LEC14, a Dominant Chinese Hamster Ovary Glycosylation Mutant Expresses Complex N-Glycans with a New N-Acetylglucosamine Residue in the Core Region*

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The Chinese hamster ovary (CHO) glycosylation mutant, LEC14, was previously selected for resistance to pea lectin (Pisum sativum agglutinin) and shown to behave dominantly. The lectin resistance properties of LEC14 cells are related to, but distinct from, those of LEC18, a dominant Chinese hamster ovary mutant that synthesizes complex N-glycans with a novel O-6-linked GlcNAc residue in the core region (Raju, T. S., Ray, M., and Stanley, P. (1995) J. Biol. Chem. 270, 30294–30302). Detailed structural studies of a complex N-glycan fraction from LEC14 cells have revealed yet another novel modification of the core region. [3H]Glc-labeled LEC14 cellular glycopeptides were desialylated, and the fraction that did not bind to concanavalin A-Sepharose was found to have an increased proportion of species that bound to tomato-agarose, and to ricin-agarose. 1H NMR spectroscopy and methylation linkage analysis of the tomato and ricin-bound fractions purified from ~10^10 LEC14 cells showed they were complex N-glycans containing a 2,3,6-trisubstituted core Man residue. To examine the core region more closely, these N-glycans were digested with mixtures of β-D-galactosidases and N-acetyl-β-D-glucosaminidases to obtain core glycopeptides. The latter were largely unbound by concanavalin A-Sepharose or pea lectin-agarose. 1H NMR spectroscopy and electrospray ionization-mass spectrometry showed that the LEC14 core glycopeptides contain a new GlcNAc residue that substitutes the core β(1,4)-Man residue at O-2 to give the following novel, N-linked core structure.

The core region of N-linked glycans is derived from the first five sugars (Man_3GlcNAc_2) attached to dolichol-phosphate during the synthesis of the Glc_3Man_2GlcNAc_2 intermediate that is transferred to Asn-X-(Ser/Thr) residues in glycoproteins (1). In plants and some lower organisms, this simple core structure can be modified by the addition of Fuc to the GlcNAc attached to Asn (Asn-GlcNAc) in α(1,6)- and/or α(1,3)-linkage (2-6), or Xyl in β(1,2)-linkage to the β(1,4)-Man residue (7-15). In mammals, the core may be modified by the addition of Fuc to the Asn-GlcNAc in α(1,6)-linkage (16,17); by the addition of GlcNAc to the β(1,4)-Man at the O-4 position to generate the bisecting GlcNAc (16,17); and/or by the addition of a GlcNAc at O-6 to the GlcNAc residue linked to the β(1,4)-Man residue (18). This latter type of core was discovered in the complex N-glycans unique to the LEC18 CHO mutant (18,19), which is characterized by a high level of resistance to pea lectin (PSA) and Lens culinaris agglutinin (LCA). The phenotype is dominant (19) and is the result of the de novo expression of a novel UDP-GlcNAcGlcNAc glycosyltransferase that is present in LEC18 but not in parental CHO cell extracts.2 LEC14 CHO cells have a phenotype similar to, but subtly distinct from, LEC18 cells (19). LEC14 cells are resistant to PSA and LCA, which bind core fucosylated N-glycans (20), but only 3-4-fold compared with the 15-40-fold level of resistance displayed by LEC18 cells (19). On this basis, LEC14 might be expected to be a weak version of the LEC18 phenotype. Mitigating against this conclusion, however, is the fact that LEC14 and LEC18 cells differ significantly in their respective sensitivities to the lectins L-PHA (agglutinin from Phaseolus vulgaris) and ricin. LEC14 cells are more sensitive than LEC18 and parental CHO cells to the toxicity of ricin, while LEC14 cells are more resistant than LEC18 and parental CHO cells to the toxicity of L-PHA (19). Qualitative differences in lectin resistance properties predict a different biochemical basis for each phenotype amongst the CHO glycosylation mutants (21).

In this paper we show that the biochemical basis of the LEC14 dominant phenotype is related to that of LEC18 in that complex N-glycans from LEC14 cells possess an altered core structure. The modified core in LEC14 cells is, however, novel and has not previously been observed in glycoconjugates from any source.

**EXPERIMENTAL PROCEDURES**

Materials—5-3H]Gal (31.5 Ci/mmol), [2-3H]Man (11.6 Ci/mmol), [6-3H]Glc (36.25 Ci/mmol), [U-13C]Glc (290 mCi/mmol), and

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‡ The abbreviations used are: Fuc, fucose; Xyl, xylose; CHO, Chinese hamster ovary; ConA, concanavalin A; PSA, Pism sativum (pea) agglutinin; LCA, L. culinaris agglutinin; L-PHA, P. vulgaris leukoagglutinin; RCA1, ricin agglutinin I; RCA11, ricin agglutinin II; PBS, phosphate buffered saline; HPAEC-PAD, high performance anion-exchange chromatography with pulsed electrochemical detection; GLC-MS, gas-liquid chromatography-mass spectrometry; ESI-MS, electrospray ionization-mass spectrometry.
LEC14 and parent glycopeptides differ in structure. [3H]Glc-labeled glycopeptides of LEC14 and parental cells, which did not bind to ConA-Sepharose, were desialylated and fractionated on tomato-agarose as described under “Experimental Procedures” (upper panel). The fractions bound to tomato-agarose were desalted and subjected to glycopeptide mapping by HPAEC-PAD. Elution was achieved using a gradient of 100 mM NaOH and 100 mM NaOH containing 1M NaOAc as described previously (18). Fractions of 1 min were collected and counted for radioactivity.

L-[6-3H]Fuc (16.1 Ci/mmol) were from Amersham Corp.; ConA-Sepharose and Sephadex G-25 were from Pharmacia Biotech Inc. L-PHA-Ringer Mannheim; D-(+)-Gal, D-(+)-GalNAc, D-(+)-mannoside, methyl D-(+)-glucoside, Triton X-100, N,N’,N’-triacycetylchitotriose (10 mg/ml) in PBS2−105 LEC14 cells as described previously (19).

