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Use of sialylated or sulfated derivatives and acrylamide copolymers of Galβ1,3GalNAcα- and GalNAcα- to determine the specificities of blood group T- and Tn-specific lectins and the copolymers to measure anti-T and anti-Tn antibody levels in cancer patients

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Sialylated or sulfated derivatives and acrylamide copolymers of blood group T-(Galβ1,3GalNAcα-) and Tn-(GalNAcα) haptens were studied for their interaction with the lectins of peanut (PNA), *Agaricus bisporus*-(ABA), *Helix pomatia*-(HPA) and *Vicia villosa* B_4-(VVA), using asialo Cowper's gland mucin (ACGM), which contains both T and Tn epitopes, as the coating substrate in enzyme linked lectin assay. Both T and Tn copolymers (~40 haptens) showed high affinity and strict specificity; although the T-copolymer at 0.05–0.07 μM concentration caused 50% inhibition of interaction of either PNA or ABA with ACGM, there was little inhibition of the HPA and VVA interactions even at over 100 times that concentration. The Tn-copolymer at 0.02–0.05 μM inhibited HPA or VVA interaction with ACGM by 50% but gave virtually no inhibition of PNA and ABA binding. Sialyl, sulfate or methyl group substitution on C-6 of GalNAc of the T-haptene did not prevent interaction with PNA but almost abolished interaction with ABA. In contrast, sialyl or sulfate group on C-6 and sulfate on C-3 of Gal in Galβ1,3GalNAcα- inhibited almost completely the interaction of PNA with ACGM but had only a slight effect on the interaction of ABA; C-6 substitution with either sialic acid or sulfate on GalNAcα- almost abolished the interaction of both HPA and VVA with ACGM. Preliminary studies revealed a significant depression in the serum level of anti-T (two to three-fold decrease) and anti-Tn (~ two-fold decrease) antibodies in breast cancer compared with normal control subjects when the acrylamide T- and Tn-copolymers were used as coating substrates in enzyme linked immunoassays.

Keywords: breast cancer, T-, Tn-specific lectins, *Agaricus bisporus*, *Helix pomatia*, *Vicia villosa*, antibodies

Abbreviations: PNA, peanut agglutinin; ABA, *agaricus bisporus* agglutinin; HPA, *helix pomatia* agglutinin; VVA (B4), *vicia villosa* agglutinin; ACGM, Asialo Cowper's gland mucin; CA, carcinoma; BSA, bovine serum albumin; HRP, Horseradish peroxidase; ABTS, 2,2′-azino-di (3-ethyl-benzthiazoline sulfonate); ELISA, enzyme-linked immunosorbent assay; Al, allyl; Bn, benzyl; AA, acrylamide; CP, copolymer.

Introduction

All humans possess antibodies, predominantly IgM, stimulated largely by the intestinal flora that react specifically with Thomsen-Friedenrich (T) and Tn antigens; these are present in immunoreactive form in > 85% of all human carcinomas (CA), but not in healthy and otherwise diseased tissues [1]. The major glycoprotein (leukosialin) of T-lymphoid cells has recently been shown to contain Tn epitopes [2]. The expression of Tn antigen in uterine cervical cancer was found to be closely related to metastasis to regional lymph nodes [3, 4]. Recently, Tn and sialyl Tn structures were shown to be markers for the glandular differentiation pattern in salivary gland CA [5]. The detection of T antigen serves as a useful marker in the prognosis of ovarian CA [6]. Lectins were used in several studies to detect expression of these antigens. T-antigen in bladder tumours was detected by examining biopsies with FITC-PNA [7]. Both T and Tn antigens were measured in squamous cell CA using PNA and VVA respectively [4]. A positive correlation between primary breast CA's aggressiveness and its affinity for *Helix pomatia* agglutinin which reacts with the Tn epitope, enabled an accurate prediction of early or late carcinoma recurrence and patient survival time [8–10].

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It is evident from the different affinities exhibited by lectins of the same apparent monosaccharide specificity towards different oligosaccharide chains that the concept of the lectin combining site must be extended beyond the monosaccharide unit in order to define the unique binding specificity of each lectin [11]. The present paper reports our investigation on the binding affinities of several T and Tn specific lectins towards synthetic and natural glycoconjugates as well as our preliminary data on the clinical usefulness of synthetic copolymers in demonstrating a depressed level of anti-T and anti-Tn antibodies in sera of breast cancer patients, compared with sera of normal adults.

