fuc}\alpha \end{array}$$

were also inhibitory being ~10-times less active than oligosaccharide N-1 R_L 0.71a. Preparations of LNFP II which have been extensively purified by column and paper chromatographies are known [4] to contain varying amounts of the isomeric structure lacto-*N*-fucopentaose III (LNFP III). LNFP II and LNFP III differ in having type 1 (Galβ1 → 3GlcNAc) or type 2 (Galβ1 → 4GlcNAc)-based non-reducing terminal sequences, respectively. It was important to determine whether the inhibitory activity observed with the type 1, Le^a-active oligosaccharides represents a cross-reaction or whether it is due to the presence of type 2 isomers in the preparations tested.

LNFP II and LNFP III can be separated by thin-layer chromatography (TLC) of acetylated derivatives [5]. Here, the purified isomers have been tested as inhibitors of anti-SSEA-1 in radioimmunoassays and it has been established that this antibody is specific for the type 2 sequence.

2. Materials and methods

2.1. *Materials*

LNFP preparations [6] kindly provided by Dr W. M. Watkins of this Institute were reduced [7] in the presence of tritiated sodium borohydride (Amersham International, Amersham). The anti-SSEA-1 reagent [1] was ascites fluid from a pristane-primed BALB/c mouse injected with antibody-producing hybrid cells and was kindly provided by Drs D. Solter and B. B. Knowles, Wistar Institute of Anatomy (Philadelphia PA).

2.2. *Radioimmunoassays*

The reactivities of oligosaccharides with anti-SSEA-1 were assayed in a double antibody radioimmunoassay as in [2]. In brief, serial dilutions of oligosaccharides were used as inhibitors of the bind-

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ing of anti-SSEA-1 ascites fluid (1/3000 dilution) to a radioiodinated glycoprotein extract of human meconium which had been enriched for blood group I, i and SSEA-1 activities by affinity chromatography on an anti-I immunoadsorbent column.

2.3. *Thin-layer chromatography*

TLC of oligosaccharides was performed on Whatman HPK silica gel plates (5 μm) either as non-derivatised samples in solvent system *n*-butanol/acetone/water (12:10:8, by vol.) or as reduced and acetylated [5] derivatives in solvent system butyl acetate/acetone/water (25:8:1, by vol.). For preparative TLC ~100 μg reduced, tritiated and acetylated oligosaccharide was loaded/5 cm plate. Sugar components, located using a Packard 7222 series radiochromatogram scanner (Packard Instrument Co., Downers Grove IL) were eluted with 10% water in acetone, dried and de-acetylated [5]. In analytical runs sugars were located with 0.5% orcinol in 1 M sulphuric acid spray. Stachyose (Sigma London Chemical Co., Poole) was used as a standard oligosaccharide. Lacto-*N*-tetraose and lacto-*N*-neotetraose were kindly provided by Dr W. M. Watkins of this Institute.

2.4. *Monosaccharide analyses*

Monosaccharide analyses were performed by gas–liquid chromatography of trimethylsilyl ethers of methyl glycosides [8] on a column (2100 × 0.4 cm) of 3% OV1 on chromosorb WHP (Chromatography Services, Hoylake, Merseyside) with *i*-inositol (Sigma) as internal standard and using a Pye Unicam (Pye Unicam, Cambridge) series 204 chromatograph with intergration by a Columbia Scientific Industries' Supergrator (Kemtronix UK, Compton, Berkshire).

3. Results and discussion

Two preparations of LNFP II were found to have differing inhibitory activities in radioimmunoassays with anti-SSEA-1, being ~10- (preparation 1) and 40- (preparation 2) times less active on a molar basis than oligosaccharide N-1 R_L 0.71a (fig.1).

TLC of reduced and acetylated derivatives showed that both preparations contained more than one component (fig.2). Two of these had mobilities consistent with them being the isomers LNFP II and LNFP III (R_F 0.25 and 0.33, respectively in agreement with Dr S. Hakomori, personal communication). A third component termed band IV (R_F 0.56) was unidentified. All three components were shown by GLC (after purification by TLC and de-acetylation) to have the carbohydrate composition of reduced lacto-*N*-fucopentaose isomers (galactose/fucose/*N*-acetylglucosamine/glucitol, 2.0:1.1–0.8:0.8:1.1–0.9).