Preparation of Radiolabeled Cell Surface Glycopeptides—Pro 5 and Pro LEC14.4A cells were cultured at a density of 1.5 × 10⁵ cells/mi in 10 ml of complete medium and 100 μCi of [3H]Glc was added. After 48 h at 37°C, glycopeptides were generated by exhaustive Pronase digestion as described previously (18). Serial Lentin Affinity Chromatography of Radiolabeled Glycopeptides—Glycopeptides were fractionated on ConA-Sepharose (0.5 cm × 20 cm) and PSA-agarose (0.5 cm × 20 cm) as described previously (18). Pooled glycopeptides were desalted on Bio-Gel P-2 (1.5 cm × 70 cm). To remove glycosidically bound sialic acid residues, glycopeptides were hydrolyzed with 10 mL HCl at 100°C for 1 h and desalted on Bio-Gel P-2. Desialylated glycopeptides were applied to tomato-agarose (0.25 cm × 20 cm) in phosphate-buffered saline containing 1 mL CaCl2, 1 mL MnCl2, and 0.02% azide, pH 7.4 (PBS2−), and the column was washed with ≤8 column volumes of buffer. The bound fraction was eluted with 10-15 mL of PBS2− containing N,N’,N’-triacycetylchitotriose (–10 mg/ml) and purified by ultrafiltration followed by gel filtration on a Bio-Gel P-2 column. Glycopeptides that did not bind to tomato-agarose were applied to a column of RCA-agarose (0.5 cm × 25 cm). After washing with at least 10 column volumes of PBS2−, bound glycopeptides were eluted with 100–200 mL lactose in PBS2−. Alternatively, a linear gradient of 0–100 mL lactose in PBS2− was used. Lectin affinity chromatography was carried out either at room temperature or at 4°C at flow rates of 6–10 mL/h.

Isolation of LEC14 Glycopeptides for Structural Analysis—Cellular glycopeptides were prepared from ~10¹⁰ LEC14 cells as described previously (18), purified on a column of Sephadex G-25 (5 cm × 65 cm), and fractionated on ConA-Sepharose (1.5 cm × 22 cm) into unbound and bound fractions (18). The ConA-unbound fraction was desalted on Sephadex G-25 (5 cm × 65 cm), hydrolyzed with 10 mL HCl at 80°C for 1 h to remove the glycosidically bound sialic acid residues, and desalted on Bio-Gel P-2 (18).

The desialylated glycopeptides were dissolved in 1 mL of PBS2− and applied to tomato-agarose (0.5 cm × 20 cm). The column was washed with PBS2− (–100 mL), and the tomato-bound fraction was eluted with 40 mL of N,N’,N’-triacycetylchitotriose (10 mg/ml) in PBS2− and purified by ultrafiltration followed by repeated Bio-Gel P-2 column chromatography. The pure glycopeptides were used for 1H NMR spectroscopy, monosaccharide analysis, and to isolate core glycopeptides by exoglycosidase digestion.

The tomato-bound fraction was desalted on Bio-Gel P-2 (1.5 cm × 70 cm), lyophilized, dissolved in 1 mL of PBS2−, and chromatographed on a column of RCA-agarose (0.5 cm × 20 cm). The column was eluted with ~200 mL PBS2− followed by 100 mL of 100 mL lactose in PBS2−. Pooled fractions were desalted on Bio-Gel P-2. A portion of each glycopeptide fraction was used for monosaccharide analysis. The remainder was used to record 1H NMR spectroscopy, which was subjected to methylation linkage analysis, or treated with exoglycosidases to isolate core glycopeptides.

Preparation and Characterization of Radiolabeled Core Glycopeptides—The tomato-bound glycopeptides of LEC14 and parent cells (~15000 cpm each) were separately treated with a mixture of β-1,4-galactosidase (bovine testis and/or D. pneumoniae, 25 milliunits) and N-acetyl-β-1,4-glucosaminidase (jack bean or D. pneumoniae) in McIlvaine buffer, pH 4.0–5.0 at 37°C for 24 h. The incubation was continued for another 24 h at 37°C by heating the mixture at 100°C for 5–10 min, and the resulting core glycopeptides (V0, excluded fraction; V1, included fraction) were separated from released monosaccharides on Bio-Gel P-2 (1.5 cm × 70 cm). A portion of the core glycopeptides were fractionated on ConA-Sepharose (0.5 cm × 20 cm) and/or PSA-agarose (0.5 cm × 20 cm) as described earlier. Fractions of 0.5 mL were collected and counted for radioactivity. Another portion of the core glycopeptides was hydrolyzed with 10 mL trifluoroacetic acid, and the hydrolysate was analyzed by HPAEC-PAD for monosaccharides. Core glycopeptides from the RCA-bound glyco-
peptides were similarly prepared and fractionated on ConA-Sepharose and/or PSA-agarose and analyzed for monosaccharide composition.

Structural Analyses of LEC14 Core Glycopeptides—The tomato-bound and RCA I-bound glycopeptides (250–300 mg) from LEC14 cells were treated, separately, with a mixture of b-D-galactosidase (bovine testis) and N-acetyl-b-D-glucosaminidase (jack bean) at 37°C for 96 h followed by another treatment with a mixture of D. pneumoniae b-D-galactosidase and N-acetyl-b-D-glucosaminidase in Mcllvine buffer as described above. The enzyme digestion was stopped by heating the reaction mixture at 100°C for 5–10 min. Core glycopeptide fractions were purified by repeated Bio-Gel P-2 chromatography and ion-exchange chromatography with Chelex resin to remove paramagnetic material. Pure glycopeptides (yield; 80 mg from RCAI-bound and 35 mg from tomato-bound) were analyzed by 1H NMR spectroscopy, and electrospray ionization-mass spectrometry (ESI-MS) and for monosaccharide composition by HPAEC-PAD.

