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Structural Assessment of the *N*-Linked Oligosaccharides of Cell-CAM 105 by Lectin-Agarose Affinity Chromatography

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The *N*-linked oligosaccharides of cell-CAM 105, a glycoprotein involved in the intercellular adhesion between rat hepatocytes, were studied by sequential lectin-agarose affinity chromatography of desialylated, [^{14}C]-labelled glycopeptides. These glycopeptides were obtained by extensive pronase digestion followed by *N*-[^{14}C]acetylation of the peptide moieties and desialylation by mild acid hydrolysis.

Assuming that all glycopeptides were radiolabelled to the same specific radioactivity, Concanavalin A-Sepharose chromatography indicated that the majority of the glycans (84%) were of the complex-type of which approximately half were bi-antennary structures. The remainder of the glycans comprised oligomannose-type structures and/or incomplete bi-antennary structures. *Pisum sativum* lectin-agarose chromatography revealed that part of the bi-antennary glycans contained a fucose residue α(1-6)-linked to the *N*-acetylglucosamine which is attached to asparagine. Furthermore, the presence of tri-, and tetra- and/or tri'-antennary complex-type glycans was demonstrated by chromatography on immobilized *Phaseolus vulgaris* leukoagglutinating phytohemagglutinin and *Aleuria*

Abbreviations: CAM, cell adhesion molecule; ConA, Concanavalin A; WGA, wheat germ agglutinin; PEA, *Pisum sativum* lectin; E-PHA, *Phaseolus vulgaris* erythroagglutinating phytohemagglutinin; L-PHA, *Phaseolus vulgaris* leukoagglutinating phytohemagglutinin; RCA_I, *Ricinus communis* agglutinin I; AAL, *Aleuria aurantia* lectin; mGlc, methyl-α-D-glucopyranoside; mMan, methyl-α-D-mannopyranoside; C0, W0, P0, E0, L0, R0, A0, non-retained, and Cn, Wn, Pn, En, Ln, Rn, An (n=1-4), retarded or bound glycopeptide fractions on columns of immobilized ConA, WGA, PEA, E-PHA, L-PHA, RCA_I and AAL, respectively. The fraction names are also used sequentially, e.g. C1P1, which indicates the fraction of glycopeptides that was eluted from ConA-Sepharose on position C1 and was subsequently eluted from PEA-agarose on position P1.

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aurantia lectin (AAL). AAL-agarose chromatography furthermore indicated the presence of α(1-3)-linked fucose in part of these glycopeptides, whereas no α(1-6)-linked fucose could be detected in these structures.

The degree of β-galactosylation of the complex-type glycans was investigated by chromatography on _Ricinus communis_ agglutinin-agarose. The results indicated that only part of the bi-antennary glycans were completely β-galactosylated. Similarly, at least three β-galactose residues were present in only a part of the tri-, and tetra- and/or tri'-antennary glycans.

The process whereby cells recognize and adhere to each other is essential for multicellular organisms to function properly during embryonic development and in the adult stage. The molecular basis for these intercellular contacts is still not well understood, but the involvement of specific cell-surface molecules, called cell adhesion molecules (CAMs), has been demonstrated (for reviews see [1-4]). Up to now, several of these molecules have been purified to homogeneity and their chemical structure has been investigated [5-10].

One of the molecules identified so far is cell-CAM 105, a cell-surface molecule involved in the intercellular adhesion between rat hepatocytes [11]. Cell-CAM 105 was shown to be an integral membrane glycoprotein consisting of two peptide chains, of apparent molecular masses of 110 and 105 kDa, respectively [5]. Immunohistochemical staining of adult, intact liver [12, 13] demonstrated that cell-CAM 105 was located in the bile canalicular region, whereas in perinatal liver [14] and in isolated hepatocytes [15] it was found around the cells, particularly in areas of cell-cell contact. Besides in the liver, it was also found in several epithelia, vessel endothelia, platelets and polymorphonuclear leukocytes [13].

The expression of cell-CAM 105 has been investigated both in foetal and regenerating rat liver showing that the molecule appeared rather late during embryonic development and that the amount was temporarily decreased after partial hepatectomy [14, 16]. These studies indicated that cell-CAM 105 functions primarily in terminally differentiated cells. Furthermore, cell-CAM 105 could not be detected in several chemically-induced transplantable hepatocellular carcinomas [17].

