Mitogenic leukoagglutinin from *Phaseolus vulgaris* binds to a pentasaccharide unit in *N*-acetyllactosamine-type glycoprotein glycans
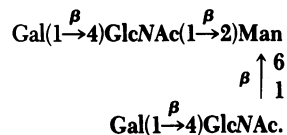
(isolectin L$_4$/oligosaccharide inhibition/lymphocyte activation)

STEN HAMMARSTRÖM*†, MARIE-LOUISE HAMMARSTRÖM (NÉE DILLNER)*, GÖRAN SUNDBLAD*, JAN ARNARP‡, AND JÖRGEN LÖNNGREN†‡

*Department of Immunology, Wenner-Gren Institute, University of Stockholm, S-106 91 Stockholm, Sweden; and ‡Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

ABSTRACT The carbohydrate binding specificity of leukoagglutinin (La; *Phaseolus vulgaris* isolectin L$_4$) was studied by using quantitative precipitation and precipitation-inhibition. A series of purified glycopeptides and synthetic oligosaccharides were used as inhibitors. The minimal structural unit required for La binding was the disaccharide GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man. Additions for this basic unit of different sugar residues gave a positive or negative contribution to binding. The most complementary structure was the pentasaccharide

$$Gal(1\overset{\beta}{\rightarrow}4)GlcNAc(1\overset{\beta}{\rightarrow}2)Man$$
$$\beta \uparrow \begin{matrix} 6 \\ 1 \end{matrix}$$
$$Gal(1\overset{\beta}{\rightarrow}4)GlcNAc.$$

This pentasaccharide unit occurs in tetraantennary *N*-acetyllactosamine-type glycoprotein glycans. Glycoproteins containing such structures were accordingly precipitated by La. Selected glycopeptides and oligosaccharides were also tested as inhibitors of La-induced DNA synthesis in human lymphocytes. The pattern of inhibition was essentially the same as that obtained by precipitation-inhibition, indicating that binding to lymphocytes via the carbohydrate binding site of the lectin is an essential step in the activation process.

Red kidney bean (*Phaseolus vulgaris*) extract contains a family of five isolectins (1–3). Each isolectin is a tetramer of M_r 115,000 (3) in which the subunits are held together by noncovalent forces (1–4). The subunits are of two different types, designated leukocyte reactive (L) and erythrocyte reactive (E), and occur in the combinations L$_4$, L$_3$E, L$_2$E$_2$, LE$_3$, and E$_4$ (1–3). L has high affinity for lymphocyte surface receptors (1–5) but little for those of erythrocytes (refs. 1 and 2; unpublished data) and is responsible for the mitogenic properties of the isolectins (1–5). E is responsible for the erythrocyte agglutinating properties (3, 6). Isolectin L$_4$ has been termed "leukoagglutinin" (La) (4).

Although La and various semipurified preparations containing mixtures of red kidney bean isolectins and unrelated substances have been used extensively as a T-lymphocyte mitogen (7, 8), little is known about the binding specificity of L. We have shown that La precipitates with carcinoembryonic antigen (CEA) and that the carbohydrate moiety of CEA is responsible for this interaction (9). Recently, human Tamm–Horsfall glycoprotein was shown to precipitate with La (10). A Pronase glycopeptide from this material, as well as high concentrations of *N*-acetylgalactosamine§ (GalNAc) inhibited this interaction (10,
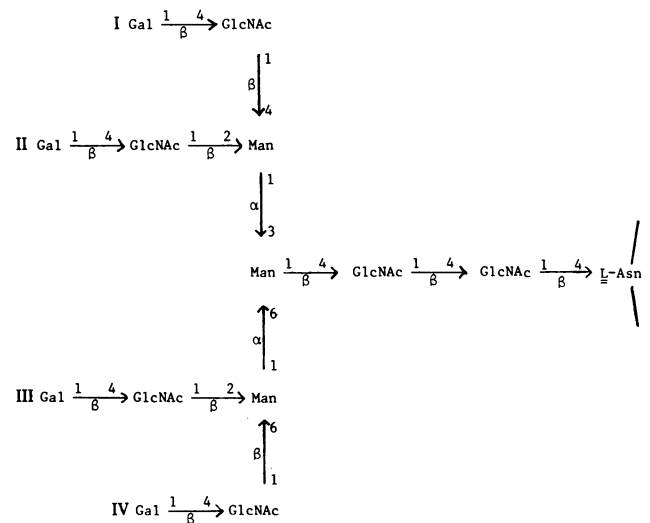

