Defining the Carbohydrate Specificities of Aplysia Gonad Lectin Exhibiting a Peculiar \(\beta\)-Galacturonic Acid Affinity*

Received for publication, September 13, 1999, and in revised form, January 10, 2000

Albert M. Wu‡‡, Shuh-Chyung Song‡‡, Yuen-Yuen Chen‡‡, and Nechama Gilboa-Garber‡¶

From the ‡Glyco-Immunochemistry Research Laboratory, Institute of Molecular and Cellular Biology, School of Medicine, Chang-Gung University, Kwei-san 33332, Taiwan and the ¶Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, 52900, Israel

Aplysia gonad lectin (AGL), which has been shown to stimulate mitogenesis in human peripheral lymphocytes, to suppress tumor cells, and to induce neurite outgrowth and improve cell viability in cultured Aplysia neurons, exhibits a peculiar galacturonic acid/galactose specificity. The carbohydrate binding site of this lectin was characterized by enzyme-linked lectino-sorbent assay and by inhibition of AGL-glycan interactions. Examination of the lectin binding with 34 glycans revealed that it reacted strongly with the following glycoforms: most human blood group precursor (equivalent) glycoproteins (gps), two Galα1→4Gal-containing gps, and two \(\alpha\)-galacturonic acid (GalUA)-containing polysaccharides (pectins from apple and citrus fruits), but poorly with most human blood group A and H active and sialylated gps. Among the GalUA and mammalian saccharides tested for inhibition of AGL-glycan binding, GalUA mono- to trisaccharides were the most potent ones. They were \(8.5 \times 10^4\) times more active than Gal and about \(1.5 \times 10^5\) more active than the human blood group Pk active disaccharide (E, Galα1→4Gal). This disaccharide was 6, 28, and 120 times more efficient than Galβ1→3GalNAcI, Galβ1→3GalNAc(T), and Galβ1→4GlcNAc (II), respectively, and 35 and 80 times more active than melibiose (Galβ1→6Glc) and human blood group B active disaccharide (Galα1→3Gal), respectively, showing that the decreasing order of the lectin affinity toward \(\alpha\)-anomers of Gal is \(\alpha\)-1→4 \(>\) \(\alpha\)-1→6 \(>\) \(\alpha\)-1→3. From the data provided, the carbohydrate specificity of AGL can be defined as GalUAα1→4 trisaccharides to mono GalUA > branched or cluster forms of E, I, and II \(\gg\) monomeric E, I, and II, whereas GalNAc is inactive.

The reproductive organs of various molluscs are rich in lectin activity (1, 2). Extracts of gonads and fertilized eggs of Aplysia contain a \(\beta\)-galacturonic acid and \(\alpha\)-galactose-binding lectin (1, 2). This lectin (Aplysia gonad lectin, AGL) reacts with marine microorganisms (3) and strongly agglutinates human papain-treated erythrocytes regardless of ABO blood groups (2). It was purified by heating to 70 °C, precipitated with ammonium sulfate, and affinity chromatographed on Sepharose 4B (2). The purified lectin is a glycoprotein of molecular mass of about 65 kDa, composed of two identical subunits. It was shown to induce mitogenic stimulation and interleukin-2 formation in human lymphocytes (4), to suppress tumorigenicity of Lewis lung carcinoma cells (5), to modulate neurite outgrowth in cultured Aplysia neurons, and to increase neurite viability in vitro (6). AGL was also shown to be useful for ultrastructural characterization of galacturonic acid in plants and fungi (7) and for differentiation between I and type human erythrocytes (8). Moreover, it was recently found to be useful for typing of halophilic Archaea and for the study of their S-layer structure (9). Although AGL has been shown to be specific for GalUA and Gal, its detailed carbohydrate specificity has not been established. It is important to elucidate the detailed carbohydrate specificity of this lectin, because it may function as a signaling adhesion molecule and has potential as a tool in experimental glycobiology, biochemistry, and immunochemistry (4–9). In the present study, we defined the glycan affinity of this lectin by both enzyme-linked biotin/avidin-mediated microtiter plate lectin assay (ELLSA) and also by examination of the inhibition of AGL-glycan interaction (10, 11). The great advantage of this method is that the amount of lectin and glycoform required is about 1/10 to 1/1000 of that required for the quantitative precipitin assay (12, 13). The results show that the carbohydrate affinity hierarchy of this lectin can be regarded as: tri-GalUAα1→4 to mono-GalUA > branched and/or clusters of E(Galα1→4Gal), I(Galβ1→3GlcNAc), and/or II(Galβ1→4GlcNAc) \(\gg\) monomeric E and I \(>\) II, although GalNAc is inactive.

