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Carbohydrate-structure-dependent recognition of desialylated serum glycoproteins in the liver and leucocytes

Two complementary systems*

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Oligosaccharides with four different types of branching were prepared from purified human transferrin, α_2-macroglobulin, caeruloplasmin and α_1-acid glycoprotein and labelled with $NaBH_3{}^3H$. Binding of these oligosaccharides to rat liver plasma membrane, rat leucocytes, pig liver plasma membranes and pig leucocyte plasma membranes was investigated. A striking dependence of binding on oligosaccharide branching was observed. The values of apparent association constants K_a at 4°C vary from $10^6\,M^{-1}$ (biantennary structure) to $10^9\,M^{-1}$ (tetra-antennary structure) in the liver, whereas in the leucocytes the K_a values were found to be of reversed order, from $1.8\times10^9\,M^{-1}$ for biantennary to $2.2\times10^6\,M^{-1}$ for tetra-antennary structures. The binding is completely inhibited by 150mM-D-galactose, but 150mM-D-mannose has almost no effect on binding. Leucocyte plasma membranes bind preferentially ^{125}I-asialoglycoproteins with biantennary oligosaccharides, thus completing the specificity pattern of the hepatic recognition system for desialylated glycoproteins. Possible physiological roles of these two complementary recognition systems under normal and pathological conditions are discussed.

Blood serum glycoproteins contain *N*-glycosidically linked carbohydrate moieties with bi-, tri- or tetra-antennary arrangements (Fig. 1) as integral part of their molecules (Montreuil, 1980). Oligosaccharide moieties are involved in the transport of glycoprotein molecules to the target cellular compartments (Olden *et al.*, 1982) as well as in the initial phase of glycoprotein catabolism. All these transport functions are performed through specific interactions between glycoprotein glycans and integral membrane lectins of various sugar specificities described as 'carbohydrate-recognition systems' on the surface of hepatocytes, cells of the reticuloendothelial system, fibroblasts and lymphocytes (Berger *et al.*, 1982).

The catabolism of glycoproteins in the blood is most often initiated by splitting the terminal sialic acid residues from their respective structures (desialylation of glycoproteins). In mammals the β-D-galactose residues occur as subterminal sugars of glycoprotein glycans, and the defective molecules are rapidly recognized by galactose-specific membrane recognition systems and catabolized. There are two such recognition systems identified so far, one in hepatocytes and the other in leucocytes.

The liver membrane lectin specific for D-galactose and *N*-acetyl-D-galactosamine described as liver receptor for desialylated serum glycoproteins by Morell *et al.* (1968) was one of the first membrane lectins whose biological functions were determined (Ashwell & Harford, 1982). Physicochemical properties of the lectin, including subunit structure, have been extensively studied (Kawasaki & Ashwell, 1976). The topology of the binding site was examined in considerable detail by determining the inhibition constants of various monosaccharides and oligosaccharides (Sarkar *et al.*, 1979). But, whatever the sugar specificities

Abbreviations used for the designation of oligosaccharides are given in Fig. 1.

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towards individual monosaccharides may be, they cannot explain profound differences in the values of association constants for blood asialoglycoproteins ranging from 10^6 M^{-1} to 10^9 M^{-1}. Partial explanation of this phenomenon came from binding experiments with both natural glycopeptides (Zinn *et al.*, 1978; Baenziger & Maynard, 1980) and synthetic oligomers of D-galactose (Connolly *et al.*, 1982). In these studies the importance of proper spatial arrangements of galactose residues for binding of the ligand to liver lectin and its internalization by pinocytosis (Baenziger & Fiete, 1980) was established.

The second galactose-specific recognition system was identified in lymphocytes (Kieda *et al.*, 1978) and has not yet been characterized in full detail (Decker, 1980). The existence of a second system for desialylated glycoprotein catabolism was discovered during examination of the catabolism of desialylated human transferrin, which was found not to be rapidly catabolized by liver but to be taken up by rabbit bone marrow (Regoeczi *et al.*, 1980).

The aim of the present study was to explain striking differences in the rate of catabolism of individual desialylated serum glycoproteins in the liver (Morell *et al.*, 1971) in terms of their carbohydrate structure. Introduction of suitable methods made it possible to include also the lymphatic recognition system in our study. Both systems were shown to be complementary in their requirements towards oligosaccharides with various types of branching.

Materials and methods

Materials

Hydrazinium hydroxide, CNBr, D-galactose, D-mannose and *N*-acetylneuraminic acid were from Merck, Darmstadt, West Germany. The homogeneity of sugars was evaluated by paper chromatography in butan-1-ol/propan-1-ol/0.1M-HCl (1:2:1, by vol.). Hepes was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. $Na^{125}I$ and $NaBH_3{}^3H$ were provided by the Institute for Research, Production and Utilization of Radioisotopes, Praha, Czechoslovakia. All other chemicals were of the highest purity available.

