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The carbohydrate chains of the β subunit of human chorionic gonadotropin produced by the choriocarcinoma cell line BeWo

Novel O-linked and novel bisecting-GlcNAc-containing N-linked carbohydrates

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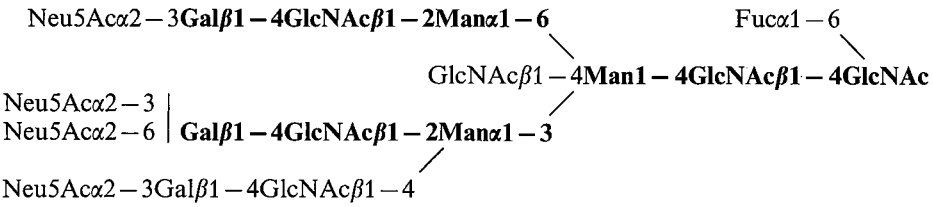
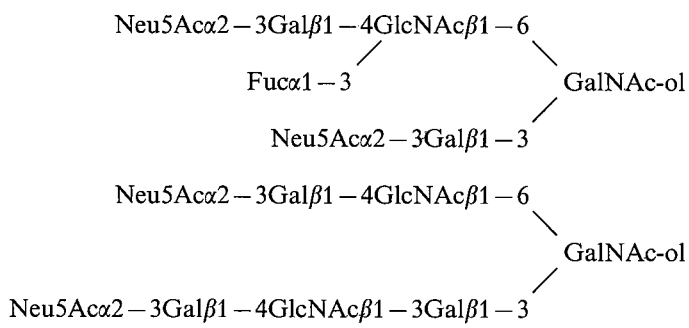
The N-linked carbohydrate chains of the β subunit of human chorionic gonadotropin (hCG-β) isolated from the culture fluid of the choriocarcinoma cell line BeWo were released enzymatically by peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase F. Subsequently, the O-linked oligosaccharides were split off from the *N*-deglycosylated protein by mild alkaline borohydride treatment. The carbohydrate chains were purified in their intact sialylated forms by FPLC anion-exchange chromatography on Mono Q, HPLC on Lichrosorb-NH_2, and high-pH anion-exchange chromatography on CarboPac PA1.

^{1}H-NMR spectroscopic analysis of the major fractions demonstrates the occurrence of the following sialylated diantennary and triantennary N-linked oligosaccharides. Residues not written in bold letters are variably present.



The incidence of triantennary carbohydrate chains is much higher than in normal urinary hCG-β (26% vs 2%). The same holds for the α1–6-fucosylation of the asparagine-bound GlcNAc (95% vs 42%). The presence of a bisecting GlcNAc and the occurrence of α2–6-linked Neu5Ac in the most abundant *N*-glycans, are new features for hCG-β.

The major O-linked carbohydrate chains identified are the tetrasaccharide Neu5Acα2–3Galβ1–3(Neu5Acα2–6)GalNAc-ol and the hexasaccharide Neu5Acα2–3Galβ1–4GlcNAcβ1–6(Neu5Acα2–3Galβ1–3)GalNAc-ol, both also found in normal urinary hCG. In addition, two novel *O*-glycans were characterized:



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Abbreviations. BeWo, the choriocarcinoma cell line BeWo; Fuc, L-fucose; GalNAc, *N*-acetyl-D-galactosamine; GalNAc-ol, *N*-acetyl-D-galactosaminitol; hCG, human chorionic gonadotropin; hCG-β, the β subunit of hCG; hCG-α, the α subunit of hCG; HOHAHA, homonuclear Hartmann-Hahn; HPAEC, high-pH anion-exchange chromatography; Le^x, Lewis x; MLEV, composite pulse devised by M. Levitt; Neu5Ac, *N*-acetylneuraminic acid; PAD, pulsed amperometric detection; PNGase-F, peptide-N^4-(*N*-acetyl-β-glucosaminyl)-asparagine amidase F; 2D, two-dimensional.