EXPERIMENTAL PROCEDURES

Lectin—The AGL was purified from extracts of the gonads of Aplysia depilans as described previously (2).

Biotinylation of the Lectin—For AGL biotinylation by biotinamido-caproate-N-hydroxy-succinimide ester (biotin ester, purchased from Sigma), the purified lectin preparation (200 \(\mu\)g/250 \(\mu\)l of phosphate-buffered saline) was mixed with 400 \(\mu\)l of the biotin ester solution (100 \(\mu\)g of biotin ester/200 \(\mu\)l of lectin) and left for 30 min at room temperature. The biotinylated lectin was dialyzed for 2-3 h against distilled H₂O and overnight against TBS. After dialysis, the sample volume was adjusted to 1 ml with TBS, and 20 \(\mu\)l of 5% sodium azide was added (200

*A This work was supported by Grant 676 from the Chang-Gung Medical Research Project, Kwei-san, Tao-yuan, Taiwan and Grants 86-2316-B182-001-BC and 84-2811-B182-001R from the National Science Research Project, Kwei-san, Tao-yuan, Taiwan and Grants 86-2317-B182-002-M7 and 86-2317-B182-002-M8 from the National Science Council, Taipei, Taiwan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Glyco-Immunochemistry Research Laboratory, Inst. of Molecular and Cellular Biology, Chang-Gung Medical College, Kwei-san 333, Taiwan. Tel.: 886-3-328-6966; Fax: 886-3-328-6456 (laboratory) or 886-3-328-3031 (college); E-mail: amwu@mail.cgu.edu.tw.

The abbreviations used are: AGL, Aplysia gonad lectin; GalUA, \(\beta\)-galacturonic acid; Gal, \(\alpha\)-galactopyranose; ELLSA, enzyme-linked lectino-sorbent assay; TBS, Tris-buffered saline; Glc, \(\beta\)-glucosepyranose; GlcNAc, 2-acetamido-2-deoxy-\(\beta\)-glucosepyranose; PSM, porcine salivary gp major; L-Fuc or Fuc, L-fucopyranose; GalNAc, 2-acetamido-2-deoxy-\(\beta\)-galactopyranose; gp, glycoprotein; RSL, rat sublingual gp major; OSm, ovine submandibular gp major; BSM, bovine salivary gp major; NeuNAc, N-acetylneuraminic acid. Lectin determinants that are used to classify applied lectins are expressed in bold: A (GalNAcα1→3Gal), A₁ (GalNAcα1→3[Fucα1→2Gal]), B (Galα1→3Gal), I (Galβ1→3GalNAc), II (Galβ1→3[4GlcNAc]), F (GalNAcα1→9GalNAc), L (Galβ1→4Glc), Tn (GalNAcα1→Ser/Thr), E (Galα1→4Gal), the human blood group P type disaccharide, which is also part of P, determinant, but this disaccharide is not the key sequence for its reactivity (26–28).

This paper is available on line at http://www.jbc.org/ Online ISSN: 1083-351X

Vol. 275, No. 19, Issue of May 12, pp. 14017–14024, 2000 Printed in U.S.A.
Carbohydrate Specificity of Aplysia Gonad Lectin

µg/ml AGL in 0.1% NaNO₃ (10–11).

Glycoproteins and Polysaccharides—The blood group substances were purified from human ovarian cyst fluid by the procedures as described previously (14–19). Regardless of their A, B, H, Leα, or Leβ activity, the purified water-soluble blood group substances have a similarly overall structure. They are polydisperse molecules (Mf, 2.0 × 10⁶ to 1.0 × 10⁷) of similar composition (75–85% carbohydrates, 15–20% protein). They bear multiple heterosaccharide branches attached by glycosidic linkages at their internal reducing ends to serine or threonine of the polypeptide backbone (14–22).

The P-1 fractions of cyst glycoproteins represent the non dialyzable portion of the blood group substances after mild hydrolysis at pH 1.5–2.0, 100 °C for 2 h, which removed most of the 1-fucopyranosyl end groups, as well as some blood group A and B oligosaccharide side-chains (14, 23, 24). The 1st Smith-degraded products of blood group A active substances (MSS 10% 2X, see Table I), in which almost all of the sugar groups at the nonreducing ends were removed, were prepared as described earlier (18, 20). Both P-1 fractions and 1st Smith degradation products, prepared from human ovarian cyst glycoprotein, are defined as precursor equivalent glycoproteins (Structure I, 25–28).