Experimental procedures

Hapten inhibition: These studies were carried out by an Enzyme-Linked Lectin Assay [12]. The microtitre wells (Dynatech Laboratories, Chantilly, VA) were coated with 5 μg asialo Cowper's gland mucin (ACGM) in 100 μl of 0.1 M Na_2CO_3, 0.02% NaN_3 buffer, pH 9.6, at 37°C for 3 h. The plates were tightly covered with parafilm and stored at 4°C for use within 3 weeks. After complete aspiration of the ACGM solution, plates were washed three times with washing buffer (10 mM Na_2HPO_4, 120 mM NaCl, 0.01% trimerosal, 0.05% Tween 20, pH 7.3), and blocked with 5% BSA in washing buffer for 30 min at 37°C. The plates were then washed three times in washing buffer before incubating with the respective HRP-conjugated lectins (Sigma Chemical Co.) diluted with diluent buffer (washing buffer containing 1% BSA). The optimal dilutions for HRP-lectin conjugates determined by checkerboard titration were 1:160 for ABA, PNA and HPA and 1:200 for VVA. Following incubation for 1 h at 37°C, the wells were washed three times with washing buffer. One hundred μl of peroxidase substrate ABTS (Kirkegaard & Perry Laboratories, Gaithersburg, MD) were added to the wells and the plates were incubated for 15 min at 37°C. The reaction was then stopped by the addition of 100 μl of 0.2 M citric acid and the resulting colour was read at 405 nm on a Biotech ELISA reader.

For studying the inhibition, 100 μl aliquots of optimally diluted HRP-lectin conjugate were mixed separately in duplicate with 100 μl of diluent buffer as well as serially diluted inhibitors in diluent buffer, and incubated for 1 h at 37°C. A 100 μl aliquot from each mix was transferred to the wells of microtitre plates which had been coated, blocked and washed as described above. The plates were then incubated for 1 h at 37°C, washed three times, incubated with ABTS and the developed colour was measured as above. The percentage inhibition was calculated from the mean of duplicate values which varied within 5%.

A comparison of the level of human sera anti-T and anti-Tn antibodies Sera were collected from healthy females and breast cancer patients admitted to Roswell Park Cancer Institute. The binding of human serum anti-T and anti-Tn antibodies to the respective epitope containing acrylamide copolymers was measured by an indirect enzyme immunoassay [13]. Sera from four normal adults and four breast cancer patients were tested. As a blind study, the category of each serum was not revealed to the person (Y. C.) performing the ELISA, until the assays on all sera were completed. Plates were coated with 2 μg T or Tn copolymer in 100 μl of coating buffer for 3 h at 37°C and blocked with 5% BSA in washing buffer. After rinsing the plate with washing buffer, 100 μl aliquots from serially diluted serum in PBS, 0.1% of BSA, 0.2% Tween 20 were added and incubated for 1 h at 37°C. The wells were then washed extensively with washing buffer and incubated with HRP-conjugated goat anti-human IgM (Sigma, 1:600 dilution) for 1 h at 37°C. The rest of the procedure (incubation with peroxidase substrate, termination of the reaction and absorbance reading) was performed as described in the hapten inhibition assay. All assays were carried out in duplicate.

Asialo Cowper's gland mucin (ACGM) ACGM used in the present study was isolated in an earlier study [14]. This preparation contained respectively T- (Galβ1,3GalNAcα-) and Tn- (GalNAcα-) epitopes in a ratio (approximate) of 3:7. Based on a molecular weight of 200 000, the ACGM molecule contained nearly 48 T-epitopes and 112 Tn-epitopes.

Acrylamide copolymers containing either T-(Galβ1,3GalNAcα-) or Tn-(GalNAcα-) haptenes
The copolymers from either Galβ1,3GalNAcα-O-Allyl or GalNAcα-O-Allyl and acrylamide were synthesized by following the procedure of Horejsi *et al.* [15]. Both copolymers exhibited an average molecular weight of 40 000, as determined by gel filtration on Biogel P60 with dextran (39 200 Da) as the marker; both copolymers contained not less than 40 haptene groups per molecule as evident from a determination of Gal and GalNAc in T- copolymer and GalNAc in Tn-copolymer as detailed for ACGM in our previous investigation [14].

Natural glycoconjugates The isolation of fetuin triantennary asialoglycopeptide and bovine IgG diantennary glycopeptide was described earlier [16]. Anti-freeze glycoprotein was a generous gift from Dr Robert E. Feeney, University of California, Davis [17, 18].

Synthetic compounds Syntheses of neutral and some sulfated carbohydrates have been published [19, 20] and the synthetic details on sialylated and the remaining compounds will be reported elsewhere.