Pronase was purchased from Serva, Heidelberg, West Germany, and sialidase from *Clostridium perfringens* from Boehringer, Mannheim, West Germany. Concanavalin A and leucoagglutinin from *Phaseolus coccineus* were kindly provided by Dr. Filka from the Laboratory for Production and Control of Lectin Preparations, Faculty of Science, Praha, Czechoslovakia. DEAE-cellulose DE-32 and CM-cellulose CM-32 were manufactured by Whatman, Maidstone, Kent, U.K., and Ficoll, Sephadex G-25, G-50, G-100 and G-200 (fine grade) and Sepharose 2B by Pharmacia Fine Chemicals, Uppsala, Sweden. Affinity matrices were prepared by immobilization of protein on CNBr-activated Sepharose 2B in 0.1M-$(NH_4)_2CO_3$, pH9.5, with an efficiency of binding of about 85–90% (Cuatrecasas *et al.*, 1968).

Preparation of blood serum glycoproteins

A three-step method for preparation of glycoproteins was used. All operations were done at 4°C, and NaN_3 (0.02%) was added to prevent bacterial growth.

Human retroplacental serum was first fractionated with $(NH_4)_2SO_4$ at concentrations 1.6M (first fraction), 2.4M (second fraction), 2.8M (third fraction) and 4.0M (fourth fraction) at pH7.0.

α_2-Macroglobulin was purified from the first $(NH_4)_2SO_4$ fraction by chromatography on DEAE-cellulose and Sephadex G-200 as described (Okubo *et al.*, 1981). The stepwise elution from DEAE-cellulose, however, was replaced by elution with a linear gradient of 0.03–0.2M-sodium acetate buffer, pH5.6, made with an Ultrograd gradient mixer (LKB Produkter, Bromma, Sweden).

Caeruloplasmin was isolated from the second $(NH_4)_2SO_4$ fraction by chromatography on DEAE-cellulose in 0.005M-sodium phosphate buffer, pH6.0. Elution was done with a linear gradient of 0.005–0.2M-sodium phosphate buffer, pH6.0. The final purification was achieved by Sephadex G-100 and hydroxyapatite adsorption chromatography (Broman & Kjellin, 1964).

Transferrin was prepared from the third $(NH_4)_2SO_4$ fraction by chromatography on CM-cellulose and DEAE-cellulose as previously described (Regoeczi *et al.*, 1974).

α_1-Acid glycoprotein was purified from the last $(NH_4)_2SO_4$ fraction by the combination of DEAE-cellulose and CM-cellulose chromatography (Kalous & Poncová, 1965). Transcortin was purified from the second $(NH_4)_2SO_4$ fraction by affinity chromatography (Mickelson & Westphal, 1979).

The purity of blood serum glycoproteins was checked by alkaline electrophoresis and isoelectric focusing in polyacrylamide gel and immunoelectrophoresis in agarose gel. Electrophoresis in polyacrylamide gel was conducted in an apparatus designed by Davis (1964) in the discontinuous buffer system of Steward *et al.* (1964). Isoelectric focusing was run in a thin layer (0.5mm) of polyacrylamide gel (5% of acrylamide and 1.5% of Ampholine, pH3.5–9.5, from LKB Produkter) in an Ultrophor apparatus (LKB Produkter) for 1.5h at a constant power 30W (Macrodrive Constant Power Supply; LKB Produkter) and temperature 10°C. Immuno-

electrophoresis was performed by using the procedure of Grabar & Williams (1955) with horse antibodies against human plasma proteins (ÚSOL, Praha, Czechoslovakia). All gels were stained in 0.1% Coomassie Brilliant Blue R-250 (LKB Produkter) and destained in methanol/acetic acid/water (4:1:5, by vol.).

Glycoproteins (10mg each) were desialylated by the use of immobilized *Clostridium perfringens* sialidase (Parker *et al.*, 1977). The degree of desialylation was assayed by measuring sialic acid contents in the freeze-dried preparations (Svennerholm, 1957). Glycoproteins and asialoglycoproteins were iodinated with ^{125}I by the chloramine-T method (Greenwood *et al.*, 1963).