The human blood group P-active substance, purified from sheep hydatid cyst glycoprotein (29, 30), was kindly provided by Dr. W. M. Watkins (University of London, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK). The mucus glycoprotein (native bird nest glycoprotein), the so-called nest-cementing substance (Structure II), from the salivary gland of Chinese swiflets (genus Collocalia), was extracted with distilled H₂O at 60 °C for 20 min from the commercial bird nest substance (Kim Hing Co., Singapore) (31, 32).

The Pneumococcus type XIV polysaccharide was prepared as described previously (33, 34). Fetuin (Life Technologies, Inc.), which is the major glycoprotein in fetal calf serum (35), has a molecular mass of 48,400 with the following composition: 78% amino acids, 8.7% sialic acid, 6.3% hexosamines, and 3.8% neutral sugars (36). It bears six oligosaccharide side-chains/molecule, three of them (of two types) are O-glycosyl-linked to Ser or Thr residues of the protein core, and the other three are N-glycosyl-linked to asparagine (37–39).

The rat sublingual glycoprotein (RLS) was prepared by the method of Moschera and Pigman (40). Its molecular mass is 2.2 × 10⁶ and it contains 81% carbohydrates (40). The carbohydrate side-chains (Structure III) are O-glycosyl-linked to Ser or Thr residues of the protein core. The established structure has 9, 10, 12, 13, and 15 sugar residues with average molecular mass 48,400 with the following composition: 78% amino acids, 8.7% sialic acid, 6.3% hexosamines, and 3.8% neutral sugars (36). It bears six oligosaccharide side-chains/molecule, three of them (of two types) are O-glycosyl-linked to Ser or Thr residues of the protein core. The established structure has 9, 10, 12, 13, and 15 sugar residues with average molecular mass 48,400 with the following composition: 78% amino acids, 8.7% sialic acid, 6.3% hexosamines, and 3.8% neutral sugars (36).

The Microtiter Plate Lectin-Enzyme Binding Assay (ELLSA)—ELLSA was performed according to the procedures of Duk et al. (10) or as described previously (12, 13). The volume of each reagent applied to the plate was 50 µl/well, and all incubations, except for coating, were performed at 20 °C. The reagents, if not otherwise indicated, were diluted with TBS containing 0.05% Tween 20. The TBS buffer or 0.15 M NaCl containing 0.05% Tween 20 was used for washing the plates between incubations.

For inhibition studies, the serially diluted inhibitor samples were mixed with an equal volume of lectin solution containing a fixed amount of lectin. The control lectin sample was diluted 2-fold with TBS containing 0.05% Tween 20. After 30 min at 20 °C, the samples were tested in the binding assay, as described above. The inhibitory activity was estimated from the inhibition curve and expressed as the amount of inhibitor (nmol/well) giving 50% inhibition of the control lectin binding. All experiments were done in duplicate or triplicate, and the data presented are mean values of the results. The standard deviation did not exceed 10% of the mean. The results are expressed as the amount of inhibitor (nmol/well) giving 50% inhibition of the control lectin binding.
not exceed 10% and in most experiments was less than 5% of the mean value. The control wells, where either coating or addition of biotinylated lectin was omitted, gave low absorbance values (below 0.1, read against the well filled with buffer) and were used as blank. It has been shown that blocking the wells before lectin addition was not necessary when Tween 20 was used in TBS.

RESULTS AND DISCUSSION

**Lectin-Glycan Interactions**—The avidity of AGL for gps and polysaccharides, as studied by a microtiter plate ELSA, is summarized in Table II according to the interaction profiles shown in Fig. 1. AGL reacted most strongly with five human blood group precursor-equivalent gps related to Structure I (Cyst OG, Cyst Modon P-1, and Cyst MSS 1st Smith in Fig. 1; Cyst Beach P-1 in Fig. 1c; and Cyst Tighe P-1 in Fig. 1e), two Galα1-4Gal-containing gps (asialo bird nest gp and sheep hydatid cyst gp, Fig. 1h), and two GalUA-containing fruit polysaccharides (pectin A from apple and pectin C from citrus, Fig. 1a). AGL also bound well asialo rat sublingual gp (Fig. 1i).