Results and discussion

Inhibition of the interaction of PNA with ACGM Several synthetic compounds showed considerable inhibition of PNA-binding and the inhibition curves are shown in Fig. 1. The concentration of the effective compounds required for 50% inhibition and the percentage inhibition that could be reached

for less effective or ineffective compounds at the maximum concentration tested are reported in Table 1.

a) Inhibition by non-anionic (neutral) saccharides: Galβ1, 3GalNAcα-O-Allyl (the typical T-structure) showed 50% inhibition at 0.23 mM, whereas the Tn-structure GalNAcα-O-Allyl had little effect (9% at 4.00 mM). Galβ1,3GlcNAcβ-O-Allyl did not inhibit at all whereas Galβ1,4GlcNAcβ-O-Bn showed 11% inhibition at 4.0 mM. Interestingly, lactose attached β1,6 to GalNAc (Galβ1,4Glcβ1,6GalNAcα-O-Bn) showed considerable inhibition (40%) at 4.0 mM. Lotan *et al.* [21] found D-galactose and α- and β-D-galactosides to exert a much lower inhibitory effect on the haemagglutinating activity of PNA compared with Galβ1,3GalNAc. Immunochemical studies of PNA by quantitative precipitation assays by Pereira *et al.* [22]

Table 1. Discerning the intricate specificities of Blood group T- and Tn-(Galβ1,3GalNAcα- and GalNAcα)-specific lectins, from their interaction with carbohydrate structures.

Compounds	*Concentration and inhibition (%)*[a]			
	PNA	*ABA*	*HPA*	*VVA(B_4)*
Non-anionic saccharides (mM):				
(i) Galβ1,3GalNAcα-O-Allyl	0.23 (50)	1.25 (50)	4.00 (6)	4.00 (12)
(ii) Galβ1,3GlcNAcβ-O-Allyl	4.00 (0)	2.00 (0)	4.00 (3)	2.00 (8)
(iii) GalNAcα-O-Allyl	4.00 (9)	2.00 (10)	4.00 (30)	1.00 (50)
(iv) Galβ1,4GlcNAcβ-O-Bn	4.00 (11)	2.00 (0)	4.00 (2)	4.00 (0)
(v) Galβ1,4Glcβ1,6GalNAcα-O-Bn	4.00 (40)	4.00 (0)	4.00 (4)	4.00 (0)
Copolymers with acrylamide (μM):				
(i) Galβ1,3GalNAcα-O-Allyl	0.05 (50)	0.07 (50)	8.30 (10)	8.30 (0)
(ii) Galβ1,3GlcNAcβ-O-Allyl	8.30 (8)	4.15 (0)	8.30 (0)	8.30 (0)
(iii) GalNAcα-O-Allyl	8.30 (6)	4.15 (15)	0.05 (50)	0.02 (50)
(iv) Galβ-O-Allyl	8.30 (11)	4.15 (4)	8.30 (0)	8.30 (0)
Natural glycoconjugates (μM):				
(i) Fetuin triantennary asialoglycopeptide	80.0 (18)	40.0 (19)	80.0 (2)	80.0 (0)
(ii) Bovine IgG diantennary glycopeptide	90.0 (0)	45.0 (8)	90.0 (4)	90.0 (6)
(iii) Anti-freeze glycoprotein	1.9 (50)	9.8 (50)	19.6 (4)	19.6 (13)
Sulfated carbohydrates (mM):				
(i) 3-SulfoGalβ-O-Bn	4.0 (5)	2.0 (0)	4.0 (0)	4.0 (0)
(ii) 6-SulfoGalβ-O-Bn	4.0 (6)	2.0 (0)	4.0 (0)	4.0 (0)
(iii) 3′-SulfoLacNAc	4.0 (21)	4.0 (3)	4.0 (2)	4.0 (0)
(iv) 2′-SulfoLacNAc	4.0 (15)	4.0 (2)	4.0 (7)	4.0 (26)
(v) 6′-SulfoLacNAc	4.0 (5)	4.0 (0)	4.0 (4)	4.0 (17)
(vi) 6-SulfoLacNAc	4.0 (0)	4.0 (0)	4.0 (4)	4.0 (0)
(vii) 3-SulfoGalβ1,3GlcNAcβ-O-Allyl	4.0 (0)	2.0 (0)	4.0 (0)	4.0 (0)
(viii) 3-SulfoGalβ1,3GalNAcα-O-Allyl	4.0 (4)	2.0 (50)	4.0 (1)	4.0 (5)
(ix) 6-SulfoGalβ1,3GalNAc-α-O-Allyl	2.00 (7)	1.75 (50)	4.00 (0)	2.00 (0)
(x) Galβ1,3(6-Sulfo)GalNAc-α-O-Allyl	0.71 (50)	2.00 (26)	4.00 (0)	2.00 (0)
(xi) Galβ1,3(6-O-Me)GalNAc-α-O-Allyl[b]	0.18 (50)	2.00 (23)	1.00 (3)	2.00 (0)
(xii) 6-SulfoGalβ1,3(6-O-Me)GalNAc-α-O-Allyl	2.00 (19)	2.00 (17)	2.00 (2)	2.00 (0)
(xiii) 6-SulfoGalNAcα-O-ONP	2.00 (10)	2.00 (5)	4.00 (0)	2.00 (6)
Sialylated carbohydrates (mM):				
(i) Galβ1,3(NeuAcα2,6)GalNAcα-O-Bn	1.75 (50)	2.00 (10)	4.00 (0)	2.00 (0)
(ii) NeuAcα2,6Galβ1,3GalNAcα-O-Bn	2.00 (0)	2.00 (36)	4.00 (0)	2.00 (0)
(iii) NeuAcα2,6Galβ1,3GlcNAcβ-O-Bn	2.00 (0)	2.00 (0)	4.00 (0)	2.00 (11)
(iv) Galβ1,3(NeuAcα2,6)GlcNAcβ-O-Bn	2.00 (0)	2.00 (0)	4.00 (0)	2.00 (8)
(v) NeuAcα2,6GalNAcα-O-Bn	2.00 (0)	2.00 (0)	4.00 (0)	2.00 (5)