FIG. 1. Structure of tetraantennary asialo-glycan of orosomucoid. Roman numerals denote antennae numbers.

11). The Pronase glycopeptide was also an inhibitor of La-induced lymphocyte transformation (11). Studies by Kaifu and Osawa (12) showed that the trisaccharide Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc-(1$\overset{\beta}{\rightarrow}$2)Man was an inhibitor of type O erythrocyte *P. vulgaris* lectin hemagglutination. This finding is in line with earlier studies by Kornfeld and Kornfeld (13) indicating that complex carbohydrates containing the sequence *N*-acetylneuraminic acid (NANA)$\rightarrow\beta$Gal$\rightarrow\beta$GlcNAc$\rightarrow$Man inhibit hemagglutination. However, the latter findings presumably define the binding specificity of E because L is devoid of erythroagglutinating properties (1, 2).

To elucidate the carbohydrate-binding specificity of L, we have used a series of glycopeptides and synthetic oligosaccharides as inhibitors of precipitation between La and CEA and of La-induced activation of human lymphocytes. The carbohydrates represent different parts of the tetraantennary structure of *N*-acetyllactosamine-type glycoprotein glycans (Fig. 1).

MATERIAL AND METHODS

La from *P. vulgaris*, purified as described by Weber (14), was obtained from Pharmacia. The procedure includes SE-Sephadex chromatography under three different conditions of pH and ionic strength and gel filtration on Sephadex G-200. La gave a single band on NaDodSO₄/polyacrylamide gel electrophoresis under reducing conditions (M_r, ≈31,000). It was homogeneous on electrophoresis at pH 4.5, demonstrating that only L was present.

CEA was purified from individual large bowel tumor metastases to the liver as described (15). Highly purified orosomucoid (α_1 acid glycoprotein), fetuin, and antithrombin III (AT III) were gifts from H. P. Ekre (Kabi AB, Stockholm), S. Svensson (Lund Hospital, Lund), and O. Larm (School of Veterinary Medicine, Uppsala), respectively. Human transferrin, further purified by gel filtration on Sephadex G-150, was from Sigma. *Salmonella thompson* C1 polysaccharide and *Shigella flexneri* Y polysaccharide were available from earlier studies; these materials contain the structural elements →2)Man(1→3)GlcNAc(1→2)Man(1→ (unpublished data) and →3)GlcNAc(1→2)-L-Rha(1→ (16), respectively. *p*-Azophenyl-2-acetamido-2-deoxy-β-galactopyranoside–bovine serum albumin (15–20 sugar units per albumin molecule) conjugate was a gift from I. J. Goldstein (University of Michigan, Ann Arbor).