Monosaccharide Analysis and Glycopeptide Mapping by HPAEC-PAD—Monosaccharide analysis of glycopeptides was carried out by HPAEC-PAD as described previously (18). Briefly, the glycopeptides (2–5 µg or 2000–4000 cpm) were hydrolyzed in 2 M trifluoroacetic acid or 4 M hydrochloric acid (100–200 µl) at 100°C for 4–5 h, dried, redissolved in water, and analyzed by HPAEC-PAD (22). Glycopeptide mapping was carried out by HPAEC-PAD using either CarboPac PA1 (4 mm × 250 mm) or CarboPac PA100 (4 mm × 250 mm) columns as described previously (18).

500 MHz 1H NMR Spectroscopy—1H NMR spectroscopy of glycopeptide samples was carried out using a 500 MHz Varian Spectrometer at 23, 30, or 42°C with a sweep width of 4000 Hz, a cycle delay time of 3 s, and 90° pulses. Acetone was used as internal standard, and chemical shifts were expressed in parts/million based on acetone being at 2.225 ppm. The samples were purified and prepared for 1H NMR spectroscopy as described previously (18).

Methylation Linkage Analysis—Glycopeptides (~100 µg) were permethylated according to Hakomori (23). The permethylated product was purified by dialysis, hydrolyzed with 2 M trifluoroacetic acid, reduced with sodium borodeuteride, acetylated with pyridine-acetic anhydride, and analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) as described previously (24). Furthermore, the trifluoroacetic acid hydrolyzate was hydrolyzed with acetic acid/sulfuric acid to achieve complete hydrolysis of glycosidic bonds, reduced with sodium borodeuteride, acetylated with pyridine-acetic anhydride, and analyzed by GLC-MS. GLC-MS was performed with a Hewlett-Packard 5890 series II gas liquid chromatograph, using a DB-5 column (J&W Scientific), coupled to a Hewlett-Packard 5871 MSD mass spectrometer.

Mass Spectrometry—An API-III triple-quadrupole mass spectrometer (PE-SCIEX, Ontario, Canada) was used to perform electrospray ionization-mass spectrometry (ESI-MS) of glycopeptides. The SCIEX IonSpray interface with nitrogen as the nebulizer gas, an ion spray voltage of 3300 and the orifice at 70 V was used. Harvard Apparatus syringe pump was used to infuse the sample into the mass spectrometer at 2 µl/min after diluting 1:1 with 50% acetonitrile/H2O containing 0.1% trifluoroacetic acid as described previously (18).

RESULTS

Unique Glycopeptide Fractions From LEC14 Cells—The resistance of LEC14 cells to PSA and LCA (19) is presumed to reflect the altered expression of cell surface carbohydrates (21). Consistent with this, two independent LEC14 mutants exhibited a 2–3-fold reduction in 125I-PSA binding when compared with parental CHO cells, over a broad range of PSA concentra-
To identify carbohydrates unique to LEC14 cells, metabolically labeled cellular glycopeptides were compared by serial lectin affinity chromatography. No significant differences in profile were observed when [3H]Glc-labeled glycopeptides from parental or LEC14 CHO cells were fractionated on a ConA-Sepharose column (25); about 90% of the glycopeptides passed through ConA-Sepharose, 5% eluted in the biantennary fraction, and 5% eluted in the hybrid and oligomannosyl fraction. Glycopeptides that did not bind to ConA-Sepharose were subjected to further lectin affinity chromatography after removing terminal sialic acid residues by mild acid hydrolysis. On a tomato-agarose column (26), less parental CHO glycopeptides (15%) bound and were eluted with N,N',N-triacetylchitotriose than LEC14 glycopeptides (45%, Fig. 1). A further difference between parental and LEC14 glycopeptides was uncovered when the glycopeptides that did not bind to tomato-agarose were fractionated on RCAI-agarose (26, 27). The fraction from LEC14, which bound to RCAI-agarose eluted at 70 mM lactose, whereas the equivalent fraction from parental cells eluted at 30 mM lactose (Fig. 2), indicating a structural difference between these glycopeptides. Also, 2-3-fold more LEC14 glycopeptides bound to RCAI-agarose compared with parental glycopeptides (Fig. 2). Glycopeptide mapping of toma-

### Table I

| Glycosyl residue | Relative proportiona |
|------------------|----------------------|
| Fucp1→          | 0.9                  |
| GlcNAcp1→       | 1.0                  |
| →4GlcNAcp1→     | 2.8                  |
| →3,4GlcNAcp1→   | 3.2                  |
| →4,6GlcNAcp1→   | 0.8                  |
| Galp1→          | 3.7                  |
| →3Galp1→        | 4.9                  |
| →2,4Manp1→      | 0.8                  |
| →2,6Manp1→      | 0.7                  |
| →3,6Manp1→      | 1.1                  |
| →2,3,6Manp1→    | 1.1                  |
| →2,4,6Manp1→    | 0.4                  |

a Relative proportions of partially methylated alditol acetates of Fuc and Gal were obtained from the total ion chromatogram of GLC-MS of a 2 M trifluoroacetic acid hydrolysate (see "Experimental Procedures"). Raw values were corrected with the relevant response factors (24). Analysis of spectra of this hydrolysate indicated that the permethylated product underwent only partial hydrolysis. Hence, the trifluoroacetic acid hydrolysate was subjected to further hydrolysis with acetic acid/sulfuric acid to release all sugar residues. Relative proportions of partially methylated alditol acetates of GlcNAc and Man were obtained from the total ion chromatogram of GLC-MS of the latter preparation and were also corrected with the relevant response factors (24). The data from both analyses are combined in the table.

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3 M. Ray and P. Stanley, unpublished observations.
to-bound (Fig. 1) and RCA₁-bound glycopeptides (data not shown) on Dionex HPAEC-PAD showed complex profiles, indicating structural differences between the species. Monosaccharide analysis of [³H]Glc-labeled tomato-bound and RCA₁-bound glycopeptides by HPAEC-PAD revealed the presence of only the expected N-linked sugar residues (Man, Gal, GlcNAc, Fuc) in LEC14 glycopeptide fractions.