Elucidation of the chemical structure of cell-CAM 105 is a pre-requisite for understanding its action *in vivo*. Recently, several aspects of the structure have been reported [5]. The amino-acid composition has been determined, and it has been shown that phosphorylation of serine residues can occur in isolated rat hepatocytes. Both peptide chains of cell-CAM 105 appeared to be glycosylated to the same extent up to a total amount of 33%. Furthermore, analysis of the carbohydrate composition suggested all the carbohydrate to be of the *N*-linked type [5]. However, the nature of these *N*-linked oligosaccharides and their role in the adhesion process still have to be established.

In the present paper we report on the qualitative assessment of the *N*-linked oligosaccharide structures of cell-CAM 105 as purified from rat liver plasma membranes. This was performed by serial lectin-agarose affinity chromatography of glycopeptides derived from cell-CAM 105 by extensive pronase treatment.

Figure 1. Standard *N*-linked glycans used in the present study to calibrate the columns of immobilized ConA, PEA, WGA, L-PHA, E-PHA and RCA_I.

Materials and Methods

Materials

Cell-CAM 105 was purified to homogeneity from rat liver plasma membranes by a combination of immunoaffinity chromatography, gel-exclusion chromatography and ion-exchange chromatography as described previously [5]. Only the material eluted in peak III

Figure 2. Standard *N*-linked fucosylated glycans used in the present study to calibrate the AAL-Sepharose 4B column.

from the ion-exchange column (Mono Q, Pharmacia, Uppsala, Sweden) was used for the isolation of glycopeptides. Concanavalin A(ConA)-Sepharose 4B (lot No. ME 02174 or LI 00753, 10 mg protein/ml of gel) was purchased from Pharmacia. P*isum sativum* lectin(PEA)-agarose (lot No. 021301, 3-5 mg protein/ml of gel), wheat germ agglutinin(WGA)-agarose (lot No. 040808, 3-5 mg protein/ml of gel), P*haseolus vulgaris* leukoagglutinating phytohemagglutinin(L-PHA)-agarose (lot No. 031202, 3-5 mg protein/ml of gel), P*haseolus vulgaris* erythroagglutinating phytohemagglutinin(E-PHA)-agarose (lot No. 040619, 3-5 mg protein/ml of gel), and R*icinus communis* agglutinin I(RCA_I)-agarose (lot No. 030514, 3-5 mg protein/ml of gel) were obtained from E-Y Laboratories, San Mateo, CA, USA. A*leuria aurantia* lectin (AAL) was obtained from mushrooms by affinity chromatography on a Sepharose 4B-*N*-(ε-aminocaproyl-β-L-fucopyranosylamine) column and immobilized AAL (3 mg protein/ml gel) was prepared by coupling AAL to Sepharose 4B as previously described [18]. Bio-Gel P-2, P-4 (200-400 mesh) and P-30 (100-200 mesh) were obtained from Bio-Rad Laboratories, Richmond, CA, USA. Pronase (70,000 units/g) was purchased from Calbiochem-Behring Corp., La Jolla, CA, USA, and [1-^{14}C]acetic anhydride (25 mCi/mmol) from Amersham International, Amersham, UK.

For the calibration of the columns of immobilized ConA, PEA, WGA, L-PHA, E-PHA, RCA_I and AAL, NMR-characterized or HPLC-purified radioactively labelled glycans were used; the carbohydrate structures of these standard glycans are shown in Figs. 1 and 2. The asialo bi-(GP*2*), tri-(*GP3*), and tetra-antennary (*GP4*) glycopeptides were prepared originally from human α_1-acid glycoprotein and were labelled with [^{14}C]acetate in the peptide part as described previously [19]. The oligomannose-type glycopeptide *AC-D3* was prepared from ovalbumin according to [20, 21], labelled with [^{14}C]acetate in the peptide part, and subsequently identified by ^{1}H-NMR spectroscopy (Bierhuizen, M.F.A. and Van den Eijnden,

D.H., unpublished results). A fraction containing the hybrid-type glycopeptides *GP-II-A* and *GP-II-B* was prepared from ovalbumin [20, 22]. These glycopeptides were further purified by HPLC and labelled with [^{14}C]acetate in the peptide part. The complex-type bi-antennary glycopeptide *MS*, which contains a fucose substituted at C-6 of the *N*-acetylglucosamine residue attached to asparagine, and the bi-antennary oligosaccharide *GG(Gn)*, which contains a bisecting *N*-acetylglucosamine attached to the β-linked core mannose and a [^{14}C]label in its galactose residues, were kindly donated by Prof. Dirk van den Eijnden (Amsterdam, The Netherlands). The mono- and di-fucosylated bi-antennary glycopeptides *GP2-MF* and *GP2-BF* (Fig. 2) isolated from human lactotransferrin were gifts from Prof. Geneviève Spik (Lille, France). The Fucα(1-3)-substituted tri-antennary glycopeptide *GP3-MF* (Fig.2) was a gift from Prof. Bernard Fournet (Lille, France). *MS*, *GP2-MF*, *GP2-BF*, and *GP3-MF* were labelled with [^{14}C]acetate in the peptide part.