CEA, orosomucoid, fetuin, transferrin, and AT III and their Pronase glycopeptides (see below) were treated with *Clostridium perfringens* neuraminidase (Sigma, type VI, 2.5 units/mg) at enzyme/substrate ratios of 1:50 to 1:100 in 0.1 M acetate buffer (pH 4.5) to give the corresponding asialo compounds. At least 80% of the sialic acid was removed by the enzyme treatment as determined by the method of Warren (17).
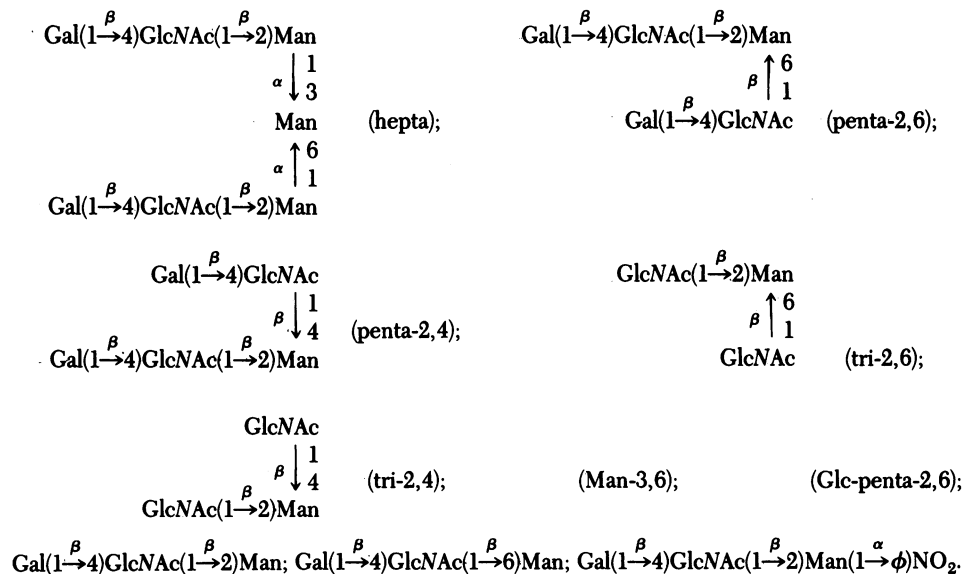
of 0.75 M urea in order to keep the reduced and alkylated products in solution. After dialysis against water, the digests were lyophilized and fractionated on Bio-Gel P-2. The carbohydrate-containing fractions [orcinol reaction (18)], which correspond to the excluded column volume, were pooled and lyophilized. This material was finally fractionated on a Bio-Gel P-4 column to remove small amounts of partially degraded glycoprotein eluting before the glycopeptides.

Pure biantennary transferrin glycopeptide was prepared by concanavalin A (Con A)-Sepharose (Pharmacia) column fractionation (19). Purified Pronase glycopeptide (30 mg) was applied to a column containing ≈500 mg of Con A. Bound material was eluted with 0.1 M methyl α-mannoside in buffer, dialyzed, and finally fractionated on a Bio-Gel P-2 column.

The purified fetuin glycopeptide fraction, containing triantennary glycopeptides (20), was similarly depleted of possible biantennary glycopeptides by Con A-Sepharose affinity chromatography in which the nonbound fraction was collected. The strucural elements of the carbohydrate moieties of the isolated glycopeptides were determined by methylation analysis using gas chromatography/mass spectrometry for the identification of partially methylated sugar residues (21, 22). The analyses indicated that CEA and orosomucoid glycopeptides were mainly of the tetraantennary type, fetuin glycopeptide was of the triantennary type, and transferrin glycopeptide was of the biantennary type, in accordance with earlier results (9, 20, 23, 24). According to amino acid analysis, all glycopeptides contained about 10% amino acid residues.

CEA and orosomucoid and their corresponding Pronase glycopeptides were also subjected to periodate oxidation and reduction with sodium borohydride as described (9).

The following synthetic oligosaccharides were used in the study:

Gal(1→4)GlcNAc(1→2)Man
     α↓3
     Man   (hepta);
     α↑6
Gal(1→4)GlcNAc(1→2)Man

Gal(1→4)GlcNAc(1→2)Man
     β↑6
Gal(1→4)GlcNAc   (penta-2,6);

Gal(1→4)GlcNAc
     β↓4
Gal(1→4)GlcNAc(1→2)Man   (penta-2,4);

GlcNAc(1→2)Man
     β↑6
GlcNAc   (tri-2,6);

GlcNAc
     β↓4
GlcNAc(1→2)Man   (tri-2,4);   (Man-3,6);   (Glc-penta-2,6);

Gal(1→4)GlcNAc(1→2)Man; Gal(1→4)GlcNAc(1→6)Man; Gal(1→4)GlcNAc(1→2)Man(1→φ)NO₂.