Analysis of LEC14 Glycopeptides by ¹H NMR Spectroscopy and Methylation Linkage Analysis—In order to characterize glycopeptides unique to LEC14 cells, the tomato-bound (Fig. 1) and RCA₁-bound fractions (Fig. 2) were prepared from ~10¹⁰ LEC14 cells. ¹H NMR spectra of LEC14 tomato-bound glycopeptides (Fig. 3) showed that they contain poly-N-acetyllactosamine chains (28) and a core Fuc residue (16, 17). Chemical shifts at 23 °C of 4.697, 4.469 and 4.149 ppm could be assigned to H-1 of β-GlcNAc, H-1 of β-Gal, and H-4 of Gal, respectively (17, 28). The large resonance at 2.030 ppm from N-acetyl protons is typical of GlcNAc residues in poly-N-acetyllactosamine chains (28). The resonance at 1.198 ppm is due to CH₃ protons of a Fuc residue α(1,6)-linked to the Asn-GlcNAc (16, 17). When spectra were acquired for longer times, resonances due to core Man residues at 5.119 and 4.869 ppm could be assigned to H-1 of α(1,3)-Man and α(1,6)-Man, respectively (Fig. 3, inset). Although spectra were recorded at two temperatures in order to uncover resonances obscured by the HOD signal, a resonance corresponding to H-1 of β(1,4)-Man, which normally occurs at 4.715–4.741 ppm for tri- and tetraantennary carbohydrates (16, 17), was not observed, indicating that this residue was in an atypical core structure.

Additional evidence in support of a structural change affecting the β(1,4)-Man residue was obtained from ¹H NMR spectroscopy of RCA₁-bound glycopeptides (Fig. 4). The RCA₁-bound glycopeptides also contained poly-N-acetyllactosamine chains, as evidenced from the large resonances due to H-1 and H-4 of Gal, and H-1 of GlcNAc residues (Fig. 4), and the resonances due to core Man residues that are more prominent than in Fig. 3. Again there was no evidence for a β(1,4)-Man residue between 4.715 and 4.741 ppm. The complexity of the N-acetyl proton region in Fig. 4 compared with Fig. 3 shows that RCA₁-bound glycopeptides were more branched.

When the chemical shifts of Figs. 3 and 4 were entered into Sugabase, the ¹H NMR data base (17), no structure was given, indicating that both LEC14 glycopeptide preparations contained unique species. To examine sugar linkages in these LEC14 glycopeptides, a portion of the RCA₁-bound fraction was subjected to methylation linkage analysis. From the data in Table I, it is evident that the LEC14 glycopeptides have terminal Gal, GlcNAc, and Fuc residues and substituted Gal, GlcNAc, and Man residues. A key finding from this analysis was the presence of 4-O-methylmannose indicating the existence of a 2,3,6-trisubstituted Man residue in the RCA₁-bound glycopeptides. In some plants and lower animals, N-linked carbohydrates contain a 2,3,6-trisubstituted Man residue due to Xyl...
substitution at O-2 of the \(\beta(1,4)\)-Man core residue (7–15, 17). However, neither the methylation linkage analysis, monosaccharide analysis, nor \(^1\)H NMR spectroscopy indicated the presence of a Xyl residue, or any other novel sugar in the LEC14 glycopeptides. The combined data suggest that either a terminal Gal or GlcNAc residue is linked to the \(\beta(1,4)\)-Man at O-2. Although terminal Fuc was present, the substitution by Fuc at O-2 of \(\beta(1,4)\)-Man seemed unlikely because the proportion of Fuc was appropriate to account for the substitution of the Asn-linked GlcNAc at O-6. Consistent with this interpretation was the presence of an approximately equivalent amount of 4,6-disubstituted GlcNAc residue corresponding to the Fuc-substituted, Asn-linked GlcNAc (Table I). The remaining residues are consistent with the glycopeptides being branched, complex, poly-N-acetyllactosamine-containing N-glycans. However, the 3,4-disubstituted GlcNAc content was significant and unpredicted. It indicates that a substantial proportion of GlcNAcs in poly-N-acetyllactosamine chains are branched, being substituted at both the O-3 and O-4 positions.

### Analysis of Core Glycopeptides

**By Lectin Affinity Chromatography—**To investigate directly whether LEC14 glycopeptides have an altered core structure, \[^1\]H Glc-labeled tomato-bound (Fig. 1) and RCA1-bound (Fig. 2) glycopeptides of both parental and LEC14 cell lines were separately treated exhaustively with a mixture of bovine testis \(\beta\)-D-galactosidase and jackbean \(\text{N}-\text{acetyl}\)-\(\beta\)-D-glucosaminidase. The digestion products were fractionated on Bio-Gel P-2 into two major glycopeptide pools (Table II).

- **Fractionation on ConA-Sepharose** revealed a significant difference between the \(V_o\) glycopeptides of LEC14 and parent cells. Approximately, 65% of the parental species bound to ConA-Sepharose, whereas \(12\%\) of LEC14 \(V_o\) glycopeptides bound to this affinity column (Fig. 6). The \(V_e\) glycopeptides of LEC14 and parent cells also showed a similar difference on ConA-Sepharose (data not shown). When LEC14 \(V_o\) glycopeptides were obtained by exhaustive digestion with a mixture of \(\beta\)-D-galactosidase and \(\text{N}-\text{acetyl}\)-\(\beta\)-D-glucosaminidase from *D. pneumoniae* (29), a large proportion (\(85\%\)) did not bind to ConA-Sepharose. The corresponding \(V_e\) glycopeptides from LEC14 also did not bind to ConA-Sepharose. In addition, a large proportion of \(V_o\) and \(V_e\) glycopeptides isolated from parent CHO cells bound to this column (18). Therefore both the \(V_o\) and \(V_e\) core glycopeptides from LEC14 contain a core structure that prevents binding to ConA-Sepharose and PSA-agarose.