Enzyme. Peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase F (EC 3.5.1.52).

The glycoprotein hormone human chorionic gonadotropin (hCG) consists of two dissimilar noncovalently linked subunits denoted α and β (Ryan et al., 1988). hCG is produced by the trophoblasts of the normal placenta and of choriocarcinoma tissue (Nishimura et al., 1981; Amr et al., 1984; Cole et al., 1984) and by non-trophoblastic neoplasia (Cox, 1981; Cole et al., 1982). Both subunits of the hormone contain two *N*-glycosylated asparagine residues. In addition, the β subunit contains four *O*-glycosylated serine residues, all located in the C-terminal region (Kobata, 1988). The primary structures of the N- and O-linked carbohydrate chains of normal urinary hCG (Bahl et al., 1978; Kessler et al., 1979a, b; Endo et al., 1979; Mizuochi and Kobata, 1980; Cole et al., 1985; Damm et al., 1987, 1988) and their importance for the biological functioning of the hormone (Morell et al., 1971; Tsuruhara et al., 1972; Moyle et al., 1975; Channing et al., 1978; Kalyan et al., 1982; Shimohigashi and Chen, 1982; Keutmann et al., 1983; Calvo and Ryan, 1985; Thotakura et al., 1990; Matzuk et al., 1990) have been well established. Many studies concerning the *N*-glycans of hCG from patients with trophoblastic diseases have revealed that, when compared with normal hCG, changes occur in the carbohydrate structures, e.g. increase or decrease in sialylation, increase in fucosylation and increase in branching (Mizuochi et al., 1983, 1985; Endo et al., 1987, 1988a; Imamura et al., 1987; Amano et al., 1988; Kobata, 1988). These changes in glycosylation pattern offered an opportunity to develop a screening method which discriminates choriocarcinoma from non-malignant trophoblastic diseases (Endo et al., 1988b). Compared with normal hCG, a change in the molar ratio between different O-linked oligosaccharides has been observed in hCG from choriocarcinoma urine (Cole, 1987; Amano et al., 1988).

The BeWo cell line is a throphoblastic cell line derived from human choriocarcinoma tissue synthesizing chorionic gonadotropin (Pattillo et al., 1968; Pattillo and Gey, 1968). This cell line is a widely used *in vitro* human cell model for the study of differentiation and hormone synthesis. In view of the possibility to use the cell line for the production of hCG, attention has to be paid to its glycosylation pattern. Here, the structures of the N- and O-linked carbohydrate chains of the hormone-specific β subunit of BeWo hCG are reported and discussed in relation to the glycans derived from hCG-β isolated from the urine of healthy women and from patients suffering from trophoblastic diseases.

EXPERIMENTAL PROCEDURES

Materials

Peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase F (PNGase-F) from *Flavobacterium meningosepticum* was obtained from Boehringer Mannheim. The BeWo cell line originates from American Type Culture Collection Certified Cell Line 98. The tissue from which the cell line was originally established was a specimen which had previously been serially transplanted in the hamster check pouch (Pattillo and Gey, 1968). BeWo hCG was supplied by Diosynth b.v. (Oss, The Netherlands). Bio-Gel P-2, P-4 and P-100 (200–400 μm mesh) were purchased from Bio-Rad. Sephacryl S-200 SF was from Pharmacia and Extracti Gel D was from Pierce.