Preparation of oligosaccharides

Biantennary oligosaccharide from transferrin (Dorland *et al.*, 1977), triantennary oligosaccharides from α_2-macroglobulin and from a caeruloplasmin (Yamashita *et al.*, 1981) and tetra-antennary oligosaccharides from α_1-acid glycoprotein (Fournet *et al.*, 1978) were prepared after three cycles of Pronase digestion (Arima *et al.*, 1972). The reaction mixture after the third digestion was applied on a Sephadex G-50 column (3cm × 135cm) equilibrated with 0.1M-pyridine adjusted to pH5.5 with acetic acid. Front glycopeptide fractions enriched by glycopeptides of higher M_r were further fractionated on a DEAE-cellulose column (2cm × 40cm) at pH8.0 developed with a linear gradient of 0.01–0.2M-Tris/HCl buffer. Fractions corresponding to the most sialylated species were desialylated with immobilized sialidase (Parker *et al.*, 1977), and sialic acid was removed on a Sephadex G-25 column (2cm × 100cm). The final purification of glycopeptides was achieved by affinity chromatography on immobilized concanavalin A (Krusius *et al.*, 1976). In the case of the tetra-antennary glycopeptide from α_1-acid glycoprotein, chromatography on Dowex 50W X2 as modified by Schmid *et al.* (1977) was employed as a final purification procedure. The asialoglycopeptides obtained (10mg of each) were subjected to preparative high-voltage electrophoresis in borate (Narasimhan *et al.*, 1980) before being converted into oligosaccharides and labelled with $NaBH_3{}^3H$ as described by Mizuochi *et al.* (1978).

Characterization of oligosaccharides

Homogeneity of oligosaccharides was tested by t.l.c. on Silufol silica-gel plates (Kavalier, Sázava, Czechoslovakia) with the solvent system ethanol/butan-1-ol/pyridine/water/acetic acid (100:10:10:30:3, by vol.) and orcinol detection (Cahour *et al.*, 1983). Paper electrophoresis of purified glycopeptides was done in pyridine/acetate buffer, pH5.0 (4ml of pyridine, 1.2ml of acetic acid, water to 1 litre) in the apparatus described by Mikeš (1957) for 2h at 1500V and 30mA. Electrophoresis in borate on polyacrylamide gels was performed as previously described (Weitzman *et al.*, 1979). The sialic acid content of each glycopeptide was measured after DEAE-cellulose chromatography by the procedure of Svennerholm (1957). The monosaccharide composition of individual oligosaccharides were determined by g.l.c. (Porter, 1975) on a Perkin–Elmer Sigma 2 chromatograph. The type of branching of the triantennary oligosaccharide from α_2-macroglobulin was assessed by chromatography on immobilized *Phaseolus* leucoagglutinin (Cummings & Kornfeld, 1982).

Preparation of leucocytes

Small pieces of rat (Wistar strain) mesenterial lymph nodes aseptically removed were homogenized by gentle strokes in a glass Potter–Elvehjem homogenizer in Hanks buffered saline solution (Pospíšil *et al.*, 1978). Cell suspensions were washed three times in the same solution, counted, assayed and resuspended (see below).

Preparation of liver and leucocyte plasma membranes

Liver and leucocyte plasma membranes were prepared and characterized in accordance with the established procedures (Dorling & Le Page, 1973; Brunette & Till, 1971). Pig tissues (liver, spleen and lymph nodes from Czech White improved breed pigs) were scraped on a cooled dish and homogenized in a loose homogenizer with a rubber pestle before homogenization in a Dounce homogenizer.

Binding assays

Membranes (equiv. to 50μg of membrane protein) or leucocytes (5×10^6 cells), radiolabelled ligand and in some experiments unlabelled inhibitor (oligo- or mono-saccharide) were incubated in Eppendorf microtubes in 0.12M-NaCl/10mM-$CaCl_2$/20mM-Hepes/NaOH buffer, pH7.5, for 1h at 4°C in a total volume of 1ml. Bound and unbound ligands were separated by centrifugation through a 0.5ml layer of 10% (w/v) Ficoll at 8000*g* for 5min and counted for radioactivity separately (Debanne *et al.*, 1981). Non-specific binding was calculated from experiments performed in the presence of 10mM-Na_2EDTA instead of 10mM-$CaCl_2$. ^3H and ^{125}I radioactivities were determined by liquid-scintillation counting in a PCS scintillation cocktail (Amersham International, Amersham, Bucks., U.K.). Results were subjected to Scatchard (1949) analysis by the least-squares method on a Hewlett–Packard hp 97 S I/O calculator.

Results

In our study we used glycoprotein-derived oligosaccharides of defined structures that were described previously. Glycoproteins used as oligosaccharide sources were isolated in gram quantities by conventional methods, and their purity was checked by immunoelectrophoresis and isoelectric focusing. Nevertheless, the extreme structural diversity of glycoprotein glycans must be taken into account. This holds especially true in the case of α_1-acid glycoprotein, which can contain bi-, tri- and tetra-antennary oligosaccharides (Fig. 1) in a single molecule because each glycosylation site has its own type of heterogeneity. In this case chromatography on long columns of Dowex 50W X2 was shown to be necessary to resolve individual glycopeptides. The method used for tritiation of oligosaccharides has a relatively low efficiency (about 0.1%), but by using carrier-free $NaBH_3{}^3H$ we achieved high specific radioactivities in ^3H-oligosaccharide preparations (13–18 MBq/mg).