All other chemicals used were of analytical grade and were obtained from commercial sources.

Preparation of N-[^{14}C]Acetylated Glycopeptides from Cell-CAM 105

Glycopeptides were prepared by extensive digestion with pronase (2.6 μg/μg cell-CAM 105) in 0.1 M Tris-HCl buffer (pH 8.0), 1 mM $CaCl_2$, 0.2% Triton X-100 at 37°C for 48 h under a toluene atmosphere. The pronase had been pre-incubated for 1 h at 37°C in order to inactivate any possible endogenous glycosidase impurity. Additional amounts of pronase (2.6 μg/μg cell-CAM 105) were added at 8 h intervals. The digestion was stopped by heating the incubation mixture at 100°C for 5 min. Subsequent centrifugation at 10 000 x *g* for 30 min was performed to remove any insoluble material. The supernatant derived from approximately 160 μg cell-CAM 105 was desalted on Bio-Gel P-2 (1 x 45 cm) in water, lyophilized and labelled with [^{14}C]acetate as described previously [19]. Since it was not possible to separate the radiolabelled glycopeptides completely from contaminating non-glycopeptide radioactivity by Bio-Gel passage alone, further purification was necessary. Firstly, ConA-Sepharose was used to withdraw oligomannose- and/or hybrid-type glycopeptides (tightly bound to ConA) from the preparation. The remaining radioactive material was combined and desialylated by mild acid treatment. Then the glycopeptides containing terminal β-linked galactose residues, presumably complex-type glycopeptides, were purified from this fraction by RCA_I-agarose chromatography. The radioactive materials that did not bind to the RCA_I-agarose column were discarded. The fraction that was tightly bound to the ConA-Sepharose and the RCA_I-binding material were combined, desalted on Bio-Gel P-2 in water, and lyophilized. The final glycopeptide preparation contained 67350 dpm ^{14}C.

Lectin-Agarose Affinity Chromatography

Glycopeptides were further analysed by serial lectin-agarose affinity chromatography. The radioactivity in aliquots from each lectin column fraction was determined using a liquid scintillation method. Appropriate fractions were pooled and desalted by gel filtration in water on a column of Bio-Gel P-2 (1 x 45 cm). After reconstitution in the appropriate buffer the glycopeptide fractions were analysed for their affinity with the subsequent lectin column.

ConA-Sepharose chromatography was performed using a 2 ml column (0.6 x 7 cm; 10 ml/h; 1 ml fractions; 20°C) and 10 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.02% NaN_3 as starting buffer [23]. After application in 1 ml of starting buffer the material not bound by the lectin was eluted with approximately 10 ml of the same buffer. Specific elution of glycopeptides bound to the lectin was performed by a 45 ml slowly increasing concave gradient of 0-0.5 M methyl-α-D-glucopyranoside (mGlc) in starting buffer. Under these conditions the standard asialo bi-antennary glycopeptide *GP2* and the oligomannose-type glycopeptide *AC-D3* were eluted as C1 (0.03 M mGlc) and C2 (0.08 M mGlc), respectively.

WGA-Agarose chromatography was performed using a 5.6 ml column (0.6 x 20 cm; 10 ml/h; 1 ml fractions; 20°C) and 10 mM sodium phosphate buffer (pH 7.4), 0.15 M NaCl, 0.02% NaN_3 as starting buffer [24]. After application in 1 ml starting buffer the glycopeptides were allowed to interact with the lectin for 1 h. Elution was then started with approximately 20 ml of starting buffer. Specific elution of glycopeptides bound to the lectin (W1) was performed with 0.2 M *N*-acetylglucosamine in starting buffer. Under these conditions standard hybrid-type glycopeptides *GP-II-A* and *GP-II-B* were eluted from the column at W1.

PEA-Agarose chromatography was performed using a 4.2 ml column (0.6 x 15 cm; 5 ml/h; 1 ml fractions; 20°C) and 10 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.02% NaN_3 as starting buffer [25]. After application in 1 ml starting buffer glycopeptides were eluted with approximately 30 ml of starting buffer. Specific elution of glycopeptides bound to the lectin was performed sequentially with 10 mM mGlc and with 0.5 M methyl-α-D-mannopyranoside (mMan) in starting buffer, respectively. Under these conditions the standard core-fucosylated bi-antennary glycopeptide *MS* was eluted from the column with 10 mM mGlc.