[a] As indicated in the column 'Compounds' as mM or μM for each group of carbohydrates; all concentrations are based on the molecular weight of the compounds.

[b] Non-sulfated carbohydrate, listed in this group for convenient comparison.

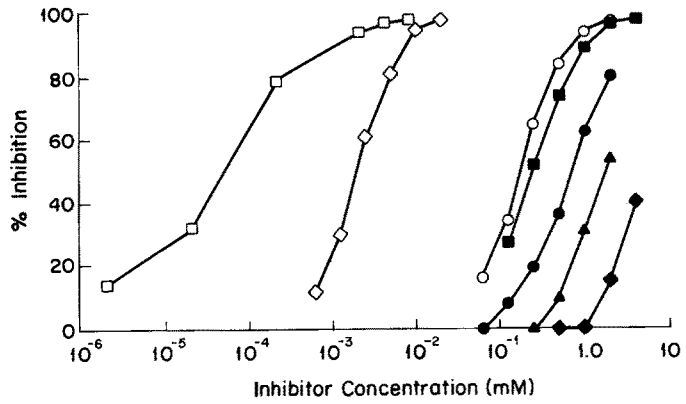
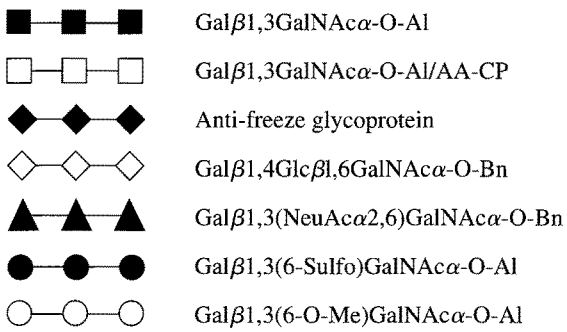


Figure 1. Influence of sialyl or sulfate group and multimeric structure of Galβ1,3GalNAcα- and GalNAcα- on the interactions of PNA with the T-epitope.



revealed that several monosaccharides and oligosaccharides were effective as inhibitors but to a small extent compared with Galβ1,3GalNAc. King and Holburn [23] reported that a radioassay for the T-antigen utilizing PNA was readily inhibitable by D-galactose and D-fucose (50% inhibition at 1.0 mM and 2.3 mM respectively). Kaifu and Osawa [24] showed that the α-glycosidic linkage of the *N*-acetylgalactosamine moiety is an important part of the receptor for ABA whereas no such requirement was observed for PNA.

b) Inhibition by the copolymers (CP) of neutral saccharides with acrylamide (AA) and by natural glycoconjugates: Galβ1,3GalNAcα-O-Al AA-CP was a very potent inhibitor of the PNA-binding to ACGM; 50% inhibition was achieved at 0.05 μM. This inhibition was very specific since the other copolymers GalNAcα-O-Allyl/AA-CP, Galβ1,3GlcNAcβ-O-Allyl/AA-CP and Galβ-O-Allyl/AA-CP at a concentration of > 100-fold (8.3 mM) did not bring any meaningful inhibition (8–11%). Among natural glycoconjugates, anti-freeze glycoprotein showed 50% inhibition at 1.9 μM. Fetuin triantennary asialoglycopeptide showed some inhibition (18%) at 80 μM whereas bovine IgG diantennary glycopeptide showed no inhibition at the concentration tested.