The homogeneity of glycopeptides and oligosaccharides was evaluated by t.l.c., high-voltage paper electrophoresis and electrophoresis in borate buffer. Oligosaccharide preparations are homogeneous in t.l.c. chromatograms developed in a previously published solvent system (Fig. 2). The weak zone accompanying the biantennary oligosaccharide (lane 1) corresponds probably to a fucosylated oligosaccharide; these were reported to move more slowly in this solvent system (Cahour *et al.*, 1983). On paper electrophoretograms some glycopeptides could be detected with ninhydrin as 'double patches', probably due to the peptide part of their molecules. This conclusion was confirmed during electrophoresis of tritiated oligosaccharides in polyacrylamide gels in Tris/borate buffer, which was reported to be one of the most sensitive techniques for testing glycopeptide and oligosaccharide heterogeneity (Fig. 3).

Monosaccharide compositions of individual oligosaccharides are presented in Table 1. Monosaccharide ratios are in good agreement with those of biantennary, triantennary and tetra-antennary oligosaccharides (Fig. 1). Trace amounts of fucose present in biantennary oligosaccharide preparation represent probably fucose residues attached to

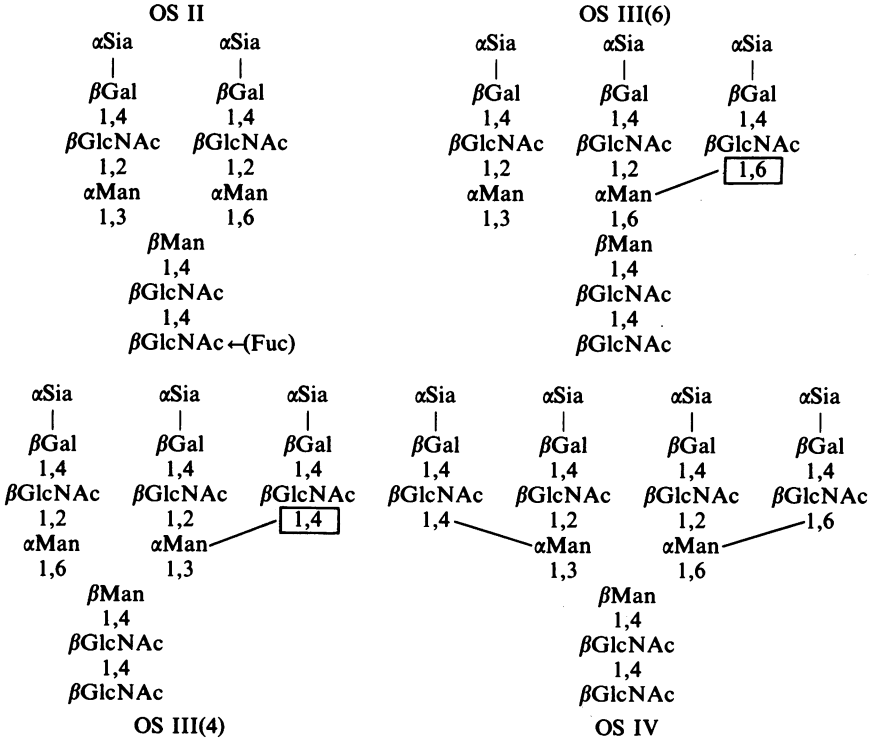

Fig. 1. *Structures of oligosaccharides used in the present study*

OS II is a biantennary oligosacharide from transferrin (Dorland *et al.*, 1977), OS III(4) is a triantennary oligosaccharide from caeruloplasmin (Yamashita *et al.*, 1981) and OS IV is tetra-antennary oligosaccharide from α_1-acid glycoprotein (Fournet *et al.*, 1978). OS III(6) is a structure proposed for triantennary oligosaccharide from human serum α_2-macroglobulin. Abbreviation: Sia, sialic acid.

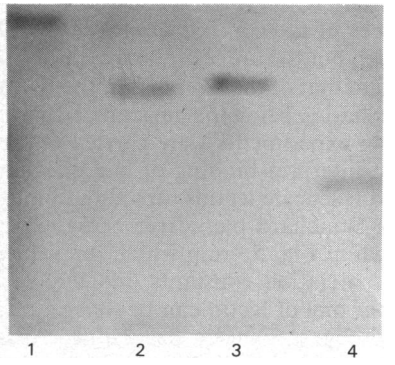

Fig. 2. *T.l.c. of individual serum glycoprotein-derived asialo-oligosaccharides*
Track 1 shows asialo-OS II from transferrin, track 2 triantennary asialo-oligosaccharide from α_2-macroglobulin, track 3 asialo-OS III(4) from caeruloplasmin and track 4 asialo-OS IV from α_1-acid glycoprotein. For experimental details see the text.