L-PHA-Agarose chromatography was performed as described previously [19]. Under these conditions the top fractions of the standard asialo tri-antennary glycopeptide *GP3* and tetra-antennary glycopeptide *GP4* were eluted at 19 ml (L1) and 30 ml (L2), respectively.

E-PHA-Agarose chromatography was performed using a 12 ml column (0.6 x 42 cm; 5 ml/h; 1 ml fractions; 20°C) and 6.7 mM potassium phosphate buffer (pH 7.4), 0.15 M NaCl, 0.02% NaN_3 as starting buffer [26]. After application in 1 ml of startingbuffer, glycopeptides were eluted with approximately 80 ml of starting buffer. Under these conditions the top fraction of the standard asialo bi-antennary oligosaccharide *GG(Gn)*, which contains a bisecting *N*-acetylglucosamine residue, was eluted from the column at 56 ml.

RCA_I-Agarose chromatography was performed using a 12 ml column (0.6 x 42 cm; 5 ml/h; 1 ml fractions; 20°C) and 10 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.02% NaN_3 as starting buffer [27]. After application in 1 ml of starting buffer glycopeptides wereallowed to interact with the lectin for 1 h. Elution was then started with 15 ml of starting buffer. Glycopeptides bound to the lectin were eluted by a 60 ml slowly increasing concave gradient of 0-5 mM galactose in starting buffer. Under these conditions the peak fraction of the standard asialo bi-antennary glycopeptide *GP2* was eluted at 0.5 mM galactose and of the standard asialo tri- and tetra-antennary glycopeptides *GP3* and *GP4* at 0.7 mM galactose.

AAL-Sepharose 4B chromatography was performed using a 4 ml column (0.5 x 20 cm; 9 ml/h; 1.5 ml fractions; 20°C) and 10 mM phosphate buffer (pH 7.2), 0.15 M NaCl as starting buffer [18]. After application in 0.5 ml of starting buffer non-retarded (A0), slightly retarded (A1) and strongly-retarded glycopeptides (A2) were eluted with standard buffer. Retained glycopeptides (A3) were eluted with 50 mM L-fucose in starting buffer. Under these conditions the Fucα(1-3)-substituted tri-antennary glycopeptide *GP3-MF* was eluted in A1, the Fucα(1-6)-substituted bi-antennary glycopeptide *GP2-MF* was eluted in A2 and the Fucα(1-6)- and Fucα(1-3)-substituted bi-antennary glycopeptide *GP2-BF* was eluted in A3.

Percentages were obtained by taking the radioactivity of the total eluate from the various lectin-agarose columns as 100%. Recovery of the radioactivity on the various columns routinely was over 85%.

Other Methods

Desialylation of glycopeptides was performed by 2 M acetic acid for 15 min at 100°C [27]. After neutralization the fractions were desalted on a column of Bio-Gel P-4 (1 x 45 cm) in 50 mM ammonium acetate (pH 5.2), 0.02% NaN_3. Under the desialylation conditions employed, at most 5% of the galactose residues present are removed (Bierhuizen, M.F.A., unpublished observation).

Assessment of molecular masses of glycopeptide fractions was performed by gel filtration on a Bio-Gel P-30 column (1.5 x 92 cm; 10 ml/h; 2 ml fractions) in 50 mM ammonium acetate (pH 5.2), 0.02% NaN_3. The column was characterized with a mixture of hemoglobin (V_o of the column), the asialotetra-antennary complex-type glycopeptide *GP4* and D-[2-^{3}H] mannose (V_t of the column) in the elution buffer.

Results

Fractionation of N-[^{14}C]Acetylated Glycopeptides of Cell-CAM 105 by ConA-Sepharose Chromatography

ConA-Sepharose was used to separate eventual tri- and tetra-antennary complex-type glycans from bi-antennary complex-type, oligomannose- and/or hybrid-type glycopeptides [28]. Under the conditions employed the standard asialo tri- and tetra-antennary complex-type glycopeptides *GP3* and *GP4* were not bound (fraction C0), the asialo bi-antennary complex-type glycopeptide *GP2* was bound (fraction C1), and the oligomannose-type glycopeptide *AC-D3*, containing six mannose residues, was tightly bound by the lectin (fraction C2).