Pronase glycopeptides of CEA, orosomucoid, fetuin, and transferrin were prepared as follows. The glycoproteins were reduced with dithiothreitol in the presence of 8 M urea, alkylated by iodoacetamide (9), and dialyzed against water and 0.01 M calcium chloride. The samples were then digested with Pronase (Calbiochem, 45,000 proteolytic units/g) at pH 8.0 (pH adjusted with NaOH) for 2–3 days at 37°C. A total of 30–40 mg of Pronase was added per gram of glycoprotein. Digestions of transferrin and fetuin were initially performed in the presence

Their synthesis and chemical properties have been described (25–29). The corresponding sugar alcohols were prepared by sodium borohydride reduction. All other sugars were available in the laboratory or were purchased from Pfanstiehl.

Quantitative precipitation or precipitation-inhibition analyses (18) were performed by a microprecipitin technique using the ninhydrin procedure for nitrogen determination (18, 30). La (35 μg) and CEA (30 μg) ("equivalence mixture") in a total volume of 200 μl was used in the inhibition studies.

Activation of human peripheral blood lymphocytes or purified T lymphocytes (31) by La and Con A was measured as the incorporation of [³H]thymidine into the cells. Lymphocytes (0.2 ml; 10⁶/ml) in Hepes-buffered RPMI-1640 (GIBCO) supplemented with 0.4% human serum albumin (HSA, Kabi AB, Stockholm), 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) were incubated for 88 hr in V-shaped microplates (Linbro, New Haven, CT) in the presence of lectin. After 72 hr at 37°C, the cells were pulsed for 16 hr with [³H]thymidine (185 kBq/ml per 10⁶ lymphocytes; 3 × 10⁵ MBq/mmol; Radiochemical Centre, Amersham, England). The cells were then transferred to glass filters by means of a sample harvester (Skatron, Lierbyen, Norway) and washed with water and methanol (32). The radioactivity of the insoluble material was determined by liquid scintillation counting. Inhibition by oligosaccharides and glycopeptides was studied by incubating the lymphocytes with different concentrations of the carbohydrates and an optimal dose of lectin (8 μg of La and 1.5 μg of Con A per ml) for 88 hr and pulsing after 72 hr as described above. Cultures were set up in triplicate, and the results are given as the mean value; SD did not exceed 15%.

RESULTS

Direct Precipitation. The interactions between La (35 μg) and various glycoproteins (1–75 μg added) and polysaccharides were studied by quantitative precipitation assays. CEA (eight different preparations), asialo-CEA, orosomucoid, and asialoorosomucoid were strongly precipitated by La. A small amount of precipitate was formed from asialo-AT III and La but AT III, transferrin, asialo-transferrin, fetuin, asialo-fetuin, *Salmonella* C1 polysaccharide, *Shigella* Y polysaccharide, and βGalNAc-albumin were not precipitated by La.

Inhibition of Precipitation. The CEA/La precipitating system was used to investigate the sugar-binding specificity of La. Glycopeptides and synthetic oligosaccharides representing different parts of the tetraantennary structure (Fig. 1) were used as inhibitors. The results are shown in Figs. 2 and 3 and Table 1 and may be summarized as follows. The most potent inhibitors were the Pronase glycopeptide fractions of orosomucoid and CEA (both containing tetraantennary structure). Removal of sialic acid from these two compounds did not change their inhibition power. However, periodate oxidation and reduction abolished their ability to inhibit precipitation. Fetuin and asialo-fetuin glycopeptide (triantennary structure) also inhibited precipitation, although 10–20 times higher molar concentrations