| Curve no. in Fig. 1 | Blood group active glycoprotein purified from human ovarian cyst fluid | Human blood group determinant present | Sugar added | Site of determinant addition |
|---------------------|-----------------------------------------------------------------------|--------------------------------------|-------------|-----------------------------|
| c                   | Cyst OG 10% 2X PPT                                                   | Ii                                   | None        |                             |
| d                   | Cyst Beach P-1                                                       | Pneumococcus type XIV Polysaccharide  |             |                             |
| e                   | Cyst Tighe P-1                                                       | Antigenic determinant                |             |                             |
| c                   | Cyst Modon 1st Smith                                                 | Leβ                                  |             |                             |
| d                   | Cyst MSS 1st Smith                                                   | A or A1                              |             |                             |
| e                   | Cyst JS phenol insoluble                                             | H                                    |             |                             |
| c                   | Cyst Tighe phenol insoluble                                          | Leβ                                  |             |                             |
| c                   | Cyst MSS 1st Smith                                                   | GalNA1→3 and as in H and Leβ         | (5), (7), (9), (11) |
| c                   | Cyst Modon                                                          |                                        |             |                             |
| d                   | Cyst Beach phenol insoluble                                          | B                                    | same as H   |                             |
| d                   | Cyst Tighe 20% of 2nd 10%                                           | B                                    | Leβ, Leα or Leγ | (7), (9), (10) and/or (12) |

**Fig. 1.** Binding of AGL to microtiter plates coated with serially diluted human blood group A, B, O, P, Leα, and II active glycoproteins, sialo and asialo glycoproteins and polysaccharides. The lectin was used at a constant amount of 5 ng/well. Total volume 50 μl. A405 was recorded after 2 h of incubation.
blood group B active gp from human ovarian cyst fluid (Cyst Tij, Fig. 1d), and asialo PSM (Fig. 1i). Because the percentage of glycans adsorbed onto the microtiter plate had not been established, the amount of glycans required to reach maximum interaction could not be evaluated. However, their binding reactivities were confirmed by the inhibition of AGL-glycan interaction with various glycans, as described under “Inhibition of AGL-Glycoform Interaction by Various Glycans” (Fig. 2 and Table III).

Except the bird nest gp [Fig. 1h], the blood group A, B, H or Le\(^b\) substances and mammalian salivary gps containing Gal\(^b\)1→4GlcNAc and Gal\(^b\)1→3GalNAc masked by sialic acids were either weakly active or inactive (Fig. 1 and Table II). These included cyst MSS 10% 2x(A\(_1\)) (Fig. 1c), cyst Medon (A\(_2\)) (Fig. 1c), cyst JS phenol insoluble(H) (Fig. 1c), cyst Tighe phenol insoluble (H+Le\(^b\)) (Fig. 1c), hog gastric mucin 4 (A+H) (Fig. 1f), rat sublingual gp (Fig. 1a), and PSM (Fig. 1i). Neither native salivary gps nor asialo products containing exposed Tn determinants only (Fig. 1i) reacted with AGL.

**Inhibition of AGL-Glycoform Interaction by Various Glycans**—The abilities of various glycans to inhibit the binding of AGL with Cyst Beach P-1 glycoprotein by ELLSA were analyzed and are shown in Fig. 2 and Table III. Among the glycans tested for inhibition of that interaction, six human blood group precursor equivalent gps (curves 1, 5, 6, 9, 10, and 11 in Fig. 2 and Table III); two Gal\(^a\)1→3Gal containing glycoproteins (sheep hydatid cyst gp and asialo bird nest, curves 3 and 4 of Fig. 2 and Table III), and two GalUA-containing polysaccharides (pectin-A and pectin-C, curves 7 and 8 of Fig. 2 and Table III) were the best inhibitors, requiring less than 12 ng to inhibit 50% of the interaction. They were much more active than monomeric Gal\(^a\)1→3Gal and Gal but much weaker than GaIU (Fig. 2 and Table III). The precursor equivalent and asialo glycoproteins were much more active than their further glycosylated, native or sialylated compounds (curve 4 versus curve 19, curves 5 and 6 versus corresponding native compounds in
TABLE II
Binding of AGL lectin to human blood group A, B, H, P, and Le\(^a\) active glycoproteins (gps), sialo- and asialo glycoproteins by ELLSA