**TABLE III**

**Comparison of \(^1\)H NMR spectral chemical shifts of LEC14 \(V_o\) core glycopeptides with related core glycopeptides**

| Residue | \(29^\circ\)C | \(29^\circ\)C | \(29^\circ\)C | \(42^\circ\)C |
|---------|----------------|----------------|----------------|----------------|
| Gal-GlcNAc | H | 5.064 | 5.070 | 5.072 | 5.067 |
| NAc | 2.914 | 2.009 | 2.010 | 2.009 |
| Core-GlcNAc | H | 4.681 | 4.671 | 4.567 | Obs |
| NAc | 2.090 | 2.095 | 2.088 | 2.088 |
| \(\beta(1,4)\)Man | H | NA | 4.685 | Obs | 4.752 |
| H | 2.522 | 4.173 | 4.212 | 4.205 |
| \(\alpha(1,6)\)Mm | H | 4.916 | 5.013 | 4.876 | 4.873 |
| H | 2.522 | 4.136 | 3.956 | 3.959 |
| \(\alpha(1,3)\)Man | H | 5.117 | 5.057 | 5.107 | 5.118 |
| H | 2.487 | 4.253 | 4.119 | 4.122 |
| \(\alpha(1,6)\)Fuc | H | 4.870 | 4.881 | 4.857 | 4.856 |
| CH3 | 1.196 | 1.204 | 1.199 | 1.210 |
| GlcNAc(1,2) | H | 4.554 | 4.548 | 4.537 | Obs |
| NAc | 2.049 | 2.051 | 2.046 | 2.047 |
| GlcNAc(1,2) | H | 4.554 | 4.548 | 4.537 | Obs |
| NAc | 2.052 | 2.051 | 2.049 | 2.051 |
| GlcNAc(1,4) | H | - | 4.466 | - | - |
| NAc | - | 2.067 | - | - |
| GlcNAc(\text{New}) | H | - | - | 4.689 | 4.680 |
| NAc | - | - | 2.080 | 2.077 |

**FIG. 7.** \(^1\)H NMR spectroscopy of LEC14 \(V_o\) core glycopeptides. LEC14 \(V_o\) core glycopeptides (\(\approx 80 \mu\)g in 200 \(\mu\)l D2O) were subjected to \(^1\)H NMR spectroscopy at 500 MHz at 23 and 42 °C. Chemical shifts (ppm) were assigned based on the acetone signal at 2.225 ppm.
approximately two GlcNAc equivalents, as predicted if glycosidase digestion was complete. By contrast, LEC14 \( V _ o \) core glycopeptides contained three GlcNAc equivalents, suggesting the presence of an additional GlcNAc residue that had resisted N-acetyl-\( \beta \)-D-hexosaminidase digestion. The extra GlcNAc residue in the \( V _ o \) fraction of LEC14 cells was postulated to be the reason that most LEC14 core glycopeptides did not bind to ConA-Sepharose or PSA-agarose (Fig. 6).

Structural Analysis of LEC14 Core Glycopeptides—To investigate the structure of LEC14 N-glycan cores, the unlabeled glycopeptides shown in Figs. 3 and 4 were separately treated exhaustively with a mixture of \( \beta \)-D-galactosidases and N-acetyl-\( \beta \)-D-glucosaminidases from \( D. \) pneumoniae, bovine testis, and jack bean. The \( V _ o \) and \( V _ c \) fractions obtained by Bio-Gel P-2 chromatography (Fig. 5) were subjected to \( ^{1} \)H NMR spectroscopy at 500 MHz (Fig. 7). Spectra were recorded at 23 and 42 °C to reveal resonances obscured by the HOD peak. The \( ^{1} \)H NMR spectra shown in Fig. 7 contained resonances due to five-NAc groups (Table III), showing that the \( V _ o \) glycopeptides contained five GlcNAc residues. The resonance at 1.199 ppm due to -CH\( _3 \) of Fuc (16, 17) showed that the \( V _ o \) cores are fully fucosylated. Table III compares the chemical shifts of LEC14 \( V _ o \) glycopeptides and two closely related structures; a GlcNAc terminating, fucosylated biantennary glycopeptide (core A) and the same glycopeptide carrying a \( \beta (1,4) \)-linked bisecting GlcNAc residue (core B). It is apparent that the chemical shifts for the majority of reporter groups are different in each glycopeptide. Assignments of chemical shifts to individual protonshave been made for the LEC14 \( V _ o \) glycopeptides on the basis of the similarity of all chemical shifts to resonances in core A or core B, except for the novel resonances that are tentatively assigned to the new GlcNAc residue. The effect of this \( \beta \)-GlcNAc (which on the basis of methylation linkage analysis (Table I) must be linked at O-2 to the \( \beta (1,4) \)-Man residue) on the chemical shifts of adjacent residues is profound, in a manner analogous to, but different from, the effects of the bisecting GlcNAc (16, 30). Compared with core A, the only reporter groups unchanged are the -NAc resonances and the -CH\( _3 \) resonance of Fuc. All other reporter groups in LEC14 \( V _ o \) glycopeptides are close to a related reporter group in the two previously assigned cores, and coupling constants, as well as intensities, are consistent with the assignments given in Table III. The chemical shifts obtained at 42 °C differed by \( <0.006 \) ppm for each reporter group.

The composition of the \( V _ o \) glycopeptides was confirmed by ESI-MS (Fig. 8). This spectrum was obtained at 70 V, under conditions that cause fragmentation of core glycopeptides (18, 31). The ion at atomic mass unit 1780.2 corresponds to a molecular ion (MH\( ^+ \)) containing five HexNAc, three Hex, one deoxyHex, and one Asn residue. The monosodiated molecular ion (MNa\( ^+ \)) at atomic mass unit 1803.0 confirms this composition. The major \( V _ o \) glycopeptide underwent fragmentation from both the nonreducing end and from the Asn end, to give fragment ions that are interpretable in structural terms (Scheme 1). The ions at atomic mass unit 904.8 and 891.2 provide strong evidence that the new GlcNAc residue is attached to the \( \beta (1,4) \)-Man residue. The methylation linkage analysis (Table I) and \( ^{1} \)H NMR spectroscopy (Fig. 7; Table III), suggested that the new GlcNAc is in \( \beta (1,2) \)-linkage to the \( \beta (1,4) \)-Man residue.

