Determination of the Binding Affinity of mAb B3 for Synthetic Derivatives of Lewis Y Using FAC/MS

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Introduction

A murine monoclonal antibody (mAb) B3 of the IgG1*k* isotype reacts with the surface of mucinous carcinomas of the colon, esophagus, stomach, and ovary (1). Like other tumour-directed antibodies that show limited reactivity against normal tissues, B3 is potentially useful for cancer treatment and diagnosis (2).

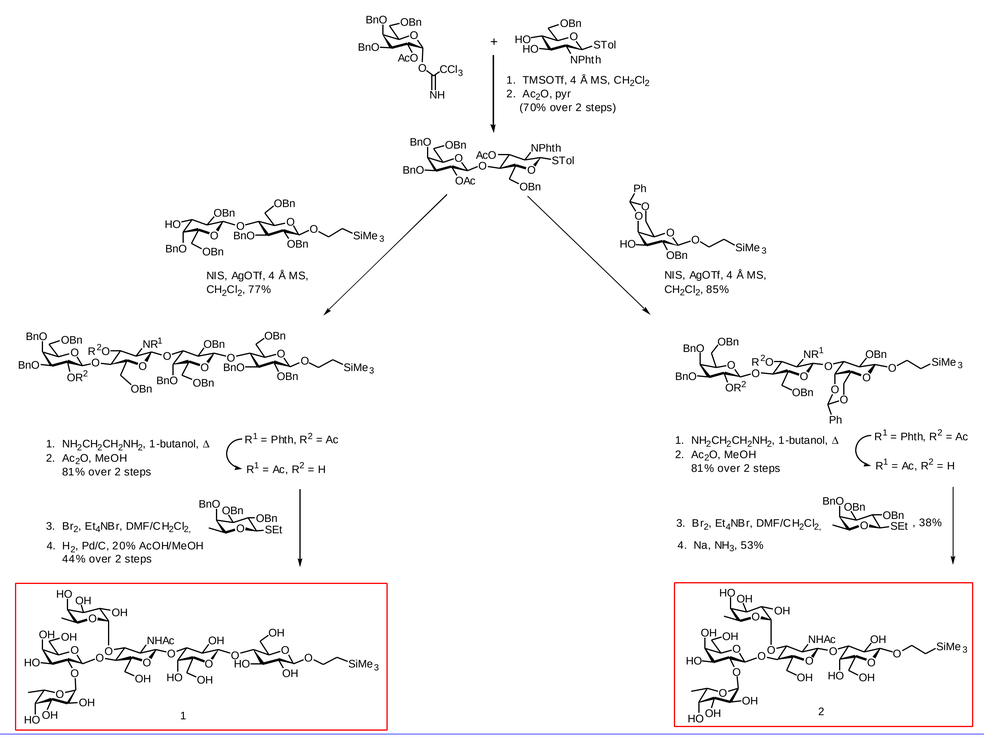
Through immunoprecipitation and ELISA experiments, it has been shown that B3 binds glycoproteins carrying oligosaccharides of the Lewis Y family (1). However, the epitope recognized by B3 has not been mapped. We wished to investigate the binding specificity of B3 and in particular the possibility that the antibody recognizes an epitope larger than the Lewis Y tetrasaccharide.

The Lewis Y tetrasaccharide is a tumour-associated antigen that is expressed on a variety of tumour cells. It may be expressed in both glycolipids and glycoproteins (3). In glycolipids, the antigen is generally carried on O3 of Gal. In glycoproteins such as *N*-glycans, the Lewis Y antigen may cap an antenna that extends from the common oligosaccharide core. These antennae frequently contain tandem repeats of LacNAc.

Bearing in mind the general structural features of glycolipids and glycoproteins, we synthesized two Lewis Y derivatives to be evaluated as ligands for B3: hexasaccharide **1** and pentasaccharide **2** (Scheme 1). Derivatives **1** and **2** represent extensions of the Lewis Y antigen from O1 of GlcNAc to lactose and galactose, respectively. These synthetic derivatives were designed to approximate the context in which the natural Lewis Y antigen might be presented to, and recognized by, B3.