| Decreasing order of activities | Fig. 1 | Glycoprotein lectin determinant\(^b\) (Blood group specificity) | Maximum \(A_{405}\) absorbance | Binding intensity\(^c\) |
|-------------------------------|-------|---------------------------------------------------------------|-----------------------------|-----------------------------|
| 1                             | c     | Cyst MSS first Smith degraded (T, Tn, I, II)                  | 2.6                         | +++                          |
| 2                             | c     | Cyst OG 10% 2X PPT (B, III/IIi)                               | 2.3                         | +++                          |
| 3                             | d     | Cyst Beach P-1 (T, Tn, I, II)                                 | 2.5                         | +++                          |
| 4                             | c     | Cyst Modon P-1 (T, Tn, I, II)                                 | 2.5                         | +++                          |
| 5                             | e     | Cyst Tighe P-1 (I, II)                                        | 2.4                         | +++                          |
| 6                             | a     | Pectin A (poly GalUA from apple)                               | 2.5                         | +++                          |
| 7                             | a     | Pectin C (poly GalUA from citrus fruits)                      | 2.5                         | +++                          |
| 8                             | b     | Asialo bird nest gp (II, E, T, F)                             | 2.4                         | +++                          |
| 9                             | h     | Asialo RSL (II)                                               | 2.4                         | +++                          |
| 10                            | f     | Hog gastric mucin no21 (I, II)                                | 1.9                         | +++                          |
| 11                            | g     | Asialo human \(\alpha\)-acid gp (II)                          | 1.9                         | +++                          |
| 12                            | d     | Cyst Tij 20% of 2nd 10% (B)                                   | 1.8                         | +++                          |
| 13                            | f     | Hog gastric mucin #14 (A, I, II)                               | 1.7                         | +++                          |
| 14                            | i     | Asialo PSM (A, A\(_h\), T, Tn)                                | 1.7                         | +++                          |
| 15                            | b     | Sheep hydatid cyst gly (E[P,])                                | 1.7                         | +++                          |
| 16                            | h     | Pneumococcus type 14 ps (II)                                  | 1.6                         | +++                          |
| 17                            | b     | Bird nest gp (Sialyl II, E, T, F)                             | 1.3                         | ++                           |
| 18                            | g     | Asialo fetuin (II, T)                                         | 0.97                        | ++                           |
| 19                            | d     | Cyst Beach phenol insoluble (B)                               | 0.86                        | +                            |
| 20                            | f     | Hog gastric no#9 (A\(_h\), H)                                 | 0.36                        | +                            |
| 21                            | c     | Cyst Modon (A\(_h\))                                         | 0.20                        | +                            |
| 22                            | e     | Cyst JS phenol insoluble (H)                                  | 0.06                        | +                            |
| 23                            | f     | Hog gastric #4 (A\(_h\), H)                                   | 0.05                        | +                            |
| 24                            | c     | Cyst MSS 10% 2x (A\(_h\), I)                                 | 0.02                        | +                            |

\(^a\) The symbols in parentheses indicating the human blood group activity and lectin determinants (26–28) are expressed in bold: F (GalNAc1→3GalNAc); A (GalNAc1→3Gal); A\(_h\) (GalNAc1→3[LFuc1→2Gal]; B (Gal1→3Gal); E (Gal1→4Gal); T (Gal1→3GalNAc); Tn (GalNAc1→Ser/Thr); III (Gal1→3GalNAc).

\(^b\) The results were interpreted according to the spectrophotometric absorbance value at 405 nm (i.e. \(A_{405}\)) after 2 h of incubation and graded as follows: ++++, O.D. >2.0; ++, O.D. 2.0–1.5; +++, O.D. 1.5–0.75; +, O.D. 0.75–0.2; and −, O.D. <0.2.

\(^c\) Glycans that showed an \(A_{405}\) nm value of less than 0.2 were: Cyst Tighe phenol insoluble (H, Le\(^b\)), bovine salivary gp (BSM) (sialyl T, Tn), asialo BSM (T, Tn), RSL (Sialyl II), human \(\alpha\)-acid gp (Sialyl mII), PSM (Sialyl A, A\(_h\), T, Tn), Fetuin (Sialyl II, T), ovine salivary gp (OSM) (Sialyl Tn), asialo OSM (Tn), and chondroitin 4-sulfate (qGlucA).

TABLE III
Amount of various glycoproteins giving 50% inhibition of AGL (5 ng/50 \(\mu\)l)-Cyst Beach P 1 gp (10 ng/50 \(\mu\)l) binding

The inhibitory activity estimated from the respective inhibition curve in Fig. 2 is expressed as the amount of inhibitor (nanogram) giving 50% inhibition. Total volume 50 \(\mu\)l. GalUA: 4 \(\times\) 10\(^4\) nanogram is equal to 2.0 \(\times\) 10\(^3\) nmol, Gal1→4Gal: 850 nanograms is equal to 2.5 nmol, Gal: 7 \(\times\) 10\(^4\) nanograms is equal to 3.9 \(\times\) 10\(^3\) nmol. Glycans that did not reach 50% inhibition are described in the legend to Fig. 2.