Table 1. *Monosaccharide composition of glycoprotein-derived oligosaccharides used in this study*
Composition is given in mol of individual monosaccharides per mole of oligosaccharide; the M_r values of biantennary (2100), triantennary (2500) and tetra-antennary (3000) oligosaccharides were estimated by using gel filtration on a Sephadex G-50 (fine grade) column (1 cm × 150 cm). Sialic acids were determined spectrophotometrically after resolution of differently sialylated oligosaccharide species on DEAE-cellulose, other monosaccharides as alditol acetates by g.l.c. as described in the Materials and methods section. —, Not detected.

	Composition (mol/mol of oligosaccharide)					
Compound	Sia	GlcNAc	Gal	Man	Fuc	GalNAc
OS II	1.8	4.01	2.08	3.18	0.18	—
OS III(6)	2.7	5.05	2.89	3.06	—	—
OS III(4)	2.8	4.97	3.01	2.95	—	—
OS IV	3.7	6.13	4.12	3.13	—	—

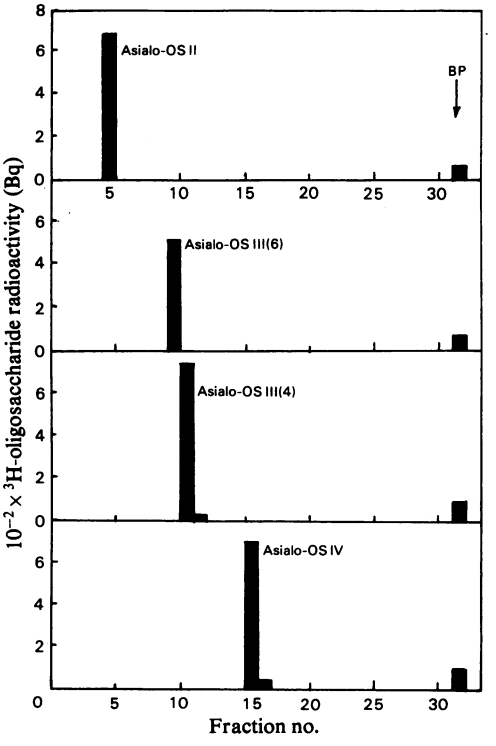

Fig. 3. *Evaluation of homogeneity of ^{3}H-oligosaccharides by polyacrylamide-gel electrophoresis in borate buffer*
Radioactivity was measured in slices 2 mm thick starting from the top of the gel rod. Gels were dissolved in 1 ml of 10% NH_3 for 4 h at 60°C, and radioactivity of the solution was determined by liquid-scintillation counting. BP shows the position of tracking dye (Bromophenol Blue).

the oligosaccharide 'core' (in parentheses in Fig. 1). Affinity chromatography on immobilized *Phaseolus* leucoagglutinin has enabled us to resolve triantennary oligosaccharides isolated from α_2-macroglobulin and caeruloplasmin. Although the triantennary oligosaccharide of caeruloplasmin was not retained on a column of leucoagglutinin–Sepharose 2B, as could be expected from the previously published structure (Yamashita *et al.*, 1981), the triantennary oligosaccharide from α_2-macroglobulin was retained in this column in the manner previously described as characteristic for oligosaccharides with the third lactosaminic branch attached to α-D-mannose by a 1→6 bond (Cummings & Kornfeld, 1982). On the basis of these results we consider OS III(6) to be the most probable structure for triantennary oligosaccharide from α_2-macroglobulin (this oligosaccharide is found rather rarely among serum glycoprotein glycans).

Fig. 4(*a*) shows the inhibition of the binding of ^{125}I-asialo-α_1-acid glycoprotein to rat liver plasma membranes by oligosaccharides and D-galactose. Large differences in the concentration of individual oligosaccharides necessary for a 50% inhibition are evident. The results support the idea of multivalent interaction between the lectin molecule and the terminal β-D-galactose residues of the ligand. Our results shown in Fig. 4(*a*) are in good agreement with the work with hepatocytes by Lee *et al.* (1983), who used both synthetic and natural ligands.

Fig. 4(*b*) presents data for the inhibition of ^{125}I-asialotransferrin binding to rat leucocytes by individual oligosaccharides. There are also great

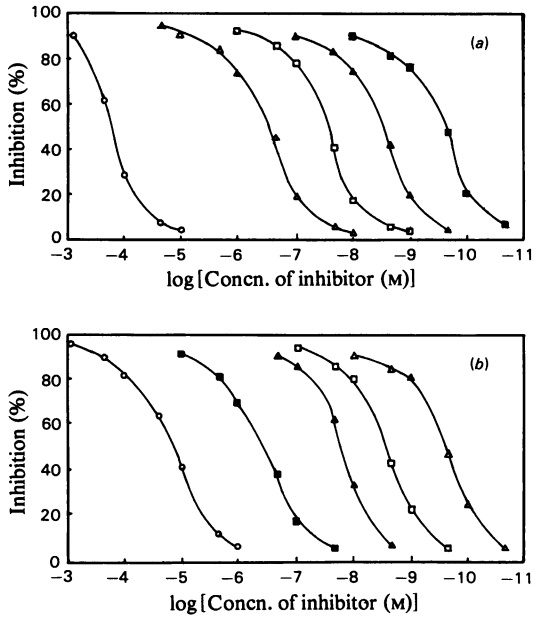

Fig. 4. *Inhibition by individual oligosaccharides of ^{125}I-asialo-α_1-acid glycoprotein binding to rat liver plasma membranes (a) and ^{125}I-asialotransferrin binding to rat leucocytes (b)*