Comparison of the dissociation constants of the larger structures **1** and **2** with that of the Lewis Y tetrasaccharide **3** should delineate the binding specificity of B3 for the reducing end of the antigen. We report here the use of frontal affinity chromatography coupled to mass spectrometry to evaluate the binding affinity of B3 for Lewis Y derivatives **1**, **2** and **3**.

Scheme 1. Synthesis of Hexa- and Pentasaccharide Derivatives of Lewis Y



Frontal Affinity Chromatography Coupled to Mass Spectrometry (4, 5)

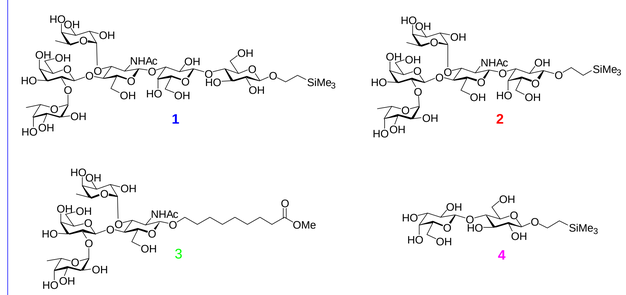
The affinity column was prepared by immobilizing biotin-labeled B3 onto a column packed with streptavidin-derivatized beads. The compounds tested as ligands are shown in Scheme 2. Lactoside **4** was included in all the analyte solutions as a void volume marker since it shows negligible reactivity with B3. The mass spectrometer was programmed to selectively detect each ligand at its *m/z* value to generate elution profiles for each of the compounds in the mixture from a single run (Figure 1).

Evaluation of mAb B3 Binding Affinity

The column capacity (B_t, number of active binding sites) was determined experimentally by infusing a series of solutions of varying concentrations of **2** with a constant concentration of void volume marker **4** through the B3 affinity column. The measured retention volumes were plotted as a function of the concentration of **2** (Figure 2). Fitting the data by the least-squares method generated values for B_t and the K_d for **2**.

Using the B_t value determined from the data shown in Figure 2, the K_d^{mix} values for **1** and **3** were determined from the data shown in Figure 1. The results are summarized in Table 1.

Scheme 2. Compounds Analyzed by FAC/MS



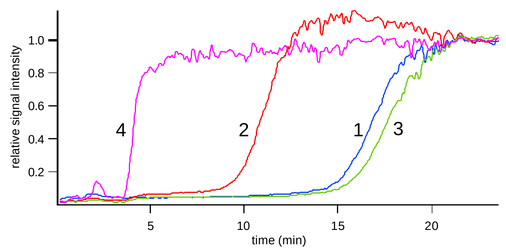


Figure 1. Four overlaid extracted-ion chromatograms showing normalized signal intensity versus time for a single run. A mixture of **1**, **2**, **3**, and **4** (Scheme 2), each present at 2.0 μM, in 10 mM NH_4OAc, 1 mM NaCl, pH 7.40, was infused at 8 μL/min through a micro-scale column of immobilized B3. The effluent from the column was analyzed by ES-MS in selected-ion monitoring (SIM) and positive mode.

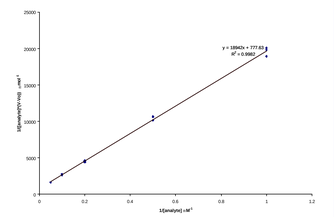


Figure 2. Plot of 1/ ([compound **2**]*(V-V_o)) versus 1/[compound **2**] used to used to determine the column binding capacity and the dissociation constant for **2**.

Table 1. K_d Values

B_t = 1.3 ± 0.2 nmol

(ca. 650 pmol mAb B3)

compound	K_d
1	12 ± 2 μM
2	24 ± 4 μM
3	11 ± 2 μM

Discussion

The K_d values for compounds **1**, **2** and **3** as determined by FAC/MS (Table 1) are of the same order of magnitude. While B3 shows slightly less affinity for pentasaccharide **2**, its affinity for **1** and **3** is essentially the same. These results suggest that in the natural glycolipid or glycoprotein antigen, sugars linked to the reducing end of the Lewis Y tetrasaccharide do not contribute significantly, if at all, to the epitope recognized by B3.

References

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