c) Inhibition by sulfated carbohydrates: When the GalNAc moiety in Galβ1,3GalNAcα- was substituted with sulfate at C-6, the inhibition was not abolished; Galβ1,3(6-Sulfo)GalNAcα-O-Allyl and Galβ1,3(6-O-Me)GalNAcα-O-Allyl brought 50% inhibition at 0.71 mM and 0.18 mM respectively. But when Gal was substituted at C-3 or C-6, (3-SulfoGalβ1,3GalNAcα-O-Allyl, 6-SulfoGalβ1,3GalNAcα-O-Allyl and 6-SulfoGalβ1,3(6-O-Me)GalNAcα-O-Allyl), the inhibition was reduced to a great extent (4%, 7% and 19% respectively). Among the other sulfated compounds, only 2′-SulfoLacNAc, 3′-SulfoLacNAc and 6-SulfoGalNAcα-O-ONP showed some inhibition (15%, 21% and 10% respectively) at the maximum concentration tested.

d) Inhibition by sialylated compounds: As seen with sulfated compounds, C-6 substitution of the GalNAc moiety in Galβ1,3GalNAcα- with sialic acid did not abolish the inhibition [Galβ1,3(NeuAcα2,6) GalNAcα-O-Bn:50% inhibition at 1.75 mM]. The other sialyl compounds were ineffective as inhibitors of the lectin binding.

Inhibition of the interaction of ABA with ACGM Figure 2 depicts the inhibition curves obtained for the compounds tested and Table 1 reports the inhibition data thus obtained.

a) Inhibition by neutral saccharides: The typical T structure Galβ1,3GalNAcα-O-Al gave 50% inhibition at 1.25 mM and this is only one fifth as effective with ABA compared with its effect on PNA; GalNAcα-O-Al brought 10% inhibition at 2.00 mM. The compound Galβ1,4Glcβ1,6GalNAcα-O-Bn in

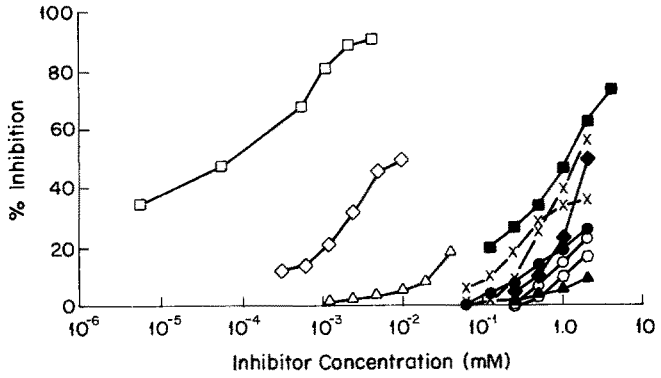
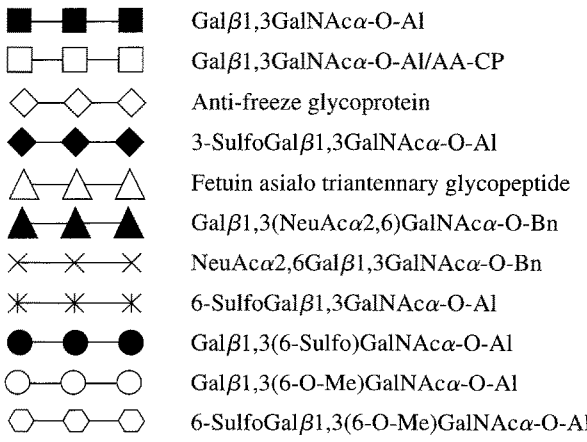


Figure 2. Influence of sialyl or sulfate group and multimeric structure of Galβ1,3GalNAcα- and GalNAcα- on the interaction of ABA with the T-epitope.



contrast to its effect on PNA (40% inhibition) did not show any inhibition on ABA. Both structures, Galβ1,3GlcNAcβ- and Galβ1,4GlcNAcβ-, were not inhibitors.

b) Inhibition by the copolymers and natural glycoconjugates: As seen with PNA, the copolymer of Galβ1,3GalNAcα-O-Al with acrylamide brought 50% inhibition of the ABA binding at 0.07 μM. The Tn haptene (GalNAcα-O) containing copolymer showed only 15% inhibition at 4.15 μM. The other copolymers had little effect. Anti-freeze glycoprotein, compared with its effect on PNA, required a five-fold higher concentration to achieve 50% inhibition with ABA. Both fetuin triantennary asialo glycopeptide and bovine IgG diantennary glycopeptide were almost ineffective (19% and 8% at 40 μM and 45 μM respectively).

c) Inhibition by sulfated carbohydrates: Several interesting differences in the binding specificities of PNA and ABA became apparent when these compounds were tested. When Gal was substituted at C-3 or C-6 (3-SulfoGalβ1,3GalNAcα-O-Al and 6-SulfoGalβ1,3GalNAcα-O-Al), the ABA binding was inhibited 50% at 2.0 mM, whereas such substitutions almost abolished the effect on PNA binding. On the other hand, C-6 substitution on GalNAc had a greater effect with ABA when compared with PNA; Galβ1,3(6-Sulfo)GalNAcα-O-Al and Galβ1,3(6-O-Me)GalNAcα-O-Al showed less inhibition (26% and 23% respectively at 2.00 mM with ABA compared to 50% inhibition at 0.71 and 0.18 mM respectively with PNA). The other sulfated compounds did not show any appreciable inhibition.

d) Inhibition by sialylated compounds: As seen with sulfated compounds, C-6 substitution of the Gal moiety in Galβ1,3GalNAcα- with sialic acid did not abolish the inhibition of ABA binding (36% inhibition at 2.00 mM) whereas C-6 substitution on the GalNAc moiety abolished to a great extent the inhibition (only 10% inhibition at 2.00 mM). These observations are in contrast to the findings with PNA, but in parallel, as noticed above with sulfated compounds.