Liver plasma membranes (corresponding to 50 μg of protein) or leucocytes (5×10^6 cells) were incubated with labelled ligand and the inhibitor in the indicated concentration. After 1 h at 4°C, bound and unbound ligands were separated and measured as described in the Materials and methods section. ○, D-Galactose; △, asialo-OS II; □, asialo-OS III(6); ▲, asialo-OS III(4); ■, asialo-OS IV. The concentrations of oligosaccharides were measured by the phenol/H_2SO_4 method (Dubois *et al.*, 1956).

differences in the concentrations needed for 50% inhibition, but the order of the inhibitory power is reversed to that of the liver lectin, the biantennary oligosaccharide being the most powerful inhibitor.

Further experiments were carried out in an attempt to interpret binding of oligosaccharides to liver and leucocyte lectins directly in binding parameters. Scatchard plots from these experiments are shown in Fig. 5, from which the values of apparent association constants and mol of ligands bound per mol of lectin can be determined for all types of oligosaccharide branching. Association constants of oligosaccharides towards membranes from lymph nodes are always somewhat higher than those measured with spleen-cell plasma membranes.

Table 2 summarizes the results of this study. From the data two major conclusions can be drawn concerning the binding and subsequent catabolism of desialylated serum glycoproteins by membrane lectins in the liver and leucocytes. (1) The binding of desialylated serum glycoproteins by membrane lectins in the liver and leucocytes depends primarily on the type of oligosaccharide structure present in each glycoprotein molecule, but the number of such oligosaccharides per glycoprotein molecule is less important (it can change the value of the association constant maximally by one order of magnitude). (2) Asialoglycoproteins having oligosaccharides OS II and OS III(6) (designated here group I oligosaccharides) are preferentially taken up and catabolized by leucocyte membrane lectins, whereas those having oligosaccharides OS III(4) and OS IV (group II oligosaccharides) are more rapidly taken up and catabolized in the liver.

Table 2. *Binding of some serum asialoglycoproteins and their oligosaccharides to the liver and leucocyte plasma membranes*

Active oligosaccharides are considered those having the highest value of the apparent dissociation constant K_a in respective recognition systems, i.e. the most-branched oligosaccharides in the case of liver plasma membranes and the least-branched oligosaccharides in the case of leucocyte plasma membranes. Values of K_a for transferrin subforms as well as their designations are taken from the paper by Debanne *et al.* (1981). Abbreviation: N.D., not determined.

Compound	No. of active oligosaccharide groups/molecule	K_a for liver plasma membrane (M^{-1})	No. of active oligosaccharide groups/molecule	K_a for leucocyte plasma membrane (M^{-1})
Asialo-OS II	1	0.85×10^6	1	1.8×10^9
Rabbit asialotransferrin	1	0.9×10^6	1	2.1×10^9
Human asialotransferrin 1	2	1.5×10^6	2	5.8×10^9
Asialo-OS III(6)	1	0.95×10^7	1	1.2×10^8
Human asialotransferrin 2	1	1.4×10^7	1	N.D.
Asialo-α_2-macroglobulin	3	2.8×10^7	4	8.1×10^8
Asialo-OS III(4)	1	1.0×10^8	1	0.9×10^7
Human asialotransferrin 3	1	1.1×10^8	1	N.D.
Asialocaeruloplasmin	1	1.3×10^8	2	2.1×10^8
Asialotranscortin	2	2.5×10^8	3	3.7×10^8
Asialo-OS IV	1	0.9×10^9	1	2.2×10^6
Asialo-α_1-acid glycoprotein	3–4	5.3×10^9	1–2	8.1×10^6