The cell-CAM 105 glycopeptides were separated by the column in four fractions: C0, 41%; C1, 43%; C2, 13%; and C3, 3% (Fig. 3, top). Fraction C3 was bound more strongly to the column than fraction C2; the peak fraction eluted at 0.2 M mGlc. These results indicated the presence of bi-antennary and other complex-type structures, whereas the presence of

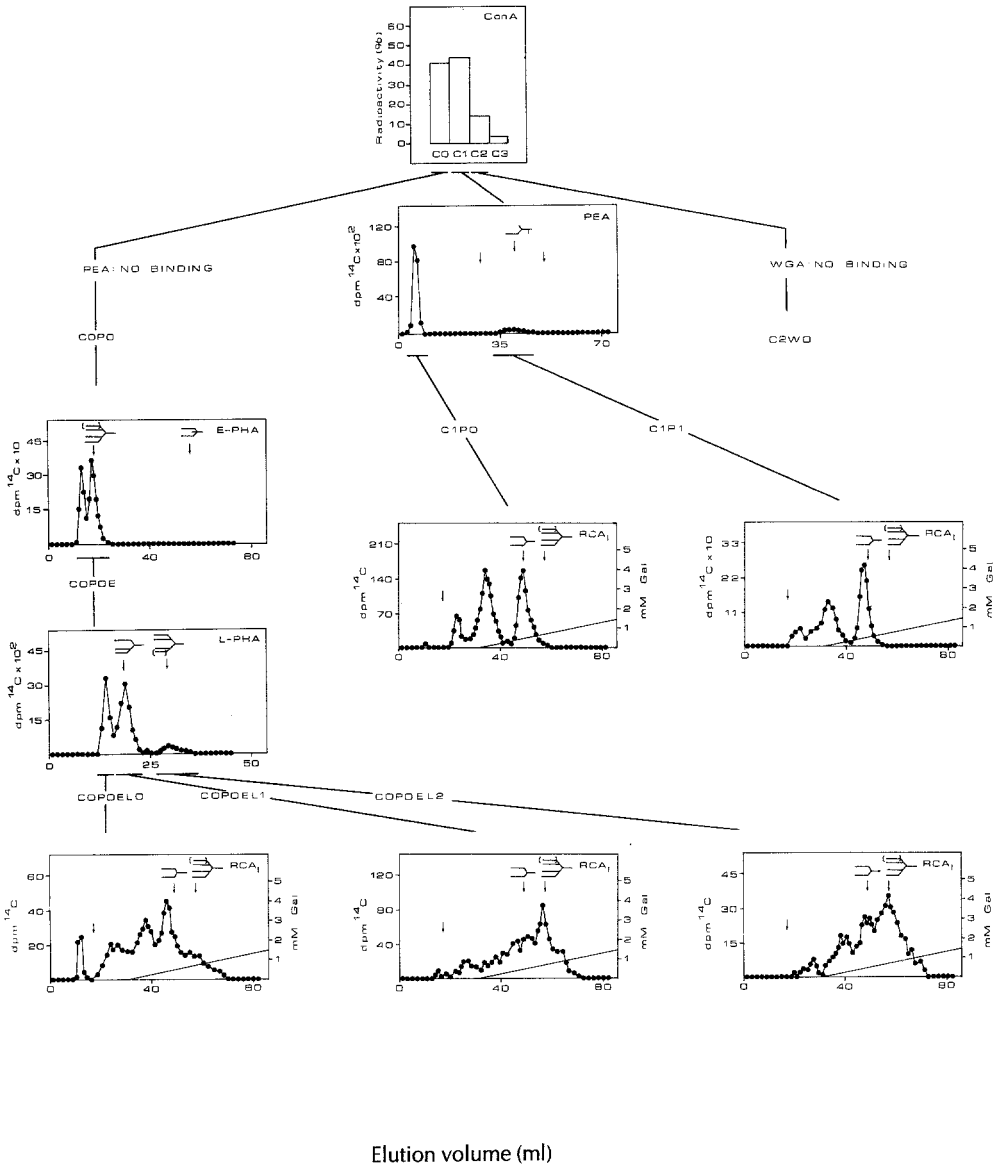

Figure 3. Lectin-agarose affinity chromatography of *N*-[^{14}C]acetylated glycopeptides from cell-CAM 105. The fractions C0, C1, C2, and C3 were prepared as described in the Materials and Methods section. Their values are expressed as percentages of the sum of the radioactivity incorporated in the various fractions. The ConA-fractions C0, C1, C2 and C3 were applied to columns of immobilized PEA, WGA, E-PHA, L-PHA and RCA_I as indicated. The linear arrows within the graphs indicate the points at which various sugars were added to elute bound glycopeptides. The elution positions of standard glycans are indicated at the upper part of each graph. The standard structures *GP2*, *MS*, *GG(Gn)*, *GP3* and *GP4* are depicted in a schematic form (see Fig. 1)

oligomannose- and/or hybrid-type structures was suggested. However, incomplete bi-antennary glycans might also be present in C2 or C3 [23].