| Curve no. | Fig. 2 | Inhibitor | Quantity giving 50% inhibition Reciprocal of relative potency\(^a\) |
|-----------|-------|-----------|---------------------------------------------------------------|-----------------------------|
|           |       |           | nanograms                                                      |                             |
| 1         | b     | Cyst OG 10% 2x ppt                                   | 0.2                         | 3.5 \(\times\) 10\(^4\)    |
| 2         | a,b   | GalUA                                               | 0.4                         | 1.8 \(\times\) 10\(^4\)    |
| 3         | a     | Sheep hydatid cyst gp                                | 0.4                         | 1.8 \(\times\) 10\(^4\)    |
| 4         | a     | Asialo bird nest gp                                  | 1.6                         | 4576.4                      |
| 5         | b     | Cyst Beach P-1                                      | 1.7                         | 4118.6                      |
| 6         | a     | Cyst Modon P-1                                      | 3.0                         | 2333.7                      |
| 7         | b     | Pectin-A                                            | 4.8                         | 1458.4                      |
| 8         | b     | Pectin-C                                            | 5.7                         | 1228.2                      |
| 9         | b     | Cyst Tighe P-1                                      | 8.0                         | 875.0                       |
| 10        | b     | Cyst Tij 20% of 2nd of 10%                         | 8.5                         | 805.8                       |
| 11        | a     | Cyst MSS 1st Smith degraded                         | 12.0                        | 583.4                       |
| 12        | b     | Cyst Beach phenol insoluble (B)                      | 20.0                        | 350.0                       |
| 13        | a     | Hog gastric mucin #21                              | 50.0                        | 140.0                       |
| 14        | b     | Asialo RSL (mII)                                   | 50.0                        | 140.0                       |
| 15        | b     | Pneumococcus type 14 ps (II)                        | 80.0                        | 87.5                        |
| 16        | a     | Asialo PSM (A, H, T, Tn)                            | 80.0                        | 87.5                        |
| 17        | a     | Hog gastric mucin #4                                | 80.0                        | 77.8                        |
| 18        | b     | Cyst 19 (B)                                        | 160.0                       | 43.8                        |
| 19        | a     | Bird nest gp                                        | 300.0                       | 23.3                        |
| 20        | a,b   | Gal1→4Gal (E)                                      | 850.0                       | 8.2                         |
| 21        | a,b   | Gal1→4Gal (E)                                      | 7 \(\times\) 10\(^3\)       | 1.0                         |

\(^a\) Reciprocal of relative potency of sugars based on weights (ng) when Gal is taken as 1.0 (49).

Fig. 2 and Table III, etc.). The decreasing order of the reactivity of these glycoforms is Cyst OG 10% 2x PPT (one of the human blood group precursor gps, Structure I, curve 1 in Fig. 2) and sheep hydatid cyst gly (blood group P\(_b\) active gp) (curve 3 in Fig. 2) > asialo bird nest gp (curve 4), five human blood group precursor gps (curves 5, 6, 9, 10, and 11) and two GalUA-containing gps; pectin-A and pectin-C (curves 7 and 8) > a blood group B active gp (curve 12); mild acid-hydrolyzed hog gastric mucin 14 (II), hog mucin 21 (II), and asialo rat sublingual gp (II, structure III) >> blood group A and H active glycoproteins and sialylated glycoproteins (Figs. 2 and 4). With several exceptions, the inhibitory reactivities of glycoforms toward AGL agree, in general, with the maximum absorbance values recorded in the binding assay (Fig. 1 and Table II).
The weak or negative reactivity of AGL with A and H active gps and most sialylated gps (Cyst Mcdon and Cyst MSS (Fig. 1c), Cyst Beach phenol insoluble (Fig. 1d), Cyst Tighe phenol insoluble (Fig. 1e)), human α₁-acid gp (Fig. 1g), RSL (Fig. 1h), and PSM (Fig. 1i) could be ascribed to the masking effects of sialic acid at the terminal Galβ1→3 to or poor adsorbance of these glycoforms onto a microwell plate.

Mild acid hydrolysis (pH 1.5, 100°C for 2 h), which removes the terminal Galβ1→3-α-GalNAc, L-Ara, GlcNAc, Glc, methyl-α-Glc, methyl-β-Glc, p-NO2-phenyl-α-GalNAc, GlcNAcβ1→4GlcNAc, and GalNAcβ1→3Gal-O-methyl. The ability of various sugars to inhibit the binding of AGL to cyst Beach P-1 gp (human blood group precursor equivalent gp purified from human ovarian cyst fluid) is shown in Fig. 3, and the amounts of ligand required for 50% inhibition of the lectin-glycan interaction are listed in Table IV. Among the oligo- and monosaccharides tested, di > tri-GalUAα1→4 to mono-GalUA were the most active, up to 8.5 x 10^4 times more active than Gal, indicating that COOH at carbon-6 is the most important factor for binding. GalUA was about 1.5 x 10^4 times more active than human blood group Pα active disaccharides (E, Galα1→4Gal), which was 6, 28, and 120 times more active than Galβ1→3GlcNAc(I), Galβ1→3GlcNAc(T), and Galβ1→4GlcNAc(II), respectively. These results show that each lectin has its own binding characteristics (26–28) and that the carbohydrate specificity of AGL can be defined as GalUAα1→4 di > trisaccharides to mono GalUA > branched or cluster forms of E, I, and II >> monomeric E(Galα1→4Gal) > I(Galβ1→3GlcNAc) > T(Galβ1→3GlcNAc) > B(Gala1→3GalNAc) > H(Galβ1→4GlcNAc), and L(lactose).