Inhibition of the interaction of HPA with ACGM The inhibition curves obtained for the compounds tested on HPA are shown in Fig. 3 and the inhibition data are reported in Table 1.

a) Inhibition by neutral saccharides: The Tn-haptene GalNAcα-Al gave 30% inhibition at 4.00 mM whereas Galβ1,3GalNAcα-O-Al did not exhibit such an effect (only 6% inhibition at 4.00 mM). The other compounds were even less effective as inhibitors of HPA binding.

b) Inhibition by the copolymer and neutral glycoconjugates: The copolymer containing the Tn-haptene (GalNAcα-O-) gave 50% inhibition at 0.05 μM whereas the other copolymers including the one containing the T-haptene were less effective. These data would imply the specific interaction of HPA with the GalNAcα- moiety and not with the Galβ1,3GalNAcα- moiety of ACGM. The above contention is also implicit from the lower effectiveness of anti-freeze glycoprotein which contains the T-haptene but not the Tn-haptene (only 4% inhibition at 19.6 μM). Fetuin triantennary asialoglycopeptide and bovine

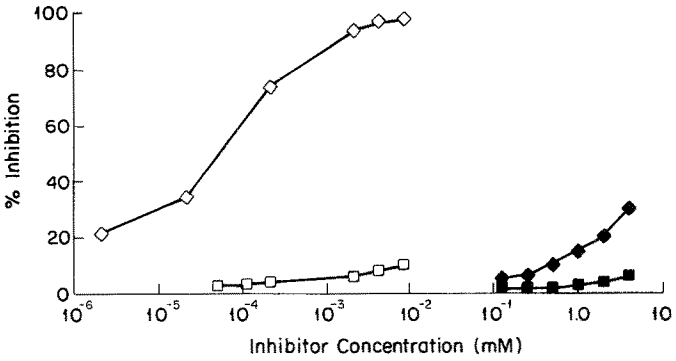
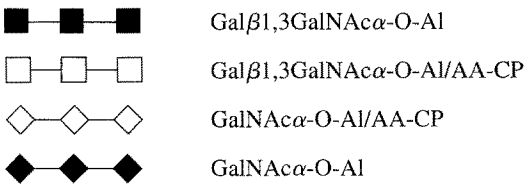


Figure 3. Influence of sialyl or sulfate group and multimetric structure of GalNAcα- and Galβ1,3GalNAcα- on the interaction of HPA with the Tn-epitope.



IgG diantennary glycopeptide were only 2% and 4% inhibitory at 80 μM and 90 μM respectively.

c) Inhibition by sulfated carbohydrates: In contrast to T specific lectins (PNA and ABA), none of the sulfated compounds were effective as inhibitors. C-6 substitution on GalNAcα- with sulfate (6-sulfoGalNAcα-O-ONP) completely abolished the inhibitory activity of the Tn-haptene.

d) Inhibition by sialylated compounds: None of the sialylated compounds including NeuAcα2,6GalNAcα-O-Bn were inhibitory. Thus, C-6 substitution on GalNAcα- with either sulfate or sialic acid abolished the interaction of Tn haptene with HPA.

Inhibition of the interaction of VVA with ACGM Figure 4 presents the inhibition curves for compounds tested and Table 1 reports the inhibition data.

a) Inhibition by neutral saccharides: The typical Tn structure GalNAcα-O-Al exhibited 50% inhibition at 1.00 mM whereas the T structure Galβ1,3GalNAcα-O-Al showed only 12% inhibition at 4.00 mM; GalNAcα-O-Al was over five-fold more effective with VVA as compared to its effect with HPA. The other neutral saccharides were mostly ineffective.

b) Inhibition by the copolymer and natural glycoconjugates: The Tn-haptene containing copolymer, at 0.015 μM, inhibited 50% of the interaction of VVA with ACGM and thus was at least three-fold more effective with VVA than with HPA. This observation confirms the finding that GalNAcα-O-Al was > five-fold more effective with VVA than with HPA. The T-haptene containing copolymer did not inhibit at all indicating the specific interaction of Tn copolymer with VVA, as observed previously in the case of HPA. The other copolymers