The nature of the glycopeptide fractions C0, C1, and C2 was further studied by serial lectin-agarose affinity chromatography. From the assessment of the molecular masses by Bio-Gel P-30 chromatography it was established that the glycopeptides in each fraction contained not more than one glycan per molecule. All [^{14}C]-labelled glycopeptides, were recovered between the elution positions of the standard asialotetra-antennary glycopeptide *GP4* and the V_t of the column.

Further Fractionation of Glycopeptide Fractions C0, C1, and C2 by Serial Lectin-Agarose Affinity Chromatography

Hybrid-type glycans could not be detected in Fraction C2 since no radioactivity was retained by WGA-agarose (Fig. 3) [24, 29]. The occurrence of hybrid-type glycans in fraction C3 could not be established due to the limiting amount of radioactivity in this minor fraction.

One-tenth of the radioactivity present in the bi-antennary fraction C1 was bound to PEA-agarose (Fig. 3, C1P1), which indicates the presence of a fucose residue in the chitobiosyl core, substituted at C-6 of the *N*-acetylglucosamine residue attached to asparagine [25]. The majority (90%) of the bi-antennary fraction apparently lacked this fucose residue (Fig. 3, C1P0).

The radioactivity present in the fraction that was not bound to ConA-Sepharose (C0) was sequentially subjected to chromatography on PEA-, E-PHA- and L-PHA-agarose. The lack of binding of this fraction to PEA-agarose [resulting in fraction C0P0 (Fig. 3)] indicated the absence of tri-antennary glycans with an α-linked mannose residue substituted at positions C-2 and C-6, and a fucose residue α(1-6)-linked to the *N*-acetylglucosamine residue that is attached to asparagine [25]. Furthermore, no complex glycans containing a bisecting *N*-acetylglucosamine attached to the β-linked core mannose could be detected upon chromatography of C0P0 on E-PHA-agarose (Fig. 3). Since the slightly retarded fraction on this immobilized lectin column co-eluted with the standard asialo tri- and tetra-antennary glycopeptides (*GP3* and *GP4* respectively), both fractions were combined, designated C0P0E and subjected to L-PHA-agarose chromatography. Three fractions were obtained, C0P0EL0, C0P0EL1, and C0P0EL2 (Fig. 3) comprising 35%, 55% and 10% of the radioactivity, respectively. Fraction C0P0EL2 co-eluted with *GP4* containing the pentasaccharide unit Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-6)Man, whereas fraction C0P0EL1 co-eluted with *GP3* lacking the Galβ1-4GlcNAcβ1-6 branch of this unit. The presence of fraction C0P0EL0 might be explained by [19, 30]: (i) the occurrence of asialo tri- and/or tetra-antennary glycans lacking the β(1-4)-linked galactose on the GlcNAcβ1-2Manα1-6 branch, which appeared to be essential for interaction with L-PHA; and (ii) the occurrence of asialo tri- and/or tetra-antennary glycans containing fucose α(1-3)-linked to *N*-acetylglucosamine on the GlcNAcβ1-2Manα1-6 branch. However, the latter possibility appeared to be less likely since the majority of the radioactivity (81%) in this fraction passed through the AAL column; only 19% of the radioactivity co-eluted with standard structures containing one α(1-3)-linked fucose residue (Table 1). Similarly, AAL-Sepharose 4B chromatography suggested that fraction C0P0EL1 consisted primarily of tri-antennary glycopeptides instead

of tetra-antennary glycans containing fucose α(1-3)-linked to *N*-acetylglucosamine on the GlcNAcβ1-6Manα1-6 branch, since 82% of the radioactivity did not interact with the immobilized AAL. The remaining part of the radioactivity (18%) co-eluted with standard structures containing one α(1-3)-linked fucose residue (Table 1). Neither in fraction C0P0EL0 nor in fraction C0P0EL1 radioactivity co-eluted with standard structures containing a fucose residue α(1-6)-linked to the *N*-acetylglucosamine which is attached to asparagine.

RCA_I-Agarose Affinity Chromatography of Complex-type Glycopeptides

The fractions containing complex-type glycans were examined for the presence of terminal β-Gal residues by RCA_I-agarose chromatography. The bi-antennary glycopeptide fractions C1P0 and C1P1 were both separated into three fractions by RCA_I-agarose (Fig. 3). Approximately 40% of the radioactivity co-eluted in both instances with the standard asialo bi-antennary glycopeptide *GP2*, containing two terminal β(1-4)-linked galactose residues. The remaining two fractions presumably represented bi-antennary structures with one β-linked galactose residue, differing in the branch to which galactose was attached [23].