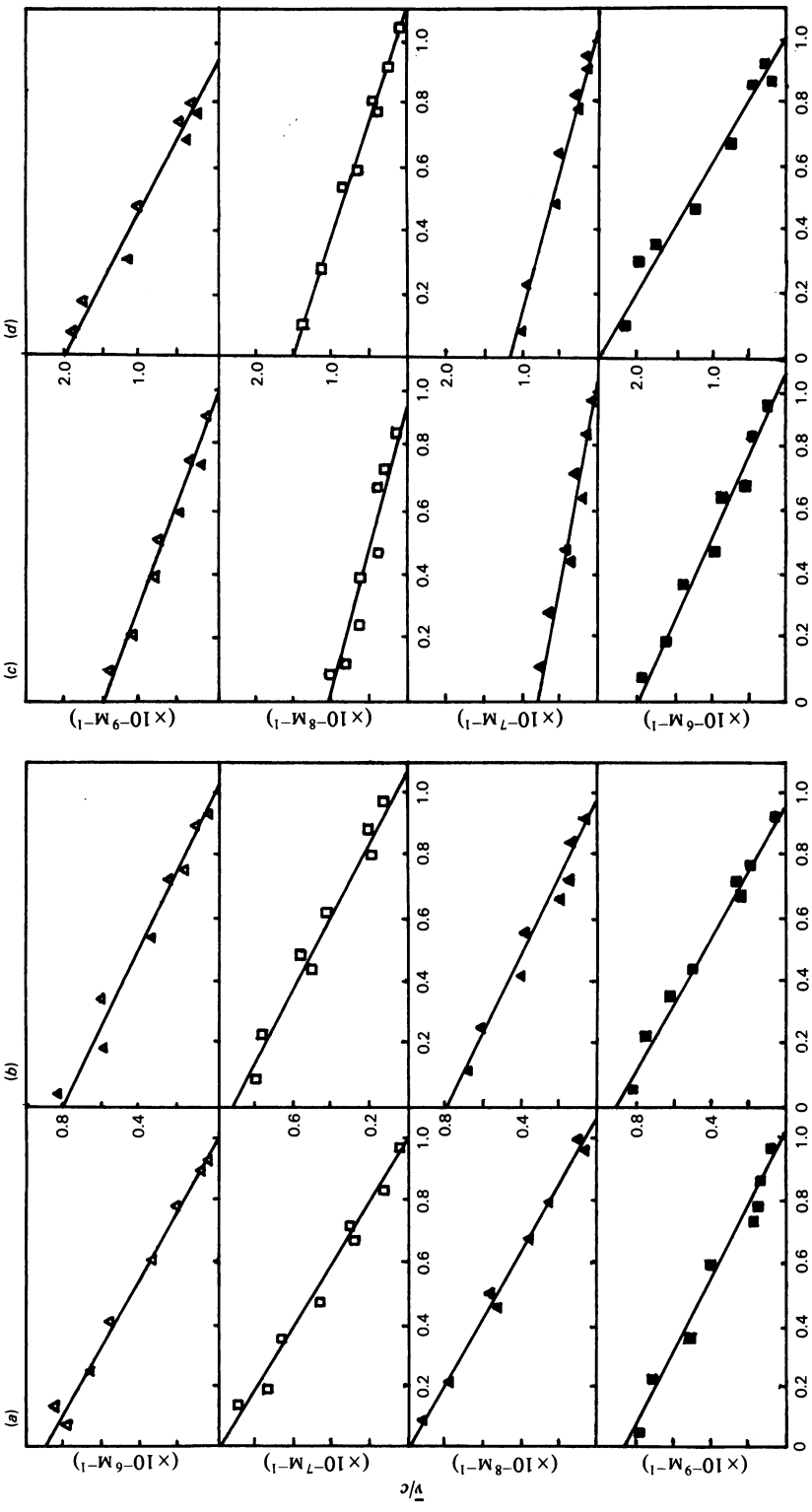

Fig. 5. *Scatchard plots for binding of individual oligosaccharides to rat liver plasma membrane (a), pig liver plasma membrane (b), pig spleen leucocyte plasma membrane (c) and pig lymph-node-leucocyte plasma membrane (d)*

Plasma membranes (corresponding to 50 μg of protein) were incubated with ^{3}H-labelled oligosaccharides in the concentration range corresponding approximately to that necessary for 50% inhibition (see Figs. 4*a* and 4*b*). After 1 h of incubation at 4°C, the bound and unbound ligands were separated and determined as described in the Materials and methods section. △, Asialo-OS II; □, asialo-OS III(6); ▲, asialo-OS III(4); ■, asialo-OS IV. $\bar{\nu}$ is the binding range (ratio of the amount of ligand bound at a given concentration) to that bound in an excess of ligand; *c* is the concentration of free ligand.

Asialoglycoproteins possessing both these types of carbohydrate structures can be catabolized in both sites according to the ratio of structures present in each individual molecule.

Discussion

The first report about the existence of two complementary recognition systems for desialylated serum glycoproteins (Regoeczi *et al.*, 1980) appeared from the investigation of asialotransferrin catabolism in rabbits. Rabbit bone marrow was identified as a tissue capable of rapid uptake of asialoglycoproteins with less-branched structures. In our work we made an attempt to describe both recognition systems in terms of carbohydrate structures required for optimal binding. The molecular mechanism of recognition seems to be quite well documented in the case of liver membrane lectin. We isolated this lectin from rat liver and performed binding experiments with both the tetramer of the lectin and the separated subunits. From these experiments absolute dependence of the ability to discriminate between oligosaccharides with different types of branching on the tetrameric arrangement of the lectin molecule was evident; after dissociation into subunits differences between the values of association constants for individual oligosaccharides disappeared and the association constants reached values typical for monoantennary ligand or D-galactose (K. Bezouška, unpublished results).