FIG. 3. Inhibition, by synthetic oligosaccharides, of precipitation of CEA with La. ▼, Penta-2,6; ▽, tri-2,6; ●, hepta; ○, hepta-ol; ◆, Gal(1→⁴)GlcNAc(1→²)Man; ■, GlcNAc(1→²)Man; ▲, penta-2,4; □, tri-2,4.

were needed than for orosomucoid glycopeptide. Asialo-transferrin glycopeptide (biantennary structure) also was an inhibitor, about twice as active as the fetuin glycopeptide. In contrast, the native glycopeptide, which contains NANA linked to the 6 position of the two subterminal Gal residues, did not inhibit precipitation. Of the synthetic oligosaccharides, the pentasaccharide corresponding to the third and the fourth branch of the

Table 1. Inhibition of La/CEA precipitation by different haptens

Compound*	IC₅₀, mM†
Orosomucoid Pronase gp	0.15
Asialo-orosomucoid Pronase gp	0.15
Asialo-orosomucoid Pronase gp IO₄⁻, BH₄⁻	>1.0
CEA Pronase gp	0.16
Asialo-CEA Pronase gp	0.15
Fetuin Pronase gp	2.0
Asialo-fetuin Pronase gp	2.4
Transferrin Pronase gp	>5.0
Asialo-transferrin Pronase gp	1.3
Hepta	5.5
Hepta-ol	5.0
Penta-2,6	2.0
Penta-2,6-ol‡	>11
Glc-penta-2,6	>18
Penta-2,4	>18
Penta-2,4-ol	>11
Tri-2,6	4.0
Tri-2,4	>27
Gal(1→⁴)GlcNAc(1→²)Man	12
Gal(1→⁴)GlcNAc(1→²)Man(1→ᵅφ-NO₂	6
Gal(1→⁴)GlcNAc(1→⁶)Man	>18
Man-3,6	>20
GlcNAc(1→²)Man	22
GlcNAc(1→²)Man-ol	>26
GlcNAc(1→²)Glc(1→ᵅOMe	>38
GlcNAc(1→⁶)Man	>40
Glc(1→²)Man	>66
Me α-Man	>102

No inhibition (≤10%) was obtained with the following compounds: *N,N',N''*-triacetylchitotriose at 6 mM; *N,N'*-diacetylchitobiose at 4.8 mM; Gal(1→⁴)GlcNAc at 5.6 mM; Gal(1→⁶)Glc at 70 mM; Gal(1→⁴)Glc at 70 mM; Gal(1→ᵅ²)Man(1→ᵅφ-NO₂ at 32.5 mM; Me α-GlcNAc at 21 mM; Me β-GlcNAc at 21 mM; Me α-GalNAc at 6.0 mM; GalNAc at 45 mM; *p*-nitrophenyl β-GalNAc at 1.0 mM; Me α-Gal at 60 mM; Me β-Gal at 30 mM; Me α-Glc at 60 mM; Me β-Glc at 60 mM.
* gp, Glycopeptide.
† > indicates 0–10% inhibition at the concentration indicated.
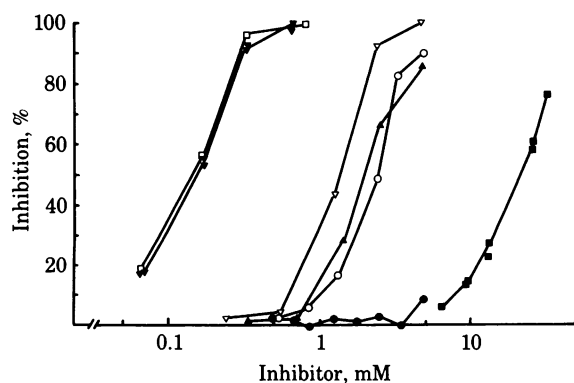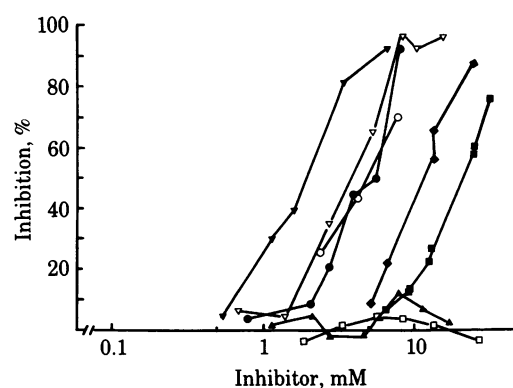‡ Alditol derived from penta-2,6.