Galβ1→4Man was about 2.5 times less active than Galβ1→3GlcNAc(I), but 6.7 and 8 times more active than Galβ1→4Glc-L and Galβ1→4GlcNAc(II), respectively. These results show that the configuration at C-2 in the subterminal hexopyranose is also important for the binding and that substitution with –NHCOCH₃ at C-2 reduces the inhibitory power. Melibiose and raffinose (Galα1→6Galβ1→2Fructo-furanoside) were almost equally active and 4.0 times more active than stachyose (Galα1→6Galα1→6Glcβ1→2Fructofuranoside), suggesting that the combining size for α1→6 oligosaccharides is probably most accessible with less than a trisaccharide structure.
times more active than melibiose (Galα1→6Glc) (curve 10 in
Fig. 3a and Table IV) and Galα1→3Glc (curve 21 in Fig. 3b
and Table IV), respectively. Hence, the decreasing order of preference
of the lectin for α-anomers of Gal is: α1→4 > α1→3 > α1→6.

Of the monosaccharide derivatives studied, phenylβGal (Fig.
3a, curve 9) was the best inhibitor 2 × 10⁴ times less active
than GalUA (Fig. 3b, curve 3), but 1.8 times more active than
Gal and 1.2 times more than p-NO₂-phenylβGal and p-NO₂-
phenylαGal. As shown in Table IV, the phenyla-derivative of
Gal was about 2 times better than the methyl-α-derivative (Fig.
3, curves 13 versus 20), whereas no significant difference was
observed between the methyl- and p-NO₂-phenyl derivatives of
β-Gal (Fig. 3a, curves 12 and 14).

GalNAc, which was tested up to a concentration exceeding
that of Gal inducing 50% inhibition by 2.4-fold (Fig. 3 and Table IV),
was inactive indicating that the N-acetamido group at C-2
of the Gal pyranose ring strongly interferes with its interaction
with AGL. β-Fuc and 1-Ara showed no inhibition up to three
times the amount of Gal giving 50% inhibition, suggesting that
the OH group at C-6 or the CH₂OH at C-6 of Gal is essential for
the inhibition curve in Fig. 3 and is expressed as the amount of inhibitor giving 50% inhibition. Total volume 50 μl.

| Curve no. | Inhibitor | Quantity giving 50% inhibition | Reciprocal of relative potencya |
|-----------|-----------|-------------------------------|--------------------------------|
| 1 | b DiGalUA (GalUAα1→4GalUA) | 6.5 × 10⁴ | 8.5 × 10⁻⁴ |
| 2 | b TriGalUA (GalUAα1→4GalUAα1→4GalUA) | 8.5 × 10⁻⁴ | 6.5 × 10⁴ |
| 3 | a,b GalUA | 1.5 × 10⁻³ | 3.7 × 10⁴ |
| 4 | b Galα1→4Gal (E) | 1.0 | 55.0 |
| 5 | b Galβ1→4GlcNac (I) | 6.0 | 9.2 |
| 6 | a Galβ1→4Man | 15.0 | 3.7 |
| 7 | a Galβ1→3N-Ara | 19.0 | 2.9 |
| 8 | b Galβ1→3GlcNac (T) | 28.0 | 2.0 |
| 9 | a Pheny1 β-Gal | 30.0 | 1.8 |
| 10 | a α-Melibiose (Galα1→6Glc) | 35.0 | 1.6 |
| 11 | a D-Raffinose (Galα1→4Glcβ1→2-Fru) | 35.0 | 1.6 |
| 12 | a MethylβGal | 35.0 | 1.6 |
| 13 | a p-NO₂-phenylαGal | 35.0 | 1.6 |
| 14 | a p-NO₂-phenylβGal | 35.0 | 1.6 |
| 15 | b Galα1→3GlcNacMethyl | 40.0 | 1.4 |
| 16 | a p-NO₂-PhenylαGalNac | 40.0 | 1.4 |
| 17 | b Galβ1→3GlcNacBenzyl (T₃) | 50.0 | 1.1 |
| 18 | a,b Gal | 55.0 | 1.0 |
| 19 | b Galβ1→3GlcNacβ1→3Galβ1→4Glc | 70.0 | 0.8 |
| 20 | a MethylαGal | 70.0 | 0.8 |
| 21 | b Galβ1→3Glc (B) | 80.0 | 0.7 |
| 22 | b Galβ1→4Glc (L) | 100.0 | 0.6 |
| 23 | b Galβ1→4GlcNac (II) | 120.0 | 0.5 |
| 24 | a Stachyose (Galα1→4Galα1→6Glcβ1→2-Fru) | 140.0 | 0.4 |

a Reciprocal of relative potency of sugars (based on molarity) with Gal taken as 1.0 (49).