Preparation of BeWo hCG-β

Mannitol, which had been added as a stabilizer to the BeWo hCG sample, was removed by gel-permeation chromatography on a column (2.5 cm × 37 cm) of Bio-Gel P-4 using as eluent 25 mM NH_4HCO_3, adjusted to pH 7 with HCl, at a flow rate of 26 ml/h, and applying detection at 206 nm. The purity of the glycoprotein material was checked by SDS/PAGE (Laemmli, 1970) on a 12.5% slab gel. BeWo hCG gave rise to two bands with apparent molecular masses of 37 kDa (Fig. 1; β, BeWo hCG-β) and 22 kDa (Fig. 1; α, BeWo hCG-α). Compared to normal urinary hCG-β, the BeWo-derived hCG-β possessed a higher apparent molecular mass ($\Delta m \approx 4$ kDa; Damm et al., 1988). Subunits were prepared from 30 mg BeWo hCG by dissolving the sample in 0.5 ml 8 M urea, adjusted to pH 6.0 with 10 mM Na_2HPO_4/NaH_2PO_4 buffer, containing 0.9% (by mass) NaCl. After incubation for 1 h at 37 °C, the subunits were separated by gel-permeation chromatography on a column (1.0 cm × 121 cm) of Sephacryl S-200 SF using the same urea buffer as eluent, but adjusted to pH 7.4, at a flow rate of 10 ml/h, and using detection at 280 nm. The fraction containing BeWo hCG-β was exhaustively dialyzed against deionized water and lyophilized. The same procedure was carried out for the α subunit, but the amount of material turned out to be too low for structural determination of the oligosaccharide chains of this subunit.

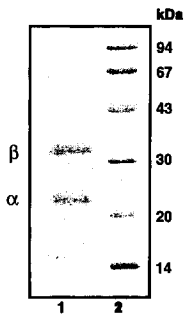


Fig. 1. SDS/PAGE of BeWO hCG on a 12.5% slab gel. The gel was stained with Coomassie brilliant blue. Lane 1, BeWo hCG; lane 2, molecular mass markers.

Liberation of the N-linked carbohydrate chains

The N-linked carbohydrate chains were released from BeWo hCG-β essentially as described by Hård et al. (1990). Briefly, BeWo hCG-β (16 mg) was dissolved in 2 ml 50 mM Tris, containing 50 mM EDTA, and adjusted to pH 8.4 with HCl. Subsequently, 40 μl 25% (mass/vol.) SDS and 10 μl 2-mercaptoethanol were added and the mixture was kept for 1 h at 40 °C. 20 μl of the non-ionic detergent Nonidet P-40 was then added, and, after thorough mixing, 4 U PNGase-F were introduced. The incubation was carried out at room temperature in an end-over-end mixer. After 4 h, a fresh aliquot of 4 U PNGase-F was added and the incubation prolonged for 16 h.

Treatment of BeWo hCG-β with PNGase-F resulted in essentially complete *N*-deglycosylation as judged from SDS/PAGE. Liberated *N*-glycans were separated from the *N*-deglycosylated protein by gel-permeation chromatography on a column (1.9 cm × 50 cm) of Bio-Gel P-100 at a flow rate of 19 ml/h, using as eluent 50 mM NH_4HCO_3, adjusted to pH 7 with HCl. This separation gave rise to five peaks, denoted V_o, A, B, C and V_t (monitoring at 206 nm; chromatogram not shown), containing *N*-deglycosylated BeWo hCG-β (V_o), released N-linked carbohydrate chains (A, B and C) and salts (V_t). Residual detergents were removed from fractions A–C on a column (1 cm × 5 cm) of Extracti-Gel D, eluted with

50 mM NH_4HCO_3. The effluents were concentrated by lyophilization and desalted on a column (1 cm × 18 cm) of Bio-Gel P-2.

Liberation of the O-linked carbohydrate chains

To release the O-linked carbohydrate chains the *N*-deglycosylated BeWo hCG-β sample was dissolved in 1 ml 0.1 M NaOH, containing 1 M $NaBH_4$, and incubated for 24 h at 37°C (Zinn et al., 1977). Then, the solution was neutralized with 4 M acetic acid and desalted on a column of Bio-Gel P-2. Residual detergents were removed by passing the sample in water through a column (1 cm × 5 cm) of Extracti-Gel D.