Molecular properties of the leucocyte D-galactose-specific lectin are documented much less than those of the liver lectin. Main obstacles in the investigation of these lectins represent difficulties in obtaining sufficient amount of homogeneous cell populations as starting material for isolation and the low stability of the lectin after disintegration of the membrane with detergents.

Physiological importance of these two complementary recognition systems may reside in their ability to differentiate between asialoglycoproteins with carbohydrate structures of either group I or group II. When considering biological roles of individual glycoproteins in the blood, we can conclude that glycoproteins with transport functions often carry oligosaccharides belonging to group II (e.g. caeruloplasmin, transcortin, α_1-acid glycoprotein, haemopexin, haptoglobin) and can be catabolized after desialylation in the liver as the central organ of metabolism, whereas glycoproteins having protective functions in the blood (immunoglobulins, fibrinogen, complement proteins) possess most frequently biantennary glycans and after desialylation can be recognized by cells of the immune system. Some of the serum glycoproteins can probably be catabolized by both these tissues: transferrin, complexes of immunoglobulins with antigens (Thornburg *et al.*, 1980) and complexes of proteinases with proteinase inhibitors (α_1-antitrypsin, α_2-macroglobulin).

There are two types of cells capable of receptor-mediated pinocytosis in the liver, namely hepatocytes and Kupffer cells. Whereas, as a rule, in hepatocytes only macromolecules of certain size are taken up, Kupffer cells can take up whole cells, for instance desialylated erythrocytes (Schlepper-Schäfer *et al.*, 1980). Also, cells of the lymphatic system not only interact with macromolecules cir-

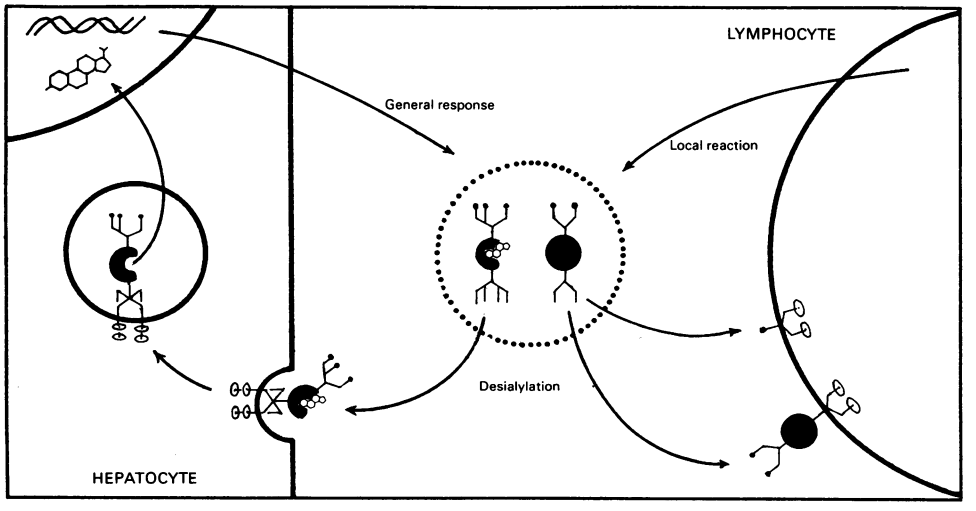

Fig. 6. *Hepatic and leucocyte recognition systems for desialylated serum glycoproteins during inflammation*

culating in the blood, but they also play an essential role in the recognition of self and non-self structures. Mixed lymphocyte reaction represents an especially powerful reaction of this type. Thus the leucocyte carbohydrate-recognition system, although much less investigated than the hepatic system, can play an important role as an additional immune-response system based upon carbohydrate–lectin interaction (Monsigny *et al.*, 1983), phylogenetically even more ancient (crustaceans, molluscs) than the antibody system (Renwrantz, 1983).

The importance of these two recognition systems may increase during some pathological events accompanied by extensive desialylation of blood serum glycoproteins (inflammation, malignant transformations). Desialylation starts in the site of tissue damage by the action of intracellular sialidases released from lysosomes of broken cells and activated by acidification. Desialylated serum glycoproteins are then transported within the whole body and may provide a signal addressed to distinct tissues. Asialoglycoproteins with group II oligosaccharides are catabolized in the liver. They may carry with them biologically active compounds that can initiate events of a general response of organism during inflammation (e.g. synthesis of 'acute-phase' glycoproteins). Asialoglycoproteins with group I oligosaccharides can interact directly with leucocytes in the blood and mediate local reaction during inflammatory process. (Fig. 6).

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