REFERENCES
1. Gilboa-Garber, N., Mizrahi, L., and Sussewein, A. J. (1984) Mar. Biol. Lett. 3, 105–114
2. Gilboa-Garber, N., Sussewein, A. J., Mizrahi, L., and Avichezer, D. (1985) FEBS Lett. 181, 267–270
3. Zipris, D., Gilboa-Garber, N., and Sussewein, A. J. (1986) Microbiol. Lett. 228, 343–349
4. Zipris, D., Gilboa-Garber, N. (1987) Develop. Comp. Immunol. 11, 501–511
5. Avichezer, D., Leibovici, J., Gilboa-Garber, N., and Michiwitz, M. (1987) in Lectures and Symposia 14th International Cancer Cong. Budapest, 1986 (Lapis, K., and Ackhardt, S., eds) Vol. 2, pp. 79–86, Karger, Basel, New York
6. Wilson, M. P., Carrow, G. M., and Levitan, I. B. (1992) J. Neurobiology 23, 729–750
7. Benhamou, N., Gilboa-Garber, N. Trudel, J., and Asselin, A. (1988) J. Histochem. Cytochem. 36, 1403–1412
8. Sudakevitz, D., Levene, C., and Gilboa-Garber, N. (1996) in Lectins: Biology, Biochemistry, Clinical Biochemistry (Van Driessche, E., Rougé, P., Beeckmans, S., and Bøgg-Hansen, T. C., eds.) Vol. 11, pp. 207–211, Textop Hellerup, Denmark
9. Gilboa-Garber, N., Mymon, H., and Oren, A. (1998) FEMS Microbiol. Lett. 163, 91–97
10. Duk, M., Lisowska, E., Wu, J. H., and Wu, A. M. (1994) Anal. Biochem. 221, 266–272
11. Lisowska, E., Duk, M., and Wu, A. M. (1996) BioMetachs 7, 115–129
12. Wu, A. M., Song, C., Chang, S. C., Wu, J. H., Chang, K. S. S., and Kabat, E. A. (1997) Glycoconjugate J. 10,161–1066
13. Chen, C. P., Song, C. S., Gilboa-Garber, N., Chang, K. S. S., and Wu, A. M. (1998) Glycoconjugate J. 8, 7–16
14. Beisser, S. M., and Kabat, E. A. (1952) J. Immunol. 68, 19–40
15. Kabat, E. A. (1956) Blood Substances: Their Chemistry and Immunochemistry, pp. 135–139, Academic Press, New York
16. Allen, P. Z., and Kabat, E. A. (1959) J. Immunol. 82, 340–357
17. Vicari, G., and Kabat, E. A. (1960) J. Immunol. 82, 821–825
18. Lloyd, K. O., and Kabat, E. A. (1968) Proc. Natl. Acad. Sci. U.S.A. 61, 1470–1477
19. Maisonnouet-McAuliffe, F., and Kabat, E. A. (1976) Arch. Biochem. Biophys. 175, 81–89
20. Wu, A. M., Kabat, E. A., Pereira, M. E. A., Gruezo, F. G., and Liao, J. (1982) Arch. Biochem. Biophys. 213, 351–394
21. Vicari, G., and Kabat, E. A. (1985) Biochemistry 24, 1470–1477
22. Wu, A. M., Kabat, E. A., Pereira, M. E. A., Gruezo, F. G., and Liao, J. (1982) Arch. Biochem. Biophys. 213, 394–421
23. Wu, A. M., and Sugii, S. (1988) Adv. Exp. Med. Biol. 228, 205–263
24. Wu, A. M., and Sugii, S. (1988) Adv. Exp. Med. Biol. 228, 351–394
25. Wu, A. M., and Sugii, S. (1988) Adv. Exp. Med. Biol. 228, 205–263
26. Leskowitz, W., and Kabat, E. A. (1954) J. Exp. Med. 98, 205–263
27. Wu, A. M., and Sugii, S., and Kabat, E. A. (1976) J. Immunol. 115, 129–139