Although only small amounts of \( V _ o \) glycopeptides were available, it was possible to obtain chemical shift information from \( ^{1} \)H NMR spectra at 23 and 42 °C (Table IV) and to perform ESI-MS on this fraction (Fig. 9). \( ^{1} \)H NMR chemical shifts could be assigned by comparison with related compounds containing Xyl-linked O-2 to the \( \beta (1,4) \)-Man or GlcNAc-linked O-6 to the core GlcNAc residue (Table IV). The spectrum at 23 °C contains three resonances due to -NAc groups (Table IV), indicating the presence of three GlcNAc residues that were assigned to the Asn-GlcNAc, core GlcNAc, and the new GlcNAc attached to \( \beta (1,4) \)-Man at O-2. The resonance at 5.105 ppm was assigned to H-1 of the \( \alpha (1,3) \)-linked core Man based on a comparison with core C (Table IV). The resonance at 4.866 ppm was assigned to H-1 of the \( \alpha (1,6) \)-linked core Man, because a similar type of shift for this residue is induced in Xyl-containing oligosaccharide (9). A resonance at 4.761 ppm was observed in the spectrum at 42 °C, which is assigned to the H-1 of the \( \beta (1,4) \)-Man residue, based on its coupling constant of 2 Hz and its relative intensity. The H-2 resonances of the core Man residues are significantly
changed compared to Core C resonances (Table IV), indicating the novelty of the core region of LEC14 Ve glycopeptides. The H-1 resonance of the Asn-linked GlcNAc in Ve glycopeptides is very similar to that of Core C. However, the H-1 resonance for the core GlcNAc residue was significantly different in LEC14 Ve glycopeptides (Table IV). The resonance at 4.680 ppm was assigned to the H-1 of the new GlcNAc attached to \( \beta(1,4) \)-Man at O-2. The coupling constant of this resonance (6.8 Hz) suggested that the new GlcNAc is in \( \beta \)-configuration.

ESI-MS analysis of the Ve glycopeptides confirmed the composition determined by \( ^1H \) NMR spectroscopy. The MH\(^+ \) ion at 1374.4 and MNa\(^+ \) ion at 1396.4 represented a species of HexNAc\(_3\)Hex\(_3\)deoxyHex\(_3\)Asn or GlcNAc\(_3\)Man\(_3\)(Fuc)Asn (Fig. 9). The molecule underwent fragmentation by three pathways as shown in Scheme 2. The ion at 691.6 atomic mass units provides critical evidence for substitution of the \( \beta(1,4) \)-Man with a GlcNAc residue. The combined data obtained on RCAI-bound, tomato-bound and on core LEC14 glycopeptides by \( ^1H \) NMR spectroscopy, methylation linkage analysis and ESI-MS show that LEC14 cells synthesize a novel N-glycan core with the structure shown in Scheme 3.

**DISCUSSION**

The structural studies described in this paper have identified a novel core of complex N-glycans (Scheme 3). These glycans from the LEC14 CHO glycosylation mutant behave as a unique species on lectin affinity chromatography (Figs. 1 and 2). Methylation linkage analysis (Table I) revealed the presence of a 2,3,6-trisubstituted Man residue and a residue of terminal GlcNAc, suggesting that a GlcNAc residue may substitute the \( \beta(1,4) \)-Man core residue. This prediction was confirmed by \( ^1H \) NMR spectroscopy (Fig. 7, Tables III and IV) and ESI-MS of core glycopeptides (Figs. 8 and 9) obtained by exoglycosidase digestions.

The GlcNAc residue linked at O-2 to the \( \beta(1,4) \)-Man residue is in an analogous position to the \( \beta(1,2) \)-Xyl found in various plant and animal glycoproteins (7–19). The presence of this GlcNAc in LEC14 glycopeptides has profound effects on the properties of the N-glycans to which it is attached. It causes branched structures with poly lactosamine chains to interact more strongly with tomato and RCA\(_I\)-bound, tomato-bound and on core LEC14 glycopeptides by \( ^1H \) NMR spectroscopy, methylation linkage analysis and ESI-MS show that LEC14 cells synthesize a novel N-glycan core with the structure shown in Scheme 3.

**TABLE IV**

Comparison of \( ^1H \) NMR spectral chemical shifts of LEC14 Ve core glycopeptides with related core glycopeptides