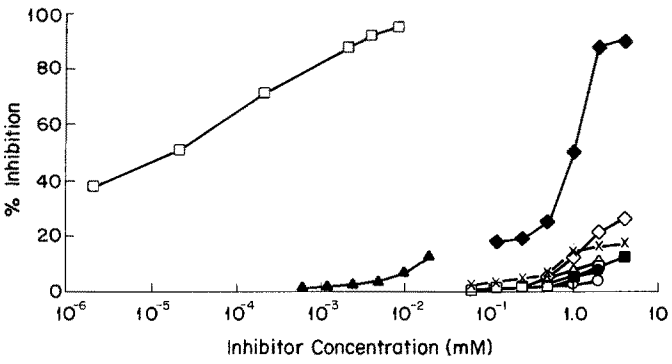
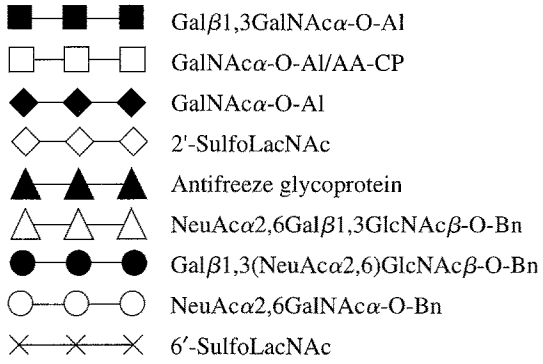


Figure 4. Influence of sialyl or sulfate groups and multimeric structure of GalNAcα- and Galβ1,3GalNAcα- on the interaction of VVA (B4) with the Tn-epitope.



were ineffective. As anticipated, none of the natural glycoconjugates tested showed any meaningful inhibition.

c) Inhibition by sulfated carbohydrates: As noted with HPA, most of the sulfated compounds did not show any inhibition. Exceptions were 2'-sulfoLacNAc, 6'-sulfoLacNAc, 3-SulfoGalβ1,3GalNAcα-O-Al and 6-sulfoGalNAcα-O-ONP showing 26%, 17%, 5% and 6% inhibition respectively.

d) Inhibition by sialylated compounds: The Tn-haptene substituted at C-6 with sialic acid (NeuAcα2,6GalNAcα-O-Bn) showed 5% inhibition at 2.00 mM; some inhibition was also noticed with NeuAcα2,6Galβ1,3GlcNAcβ-O-Bn and Galβ1,3(NeuAcα2,6)GlcNAcβ-O-Bn (11% and 8% inhibition respectively at 2.00 mM).

Anti-T and anti-Tn antibodies in normal and breast cancer sera A measurement of these antibodies was made using the acrylamide copolymers containing either T(Galβ1,3GalNAcα-) or Tn (GalNAcα-) haptenes, which have been shown above in the lectin-binding studies (Table 1) to be highly specific; they are not cross-reactive with each other in terms of T and Tn specificities. Figures 5 and 6 present anti-T and anti-Tn levels respectively in four normal and four breast cancer sera. At all levels of dilution, the normal sera showed three to four-fold the anti-T and two to three-fold the anti-Tn levels respectively that are present in cancer sera.

Presant and Kornfield [25] reported that the erythrocyte tryptic glycopeptide (MW 10 000 containing six sialylated T-

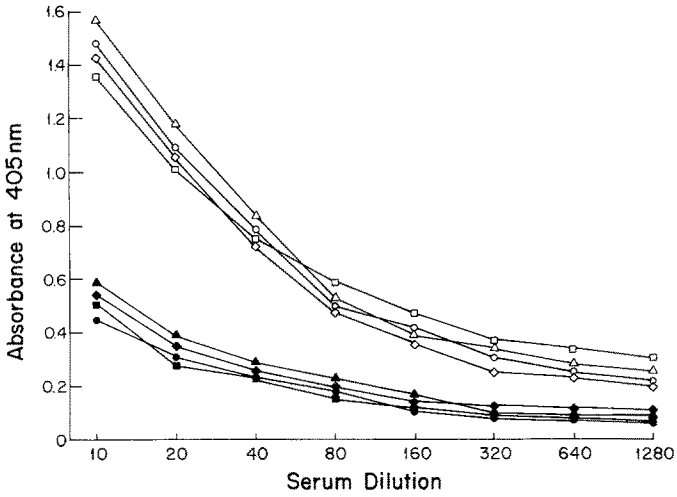
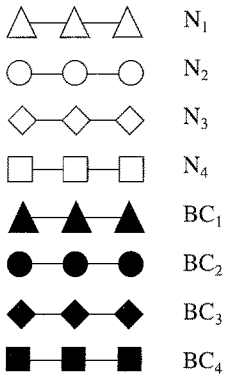