FIG. 2. Inhibition, by Pronase glycopeptides, of precipitation of CEA with La. □, CEA- and asialo-CEA Pronase glycopeptides; ▼, orosomucoid- and asialo-orosomucoid Pronase glycopeptides; ▽, asialotransferrin Pronase glycopeptide; ○, asialo-fetuin Pronase glycopeptide; ▲, fetuin Pronase glycopeptide; ●, transferrin Pronase glycopeptide; ■, GlcNAc(1→²)Man.

tetraantennary structure [penta-2,6 (Fig. 1)] was the best inhibitor. It was 2–3 times more potent as an inhibitor than the corresponding trisaccharide (tri-2,6) and the branched heptasaccharide [hepta, equal to the second and the third branch of the tetraantennary structure and including the "branching" Man residue (Fig. 1)]. There was no difference in inhibition power between reduced and original heptasaccharide, indicating that the reducing Man residue did not contribute to the binding phenomenon. Interestingly, however, the asialo-transferrin glycopeptide was about 4 times more active as an inhibitor than the synthetic heptasaccharide. The penta- and trisaccharides (penta-2,4 and tri-2,4) corresponding to the first and the second branch of the tetraantennary structure did not inhibit precipitation even at high concentrations. The minimal inhibitory unit was the disaccharide GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man. The presence of a β-galactosyl group in the 4 position of GlcNAc increased binding about 2-fold. Reduction of the Man residue to its alditol or changing GlcNAc to Glc in this disaccharide abolished binding. Similarly the pentasaccharide Glc-penta-2,6 did not inhibit precipitation. The importance of the 1→2 linkage between GlcNAc and Man was shown by the lack of inhibition with the compounds GlcNAc(1$\overset{\beta}{\rightarrow}$6)Man and Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc(1$\overset{\beta}{\rightarrow}$6)Man. Oligosaccharides corresponding to areas of the tetraantennary structure not involving the GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man sequence—e.g., Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc or Man-3,6—did not inhibit precipitation. Similarly, no simple sugar or methylglycoside including GalNAc inhibited precipitation.

Inhibition of La-Induced Lymphocyte Activation. La is a potent T-lymphocyte mitogen. To learn whether the proliferative response induced in lymphocytes by the lectin was dependent on its carbohydrate-binding site we studied the effect of selected sugar haptens on La-induced incorporation of [^{3}H]thymidine into lymphocytes. Either peripheral blood lymphocytes or, in some experiments, purified T lymphocytes were incubated with an optimal dose of La [8 μg/ml per 10^6 lymphocytes (4)] in the presence of different concentrations of inhibitor. A typical experiment is shown in Fig. 4, and the results of all experiments are summarized in Table 2. None of the sugar haptens was toxic for lymphocytes at the concentrations used in the experiments (Fig. 4; Table 2), as determined by the re-
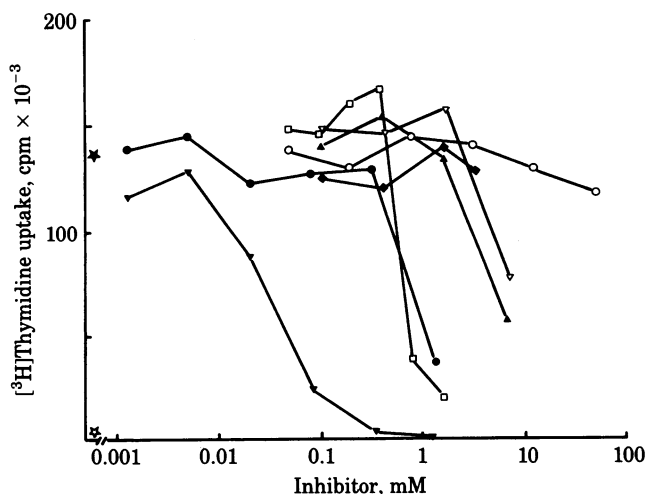