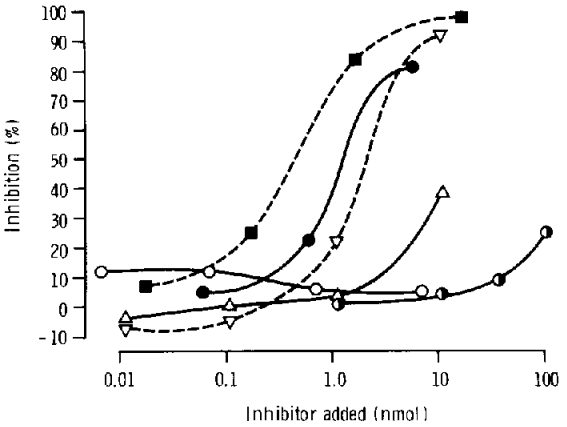


Fig.1. Inhibition by oligosaccharides of the binding of anti-SSEA-1 to ^{125}I-labelled meconium glycoproteins. The symbols used, the designations and the structures of the oligosaccharides are:

(■) oligosaccharide N-1 R_L 0.71a

Galβ1 → 4GlcNAcβ1 → 6(?)-3hexenetetrol
↑ 1,3
Fucα

(●) lacto-*N*-fucopentaose III (LNFP III)

Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glc
↑ 1,3
Fucα

(◑) lacto-*N*-fucopentaose II (LNFP II)

Galβ1 → 3GlcNAcβ1 → 3Galβ1 → 4Glc
↑ 1,4
Fucα

(○) band IV; (▽,△) preparations 1 and 2, respectively of lacto-*N*-fucopentaose II before fractionation. The inhibition data for N-1 R_L 0.71a and preparation 1 (– – –) have been taken from [2].

The differences in the activities of N-1 R_L 0.71a and LNFP III are probably not due to experimental error. Although the inhibition data were taken from two different assays there is little assay-to-assay variation. The other possible source of error, that of the quantitation of small amounts of oligosaccharides, was also not apparent. Inhibitor concentrations determined by hexose analysis ([11] using galactose as standard) and GLC were in agreement with each other and with those calculated from the dry weight where available (N-1 R_L 0.71a and LNFP II preparation 2 before fractionation).

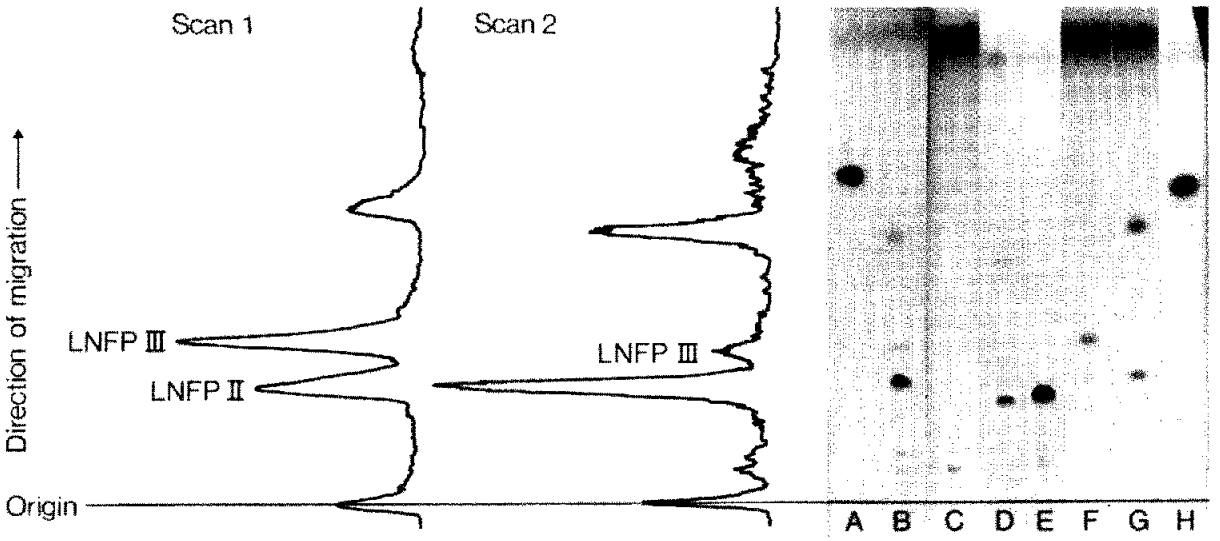


Fig.2. Thin-layer chromatography of reduced, tritiated and acetylated oligosaccharides. Scans 1 and 2 are radiochromatograms of LNFP preparations 1 and 2, respectively. Lanes A–H are oligosaccharides revealed by charring with orcinol/sulphuric acid. The lanes are taken from 3 separate chromatograms as follows: (A,B); (C,F,G); (D,E,H). Lanes (A,H) are stachyose (neutral tetrasaccharide); lane (B) corresponds to scan 2; lanes (C,E–G) are preparative TLC products of LNFP preparation 2. The products in lane (C) gave those shown in lane (D) on re-acetylation.