Approximately 40 and 60% of the tri- and/or tetra-antennary glycans containing fractions C0P0EL1 and C0P0EL2, respectively, co-eluted with the standard glycopeptides *GP3* and *GP4*, indicating the presence of at least three terminal β-linked galactose residues (Fig. 3). Furthermore, approximately 30 and 20%, respectively, of the radioactivity in these fractions co-eluted with the standard glycopeptide *GP2*, containing two terminal β(1-4)-linked galactose residues. Less than two terminal β-Gal residues appeared to be present in the remainder of the fractions, 30% and 20%, respectively.

The RCA_I-agarose elution profile obtained for the fraction C0P0EL0 indicated that approximately 60% of the fraction contained less than two, 30% contained two and not more than 10% contained three or more β-linked galactose residues.

Discussion

Glycans of the *N*-glycosidic type have a common core with the structure Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ1-*N*-asparagine. Dependent on the sugars added to this core structure three different types can be distinguished. Oligomannose-type structures contain only additional mannose residues, whereas in complex-type structures *N*-acetylglucosamine, galactose, fucose and sialic acid can be added. Finally, a hybrid-type of these structures can occur. Analysis of the carbohydrate composition of cell-CAM 105 denoted the presence of galactose, fucose, mannose, *N*-acetylglucosamine and *N*-acetylneuraminic acid, but no galactosamine [5]. The absence of galactosamine suggested that only *N*-linked oligosaccharides were present.

In this study lectin-agarose affinity chromatography was used to investigate the *N*-linked oligosaccharides from cell-CAM 105. The presence of bi-antennary complex-, and other complex-type glycans was indicated by ConA-Sepharose chromatography, whereas the absence of hybrid-type and complex-type glycans containing a bisecting *N*-acetylglucosa-

Table 1. Behaviour of glycopeptide fractions C0P0EL0 and C0P0EL1 on AAL-Sepharose 4B. The column of immobilized AAL was calibrated with appropriate standard *N*-linked complex-type glycopeptides containing *N*-acetylglucosamine residues substituted by α(1-3)- and/or α(1-6)-linked fucose residues (see the Materials and Methods section). A0, non-retained fraction; A1, slightly retarded fraction; A2, strongly retarded fraction; A3, retained fraction eluted specifically with fucose.

Elution position	Type of substitution of GlcNAc by Fuc in the co-eluting standard *N*-linked glycopeptide	Relative distribution of radioactivity (%)	
		C0P0EL0	C0P0EL1
A0	-	81	82
A1	Fucα1-3	19	18
A2	Fucα1-6	n.d.[a]	n.d.
A3	Fucα1-3 plus Fucα1-6	n.d.	n.d.

[a] n.d., not detectable.

mine residue was demonstrated by WGA- and E-PHA-agarose chromatography, respectively. The structures proposed to be present are based on the carbohydrate-binding specificities of the lectins, taking into account what is known about *N*-glycosylation in rat liver [31, 32]. The tentative assignment of oligosaccharide structures to cell-CAM 105 is shown in Table 2.

The majority of the glycopeptides comprised complex-type glycans, according to the relative distribution of radioactivity in the various lectin-binding fractions. Bi-antennary and other complex-type glycans apparently were present in about equal amounts. A minority (16%) represented oligomannose-type and/or incomplete bi-antennary structures. This correlation of the incorporated amount of radioactivity to the relative occurrence of glycan chains, is based on the assumption that all glycopeptides were labelled equally with [1^4C]acetate. Recently, we showed that glycopeptides from human α_1-acid glycoprotein, prepared in a similar manner, contained only a few amino-acid residues and were all radiolabelled to the same extent [33]. The observed molecular masses for the glycopeptides prepared from cell-CAM 105 were in the same range or lower than the masses obtained for the glycopeptides of α_1-acid glycoprotein, suggesting also the presence of a short peptide chain. Furthermore, we noticed that the ratio of the bi-antennary to the other complex-type glycans for the *N*-[^{14}C]acetylated cell-CAM 105 glycopeptides (Fig. 3) was the same as the corresponding ratio for the total membrane-bound glycopeptides from rat hepatocytes, metabolically labelled with [2-^{3}H]mannose [34]. Although the radiolabelling of more than one amino-acid residue per glycopeptide with [^{14}C]acetate therefore appears to be less probable, it cannot be completely excluded.