FIG. 4. Inhibition, by Pronase glycopeptides and oligosaccharides, of La-induced DNA synthesis in human blood lymphocytes. ★, Stimulation with La only at 8 μg/ml. Inhibitors: ▼, orosomucoid Pronase glycopeptide; ●, asialo-fetuin Pronase glycopeptide; □, hepta; ▲, tri-2,6; ▽, Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man; ◆, tri-2,4; ○, methyl α-Man.

Table 2. Inhibition of La-induced DNA synthesis in human blood lymphocytes by different sugar haptens

Compound*	Experiments, no.	IC$_{50}$, mM† Mean	IC$_{50}$, mM† Range
Orosomucoid Pronase gp	5	0.14	0.05–0.25
Asialo-CEA Pronase gp	1	0.22	
Asialo-fetuin Pronase gp	1	1.1	
Hepta	4	0.6	0.3–0.9
Penta-2,6	2	3.2	3.0–3.3
Tri-2,6	4	4.4	3.0–6.0
Tri-2,4	3	—	>2.5->4.0
Gal(1→4)GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man	3	2.4	0.8–5.0
GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man	4	2.6	1.0–6.2
GlcNAc(1$\overset{\beta}{\rightarrow}$6)Man	2		>14
Me α-Man	4		>5->50
GalNAc	1		>40
Gal	3		>3->11

* gp, Glycopeptide.
† >, Inhibition between −15% and 15% at the concentration indicated.

lease of ^{51}Cr from labeled lymphocytes (5). The orosomucoid and the asialo-CEA Pronase glycopeptides were the most potent inhibitors, followed by the heptasaccharide and the asialo-fetuin Pronase glycopeptide and then by the oligosaccharides Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man, GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man, penta-2,6, and tri-2,6. No other sugar, including tri-2,4 and Gal(1$\overset{\beta}{\rightarrow}$6)Man, inhibited La-induced lymphocyte proliferation. With three exceptions the relative potencies of the sugar haptens as inhibitors were similar, irrespective of whether their potency was determined in this assay or by inhibition of precipitation (Table 1). The three exceptions were hepta, Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man, and GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man; all were relatively more potent as inhibitors of lymphocyte activation than of precipitation.

The heptasaccharide was also a potent inhibitor of Con A-induced lymphocyte activation (1.5 μg of Con A per ml per 10^6 lymphocytes was the optimal dose) giving 50% inhibition at 0.75 mM, compared to 45 mM for Me α-Man.
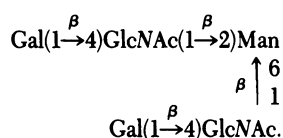
DISCUSSION