| Assignment | LEC14 Ve | LEC18 Ve | Core C | Core D |
|------------|----------|----------|--------|--------|
| Asn-GlcNAc | H\(^1\) | 5.073 | 5.189(\(\alpha\)) | 4.703(\(\beta\)) | 5.5085 | 5.507 | 5.067 |
| NAc        | 2.012 | 2.039 | 2.018 | 2.013 | 2.016 |
| Core GlcNAc| H\(^1\) | 4.592 | 4.615(\(\alpha\)) | 4.607(\(\beta\)) | 4.565 | 4.567 | Obs |
| NAc        | 2.091 | 2.074 | 2.079 | 2.080 | 2.089 |
| \(\alpha(1,6)\)Fuc | H\(^1\) | 4.87 | - | 4.903 | 4.857 | 4.856 |
| CH\(_3\)   | 1.210 | - | 1.212 | 1.199 | 1.200 |
| \(\beta(1,4)\)Man | H\(^1\) | 4.7 | 4.875 | 4.784 | Obs | 4.761 |
| H\(^2\)   | 4.256 | 4.265 | NA | 4.215 | 4.209 |
| \(\alpha(1,6)\)Man | H\(^1\) | 4.915 | 4.912 | 4.914 | 4.866 | 4.865 |
| H\(^2\)   | 3.969 | 3.982 | NA | 3.955 | 3.965 |
| \(\alpha(1,3)\)Man | H\(^1\) | 5.100 | 5.122 | 5.106 | 5.105 | 5.113 |
| H\(^2\)   | 4.069 | 4.041 | NA | 4.113 | 4.119 |
| \(\beta(1,2)\)Xyl | H\(^1\) | - | 4.464 | - | - |
| H\(^2\)   | - | 3.372 | - | - |
| GlcNAc (new) | H\(^1\) | - | - | 5.211 | 4.680 | 4.680 |
| NAc        | - | - | 2.055 | 2.044 | 2.041 |
glycopeptides. This meant that few partial digestion products were higher in LEC14 compared with parental CHO cells (Fig. 5). Interestingly, the new GlcNAc residue, which carries the bisected GlcNAc on cell surface N-glycans, is 10–20-fold more resistant to ricin and ~15-fold more sensitive to P. vulgaris erythroidagglutinin (E-PHA) compared with parental CHO cells, which have N-glycans lacking a bisecting GlcNAc (33). The presence of a bisecting GlcNAc affects the conformation of distal Gal residues, making them less accessible for recognition by ricin (34). By contrast, LEC14 cells are similar to parental CHO cells in their sensitivity to ricin and E-PHA, while LEC18 cells are slightly sensitive to ricin (19). However, the latter mutants are altered with respect to other lectins that recognize lactosamine units. LEC14 cells have glycopeptides that bind more to tomato and RCA, lectins, while LEC18 cells bind more D. stramonium lectin than parental CHO cells (18). The cores of LEC14 and LEC18 N-glycans lead to cellular resistance to PSA and LCA (19), whereas a bisecting GlcNAc in N-glycans does not confer resistance to these lectins (35). Therefore, each core generates a distinct lectin resistance phenotype that reflects differences in the corresponding cell surface carbohydrates.

The lectin resistance properties of the CHO glycosylation mutants signify either decreased binding (resistance) or increased binding (hypersensitivity) of toxic plant lectins to cell surface carbohydrates (36). The profound changes in lectin recognition that are characteristic of, and unique to, each CHO dominant mutant are generated by the de novo expression of a single glycosyltransferase in each case: LEC10 CHO mutants express N-acetylglucosaminyltransferase III (GlcNAc-TIII; Ref. 33); LEC11, LEC12, LEC29, and LEC30 CHO cells express distinct α(1,3)-fucosyltransferase activities (37–39); LEC14 and LEC18 CHO mutants also each possess a GlcNAc-α-fucosyltransferase activity that is absent from parental cells. GlcNAc-TIII and the α(1,3)-fucosyltransferases are coded for by developmentally regulated genes (40), and it is predicted that this will also be true for the new transferases of LEC14 and LEC18 cells. Considering the consequences of expressing one of these glycosyltransferases on the array of N-glycan structures present at the cell surface and their regulated expression in different tissues, it seems very likely that animal lectins exist that recognize the specific glycan changes resulting from the expression of each transferase. This paradigm may in fact operate in the case of the selectins that recognize sialyl-Lewis X determinants, as the latter may be generated by the regulated expression of an α(1,3)-fucosyltransferase (41). Analogous carbohydrate binding proteins that recognize lactosamine units in the context of different N-glycan core structures, or that recognize the various cores themselves (42), are predicted to exist and to mediate specific cell-cell adhesion events important in morphogenesis.

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REFERENCES

1. Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
2. Staudacher, E., Alltmann, F., Glossi, J., Marz, L., Schacht, H., Kamering, J. P., Hard, K., and Vliegenthart, J. F. G. (1991) Eur. J. Biochem. 199, 745–751
3. Staudacher, E., Altman, F., Marz, L., Hard, K., Kamering, J. P., and Vliegenthart, J. F. G. (1992) Glycoconjugat. J. 9, 82–85
4. Tretter, V., Altman, F., and Marz, L. (1992) Eur. J. Biochem. 199, 647–652

adjacent residues in a specific fashion (Tables III and IV). It affects the fragmentation pathways of ions generated during ESI-MS at 70 V (Schemes I and II, and since completely different fragmentation patterns were obtained with LEC18 core glycopeptides that have a GlcNAc-GlcNAc linkage in the core (18), and it appears to increase the relative resistance of β(1,2)-linked arm GlcNAc residues to cleavage by β-hexosaminidases, because after exoglycosidase digestion, the proportion of V0 partial digestion products was higher in LEC14 compared with parental glycopeptides (Fig. 5). This meant that few V0 glycopeptides were generated from LEC14 compared with parental glycopeptides (Fig. 5). Interestingly, the new GlcNAc residue, although terminal, appeared completely resistant to removal by our conditions of β-hexosaminidase treatment. The same resistance was observed with glycopeptides from LEC18 cells that contain a GlcNAc-linked O-6 to the core GlcNAc residue (18). Thus, the new GlcNAc linkages are quite resistant to the action of the tested β-hexosaminidases. Interestingly, the bisecting GlcNAc (β(1,4)-linked GlcNAc to β(1,4)-Man) is reported to be sensitive to these enzymes (32).

The two structures synthesized by mammalian cells that are most related to the new core are shown in Table III. Each core gives a unique 1H NMR spectrum that reflects profound conformational differences between the three structures (Table III). These chemical differences translate into biological phenomena at the surface of CHO cells. Thus LEC10 CHO cells, which carry the bisected GlcNAc on cell surface N-glycans, are 10–20-fold more resistant to ricin and ~15-fold more sensitive to P. vulgaris erythroidagglutinin (E-PHA) compared with parental CHO cells, which have N-glycans lacking a bisecting GlcNAc (33). The presence of a bisecting GlcNAc affects the conformation of distal Gal residues, making them less accessible for recognition by ricin (34). By contrast, LEC14 cells are similar to parental CHO cells in their sensitivity to ricin and E-PHA, while LEC18 cells are slightly sensitive to ricin (19). However, the latter mutants are altered with respect to other lectins that recognize lactosamine units. LEC14 cells have glycopeptides that bind more to tomato and RCA, lectins, while LEC18 cells bind more D. stramonium lectin than parental CHO cells (18). The cores of LEC14 and LEC18 N-glycans lead to cellular resistance to PSA and LCA (19), whereas a bisecting GlcNAc in N-glycans does not confer resistance to these lectins (35). Therefore, each core generates a distinct lectin resistance phenotype that reflects differences in the corresponding cell surface carbohydrates.