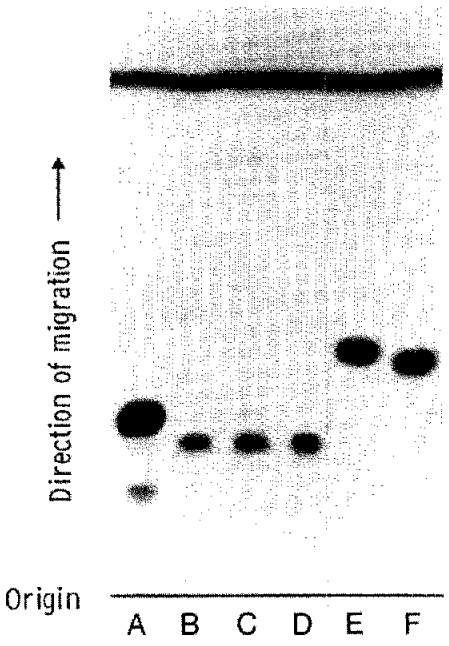


Fig.3. Thin-layer chromatography of non-derivatised (A,E,F) and reduced, purified oligosaccharides after de-acetylation (B,C,D): lane (A) stachyose (with a minor contaminant); lane (E), lacto-*N*-tetraose; lane (F) lacto-*N*-neotetraose; lane (B) band IV; lane (C) LNFP III; lane (D) LNFP II.

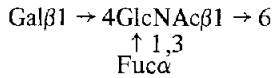
The product identified as LNFP III was the major component in preparation 1, which had the greatest inhibitory activity in the radioimmunoassay (fig.2, scan 1). Preparation 2 (scan 2) had a smaller proportion of this isomer. This latter preparation was fractionated by preparative TLC as illustrated in fig.2 (B–G). The two slowest migrating components shown in lane B were identified as partially acetylated oligosaccharides as, for example, the products in lane C were converted into the products in lane D on re-acetylation. The purified products identified as LNFP II and LNFP III are shown in lanes E and F, respectively. Preparative TLC of band IV gave a product contaminated with LNFP II (lane G). A second preparative TLC step gave band IV with no detectable contaminant (not shown).

After de-acetylation LNFP II, LNFP III and band IV had identical mobilities on TLC (fig.3 (B–D)). These pentasaccharides were clearly separable from the unfucosylated type 1- and type 2-based tetrasaccharides, lacto-*N*-tetraose (lane E) and lacto-*N*-neotetraose (lane F) respectively, which were partially separable from each other.

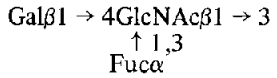
When the three purified lacto-*N*-fucopentaose isomers were tested in radioimmunoassays with anti-SSEA-1 only that identified as LNFP III had inhibitory

activity (fig.1). This oligosaccharide was ~3-times less active than oligosaccharide N-1 R_L 0.71a.

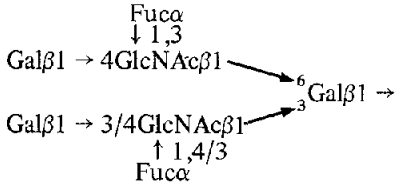
These studies clearly demonstrate the importance of separating closely related oligosaccharide isomers in determining the precise specificity of anti-carbohydrate antibodies. We have established that anti-SSEA-1, like conventional antisera against LNFP III [9], is specific for the fucosylated type 2 sequence and that it does not cross-react with the type 1-based, Le^a-active structure. Furthermore, the lower inhibitory activity of LNFP III compared with that of N-1 R_L 0.71a raises the possibility that the combining site of anti-SSEA-1 is more complementary to the sequence:



than to:



The former sequence is presumably derived [3,10] from a branched structure such as the following:



and the latter is known to occur as part of both linear and branched carbohydrate chains. Further studies with defined oligosaccharides containing internally branched sequences are required to determine the complete antigenic determinant recognised by this antibody.

Acknowledgements

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