These studies indicate that the minimal structural unit required for La binding is the disaccharide GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man. This unit is present in N-acetyllactosamine-type glycoprotein glycans of bi-, tri-, and tetraantennary type (Fig. 1; ref. 24). Of direct importance for the interaction with the binding site is the N-acetamido group in GlcNAc, the β-1,2-linkage between the two sugar residues, and the ring structure of mannose. If the mannose residue is replaced in the 6 position by GlcNAc or by N-acetyllactosamine, as in tetraantennary glycans and in tri-2,6 and penta-2,6, binding to the La site is clearly enhanced. It is interesting to note that the disaccharide GlcNAc(1$\overset{\beta}{\rightarrow}$6)Man in itself does not inhibit precipitation. When GlcNAc or N-acetyllactosamine is linked to the 4 position of this mannose residue as in triantennary glycans, there is no binding. Compounds containing Gal or NANA(2$\overset{\alpha}{\rightarrow}$3)Gal in the 4 position of GlcNAc in the disaccharide unit show unchanged or perhaps even enhanced affinity for La compared to the disaccharide. However, if the substituent is NANA(2$\overset{\alpha}{\rightarrow}$6)Gal, as in the transferrin glycopeptide, there is no binding to the site. The reason for this finding is probably that the sialic acid residue in NANA(2$\overset{\alpha}{\rightarrow}$6)Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man. . . folds back on the GlcNAc(1→2)-

Man sequence,¶ thus blocking access to the essential disaccharide unit.

The presence of mannose residues in the 3 position of GlcNAc in the disaccharide as in *Salmonella* C1 polysaccharide appears not to be compatible with binding. Whether this is also the case when the substituent in this position is L-fucose, as in several N-acetyllactosamine-type glycoproteins (24), remains to be established. The central branching mannosyl residue (Fig. 1) does not contribute to the binding phenomenon because the reduced heptasaccharide is as active as the original compound. However, it is unclear why the asialo-transferrin glycopeptide is a better inhibitor of precipitation than the heptasaccharide. Similarly, it is unknown why the glycopeptides from CEA and orosomucoid are better inhibitors than the oligosaccharide penta-2,6. One possible explanation is that the peptide moieties contribute to binding; another is that the binding sugar sequences occupy more favorable conformations in the glycopeptides compared to the free oligosaccharides.

It would appear as if the carbohydrate binding site of La is relatively large, perhaps as large as the pentasaccharide

$$Gal(1\overset{\beta}{\rightarrow}4)GlcNAc(1\overset{\beta}{\rightarrow}2)Man$$
$$\beta \Big\uparrow {}^6_1$$
$$Gal(1\overset{\beta}{\rightarrow}4)GlcNAc.$$

Extended binding sites have been demonstrated for wheat germ agglutinin (33), *Aaptos* lectins (34), and recently for *Helix pomatia* A hemagglutinin and *Dolichos biflorus* lectin (unpublished data). For precipitation, however, it does not seem necessary that the entire pentasaccharide be present in the glycoprotein. Thus, asialo-AT III, which contains four biantennary glycans identical to those in asialo-transferrin (35), was precipitated by La. However, triantennary glycoproteins—e.g., fetuin—which contain only one reactive $\rightarrow$4)GlcNAc(1$\overset{\beta}{\rightarrow}$2)-Man(1$\rightarrow$ sequence per glycan chain were not precipitated.

La-induced blastogenesis was inhibited by the same oligosaccharides and glycopeptides that were active as inhibitors of precipitation. This indicates that binding of the lectin to the lymphocyte surface via its carbohydrate binding site is an essential early event in the process of lymphocyte activation. Presumably, La binding to the lymphocyte plasma membrane is mediated by carbohydrate structures similar to those discussed above. In this context it is interesting to note that La binds to almost the same T-lymphocyte surface glycoproteins as does Con A (5), another mitogenic lectin. We note that the relative potency, as inhibitors, of some compounds differed slightly in the biological assay compared to precipitation inhibition. An explanation may be that some of the compounds also interact with the lymphocyte surface.

Because the trisaccharide Gal(1$\overset{\beta}{\rightarrow}$4)GlcNAc(1$\overset{\beta}{\rightarrow}$2)Man was an inhibitor of *P. vulgaris* lectin/erythrocyte agglutination (12), it is obvious that the specificity of E must be grossly similar to that of L.

¶ Montreuil, J., Ninth Aharon Katzir-Katchalsky Conference on Carbohydrate–Protein Interactions, Kiryat Anavim, March 1981, ed. Sharon, N. (abstr.), p. 2.

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