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REFERENCES

1. Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
2. Staudacher, E., Altman, F., Glossi, J., Marz, L., Schacht, H., Kamering, J. P., Hard, K., and Vliegenthart, J. F. G. (1991) Eur. J. Biochem. 199, 745–751
3. Staudacher, E., Altman, F., Marz, L., Hard, K., Kamering, J. P., and Vliegenthart, J. F. G. (1992) Glycoconjugat. J. 9, 82–85
4. Tretter, V., Altman, F., and Marz, L. (1992) Eur. J. Biochem. 199, 647–652
5. Kubelka, V., Altmann, F., Staudacher, E., Tretter, V., Marz, L., Hard, K., Kamerling, J. P., and Vliegenthart, J. F. G. (1993) Eur. J. Biochem. 213, 1193–1204
6. Haslam, S. M., Reason, A. J., Morris, H. R., and Dell, A. (1994) Glycobiology 4, 105–111
7. Ashford, D., Dwek, R. A., Welph, J. K., Amatayakul, S., Homans, S. W., Lis, H., Taylor, G. N., Sharon, N., and Rademacher, T. W. (1987) Eur. J. Biochem. 166, 311–320
8. Fournet, B., Leroy, Y., Wieruszeski, J. M., Montreuil, J., Poretz, R. D., and Goldberg, R. (1987) Eur. J. Biochem. 166, 321–324
9. D’Andrea, G., Bouwstra, J. B., Kamerling, J. P., and Vliegenthart, J. F. G. (1988) Glycoconj. J. 5, 151–157
10. Kamerling, J. P. (1991) Pure Appl. Chem. 63, 465–472
11. Bouwstra, J. B., Spoelstra, E. C., De Waard, P., Leeflang, B. R., Kamerling, J. P., and Vliegenthart, J. F. G. (1990) Eur. J. Biochem. 190, 113–122
12. van Kuik, J. A., van Halbeek, H., Kamerling, J. P., and Vliegenthart, J. F. G. (1985) J. Biol. Chem. 260, 13984–13988
13. Lammerse, J. P. M. (1994) Structural and Conformational Analysis of Xylose-containing Glycoprotein N-glycans, Ph.D. Dissertation, Utrecht University
14. van Kuik, J. A., Sijbesma, R. P., Kamerling, J. P., Vliegenthart, J. F. G., and Wood, E. S. (1986) Eur. J. Biochem. 160, 621–625
15. van Kuik, J. A., Sijbesma, R. P., Kamerling, J. P., Vliegenthart, J. F. G., and Wood, E. S. (1987) Eur. J. Biochem. 169, 399–411
16. Vliegenthart, J. F. G., Dorland, L., and van Halbeek, H. (1983) Adv. Carbohydr. Chem. Biochem. 41, 209–374
17. van Kuik, A., and Vliegenthart, J. F. G. (1993) Trends Food Sci. Technol. 4, 73–76
18. Raju, T. S., Ray, M. K., and Stanley, P. (1995) J. Biol. Chem. 270, 30294–30302
19. Rijka, J., and Stanley, P. (1986) Som. Cell Mol. Genet. 12, 51–62
20. Kornfeld, K., Reitman, M. L., and Kornfeld, R. (1981) J. Biol. Chem. 256, 6633–6640
21. Stanley, P. (1984) Ann. Rev. Genet. 18, 525–552
22. Hardy, M. R., and Townsend, R. R. (1994) Methods Enzymol. 230, 208–225
23. Hakomori, S.-I. (1964) J. Biochem. (Tokyo) 55, 205–209
24. York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., and Albersheim, P. (1984) Methods Enzymol. 118, 3–40
25. Baenziger, J. U., and Fiete, D. (1979) J. Biol. Chem. 254, 2400–2407
26. Merkle, R. K., and Cummings, R. D. (1987) J. Biol. Chem. 262, 8179–8189
27. Green, E. D., Brodbelt, R. M., and Baenziger, J. U. (1987) J. Biol. Chem. 262, 12030–12039
28. Strecker, G., Wieruszeski, J. M., Michalski, J. C., and Montreuil, J. (1989) Glycoconj. J. 6, 67–83
29. Kobata, A. (1979) Anal. Biochem. 100, 1–14
30. Grey, A. A., Narasimhan, S., Brisson, J. R., Schacter, H., and Carver, J. P. (1982) Can. J. Biochem. 60, 1123–1131
31. Reinhold, V. N., Reinhold, B. B., and Costello, C. E. (1995) Anal. Chem. 67, 1772–1784
32. Edge, C. J., Rademacher, T. W., Wormald, M. R., Parekh, R. B., Butters, T. D., Wing, D. R., and Dwek, R. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6338–6342
33. Campbell, C., and Stanley, P. (1984) J. Biol. Chem. 259, 13370–13378
34. Stanley, P., Sundaram, S., and Sallustio, S. (1991) Glycobiology 1, 307–314
35. Stanley, P. (1983) Methods Enzymol. 96, 157–184
36. Stanley, P., and Carver, J. P. (1977) Adv. Exp. Med. Biol. 84, 265–282
37. Campbell, C., and Stanley, P. (1984) J. Biol. Chem. 259, 11208–11214
38. Howard, D. R., Fukuda, M., Fukuda, M. N., and Stanley, P. (1985) J. Biol. Chem. 262, 16830–16837
39. Potvin, B., and Stanley, P. (1991) Cell Regul. 2, 989–1000
40. Kleene, R., and Berger, E. G. (1993) Biochim. Biophys. Acta 1154, 283–325
41. Lasky, L. A. (1995) Annu. Rev. Biochem. 64, 113–139
42. Scheiffele, P., Peranen, J., and Simons, K. (1995) Nature 378, 96–98
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