Figure 5. A comparison of the serum anti-T antibody levels between normal (N) and breast cancer (BC) patients, using acrylamide copolymer as the haptene.



epitopes) as compared to its asialoglycopeptide and Gal, β1, 3GalNAc, was 1/8 and 65-fold as active, respectively, towards ABA. The present study found that the T-copolymer (MW 40 000 containing ~40 epitopes) was ~18 000-fold more active with ABA compared with Galβ1,3GalNAcα-O-Al. Hence, the erythrocyte asialo glycopeptide and the T-copolymer were respectively 87-fold and 450-fold more active than Galβ1,3GalNAcα-O-Al when the calculation is done on the basis of epitope concentration. Lotan *et al.* [21] observed that T-antigen (desialylated O,NN blood group substance; MW 550 000) was 250 times more active towards PNA than was Galβ1,3GalNAc, when the activities were expressed on the basis of epitope concentration. The T-copolymer was 250 000-fold more active than Galβ1,3GalNAcα-O-Al towards PNA, on molar basis and 625-fold more active on epitope concentration basis. Hammarstrom and Kabat [26] reported that 1100 nmol of GalNAc were needed for 50% inhibition of the HPA precipitation with 3.7 μg of asialo OSM [MW 620 000; 3.7 μg (6 pmol) in 200 μl = 0.03 μM], indicating that asialo OSM was ~180 000-fold more effective than GalNAc in interacting with HPA. The Tn copolymer

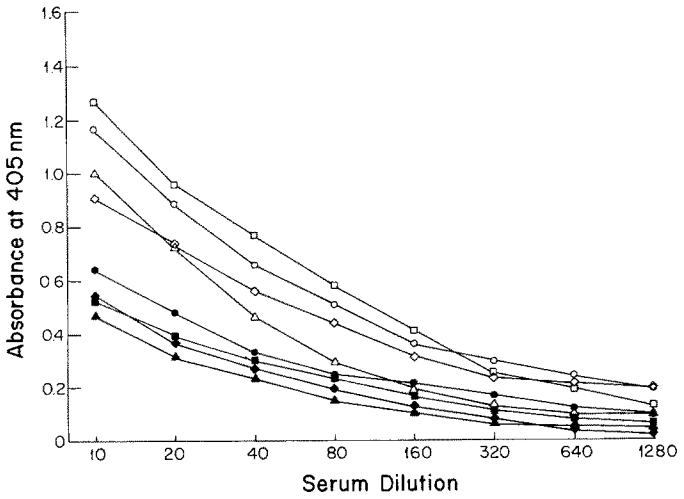


Figure 6. A comparison of the serum anti-Tn antibody levels between normal and breast cancer patients, using acrylamide copolymer as the haptene.

The sera tested here were as above (Fig. 5). (Symbols remain the same as in Fig. 5.)

(MW 40 000) of the present study at 0.05 μM and 0.02 μM respectively brought 50% inhibition of HPA and VVA (B_4) interactions; Tn-copolymer, was > 80 000-fold and 50 000-fold more effective respectively in its interaction with HPA and VVA-B_4 compared with GalNAcα-O-Al [27]. The present study has thus shown that molecules containing clusters of T- and Tn-epitopes are several orders more effective than monomeric epitopes, when considering their interaction with T- and Tn-specific lectins. The present investigations also delineated the difference in the specificities of PNA and ABA, the former being affected by C-3 or C-6 substitution on Gal and the latter, C-6 substitution on GalNAc in the T-epitope. The occurrence of sulfated mucin-type carbohydrate chains in normal colon, colon CA and metastases [28] and the identification of small oligosaccharide structures containing both sialic acid and sulfate such as 6-sulfo or 4-sulfo Galβ1,3(NeuAcα2,6) GalNAcα- in mucin glycoproteins [29] necessitate the importance of knowing such a difference in the specificities of PNA and ABA for a meaningful interpretation of histochemical and structural data obtained by using these lectins. Both Tn-specific lectins (HPA and VVA) were affected by C-6 substitution on the Tn-epitope, thus demonstrating that these lectins do not interact with sialyl Tn.

The high affinity and non-cross reactivity of T- and Tn-copolymers with each other enabled us to measure both anti-T and anti-Tn levels in the same sera. Springer and co-workers [10, 30] pointed out that an increase in Tn antigen, both in absolute terms and when compared with T, often paralleled increased malignancy; this means more depression in the level of anti-Tn than in the level of anti-T, with the progression or aggressiveness of malignancy. Thus, our present simple assay of measuring both anti-T and anti-Tn in the same sera, using T- and Tn- copolymers, which are very cheap to make, is anticipated to capture the attention of cancer clinics. Another interesting experiment on T- and Tn-copolymers would be to test them for their protection of mice against a challenge by highly metastatic carcinomas [31].

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