A small part of the bi-antennary glycans contains a fucose residue α(1-6)-linked to the *N*-acetylglucosamine which is attached to asparagine. Furthermore, RCA_I-agarose chromatography indicated that less than half of the bi-antennary glycopeptides was completely β-galactosylated. According to the carbohydrate-binding specificity of RCA_I [35], galactose

Table 2. Tentative assignment of oligosaccharide structures to cell-CAM 105.
The values, given in %, were calculated from the fractionation of the [^{14}C]acetylated glycopeptides on the immobilized lectins as indicated in Fig. 3, on the assumption that all glycopeptides have the same specific radioactivity.

Bi[a] (C1)	Tri (COPOEL1)	Tetra and/or tri′ (COPOEL2)	α(1-3)-Fucosylated/undergalactosylated tri and/or tetra[b]	Oligomannose/incomplete bi (C2 + C3)
43	18	4	19	16

[a] 10% of these bi-antennary glycans contain a fucose residue α(1-6)-linked to the *N*-acetylglucosamine which is attached to asparagine.
[b] Summation of % radioactivity in fraction COPOEL0 and the part of fraction COPOEL1 which was retarded on the AAL column.

can be present both in β(1-4)- and β(1-3)-linkage. However, β(1-3)-linkage seems less likely, since Krusius and Finne [31] established that in the complex-type *N*-linked glycans, obtained from whole rat liver tissue, no *N*-acetylglucosamine substituted solely at C-3 was detected.

The majority of the complex-type glycopeptides retarded on L-PHA-agarose represented most probably tri-antennary glycans. One-tenth of the glycans applied to the column accounted for tetra- and/or tri′-antennary glycans.

The presence of α(2-6)-linked *N*-acetylneuraminic acid at the galactose residue of the Galβ1-4GlcNAcβ1-2Manα1-6 branch has been reported to abolish the interaction of glycans with L-PHA-agarose [19, 30]. Therefore, it is reasonable to assume that at least the terminal non-substituted β(1-4)-linked galactose on this branch is present in the fractions binding to the lectin. RCA_I-agarose chromatography indicated that, besides this galactose residue, approximately half of the cell-CAM 105 glycopeptides retarded by L-PHA-agarose contained another two β-linked galactose residues. The other half of these glycopeptides appeared to be undergalactosylated. The absence of the β(1-4)-linked galactose rather than the presence of an α(1-3)-linked fucose residue on the GlcNAcβ1-2Manα1-6 branch in tri- and/or tetra-antennary glycans occurring in COPOEL0 appeared to be the main reason for the lack of interaction with L-PHA, as judged from the behaviour of COPOEL0 on both immobilized AAL and RCA_I. This considerable undergalactosylation of the glycan structures of cell CAM-105 is in good agreement with the undergalactosylation found by us for the total membrane-bound glycopeptides of rat hepatocytes [34].

The absence of both hybrid-type and complex-type glycans containing a bisecting *N*-acetylglucosamine residue was indicated by chromatography on columns of immobilized WGA and E-PHA, respectively. In addition, in a similar study towards the *N*-glycosylation of membrane-bound glycoproteins in isolated rat hepatocytes, we could detect neither of these structures [34]. These observations coincide with the recent finding that *N*-acetylglu-

cosaminyltransferase III, the enzyme responsible for the transfer of *N*-acetylglucosamine in β(1-4)-linkage to the β-linked mannose of the tri-mannosyl core, has a very low activity in microsomal membranes of rat liver [36].

ConA-Sepharose chromatography revealed the presence of a strong (C2) and a very strong binding fraction (C3), of which C2 co-eluted with our standard *AC-D3,* containing six mannose residues. Although it is tempting to assign oligomannose structures to these fractions the presence of incomplete bi-antennary glycans cannot be excluded [23]. A possible explanation for the occurrence of fraction C3 might also be the presence of an extra α-linked mannose to the C-2 hydroxyl group of the terminal α(1-6)-linked mannose of e.g. our standard *AC-D3,* since these structures are known to bind more tightly to ConA than our standard *AC-D3* [37]. Oligomannose-type glycans containing such an a-linked mannose residue have been reported in whole rat liver slices [32].

Recently, Cunningham *et al.* [7] investigated the carbohydrate chains of the liver cell adhesion molecule, L-CAM, from chick embryonic liver cells. Digestions with endoglycosidases H and F, followed by sodium dodecyl sulphate - polyacrylamide gel electrophoresis, indicated L-CAM to contain one oligomannose- and three complex-type *N*-linked glycans. However, the nature of the glycans and their role in the adhesion process have to be investigated. In this respect it is interesting to note that cell-CAM 105 also seems to contain oligomannose- and complex-type *N*-linked glycans. Whether this apparent structural resemblance of the carbohydrate chains originates from their functional role, has to be established.

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