FPLC fractionation of the N- and O-linked carbohydrate chains

The Bio-Gel P-100 carbohydrate-positive fractions containing enzymatically released N-linked oligosaccharide and chemically released *O*-glycans, were fractionated on a Mono Q HR 5/5 anion-exchange column (Pharmacia FPLC system), essentially as described (Damm et al., 1987). Identically charged FPLC subfractions from the three Bio-Gel P-100 fractions were pooled, desalted on Bio-Gel P-2 and lyophilized. Small aliquots of each pooled subfraction were checked for homogeneity on Mono Q.

HPLC subfractionation of the N- and O-linked carbohydrate chains

The carbohydrate-containing FPLC subfractions were further fractionated on a Kratos Spectroflow 400 HPLC system (ABI Analytical, Kratos Division) using a Lichrosorb-NH_2 10-µm column (0.46 cm × 25 cm, Chrompack). Samples were dissolved in a mixture of 30 mM K_2HPO_4/KH_2PO_4, pH 7.0, and acetonitrile (38:62 or 36:64, by vol.; see Results). Elutions were carried out isocratically with the same solvent mixtures at a flow rate of 2 ml/min at 25°C. Runs were monitored at 205 nm.

High-pH anion-exchange chromatography (HPAEC)

Further fractionation of some of the HPLC fractions was carried out by HPAEC, using pulsed amperometric detection (PAD; Lee, 1990; Damm et al., 1989) on a Dionex LC system consisting of a Dionex Bio LC quaternary gradient module and a PAD 2 detector, using a CarboPac PA1 pellicular anion-exchange column (0.4 cm × 25 cm, Dionex). Eluent A contained 0.1 M NaOH and eluent B contained 0.1 M NaOH and 1 M sodium acetate. Elutions were carried out at a flow rate of 1 ml/min starting with 94% (by vol.) eluent A/6% (by vol.) eluent B for 0.4 min, and going to 87% (by vol.) eluent A/13% (by vol.) eluent B in 30.4 min, followed by a steeper gradient to 75% (by vol.) eluent A/25% (by vol.) eluent B in 35 min. Detection was performed with a gold electrode and triple-pulse amperometry comprising the following pulse potentials and durations: E_1 = 0.05 V, 300 ms; E_2 = 0.65 V, 60 ms; E_3 = −0.95 V, 180 ms. Fractions were immediately neutralized by addition of 1 M HCl and lyophilized. Salts were removed by gel-permeation chromatography on Bio-Gel P-2.

^{1}H-NMR spectroscopy

Prior to ^{1}H-NMR spectroscopic analysis, the desalted samples were repeatedly treated with ^2H_2O, finally using ^2H_2O containing 99.96 atoms ^{2}H/100 atoms H (MSD Isotopes). Resolution-enhanced ^{1}H-NMR spectra were recorded on a Bruker AM-500 spectrometer (Bijvoet Center, Department of NMR Spectroscopy, Utrecht University) or on a Bruker AM-600 spectrometer (SON hf-NMR facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands) at probe temperatures of 27°C. Chemical shifts are expressed by reference to internal acetone (δ = 2.225 ppm in ^2H_2O at 27°C; Vliegenthart et al., 1983). For two-dimensional (2D) homonuclear Hartmann-Hahn (HOHAHA) measurements (Bax and Davis, 1985) a MLEV-17 mixing sequence of 40 ms was used; the 90° pulse width was 23.3 µs and the spectral width was 4000 Hz in both dimensions. The HOD signal was presaturated during 1 s. In total, 400 spectra of 2048 data points were recorded, with 80 scans/t_1 value. The 2D NMR data were processed on a VAXstation 3100 using the TRITON software package (Bijvoet Center, Department of NMR Spectroscopy, Utrecht University). The time-domain data were multiplied with a phase-shifted sine-bell. After Fourier transformation, the resulting data set of 1024 × 1024 points was baseline-corrected in both frequency domains by a fourth-order polynomal fit.

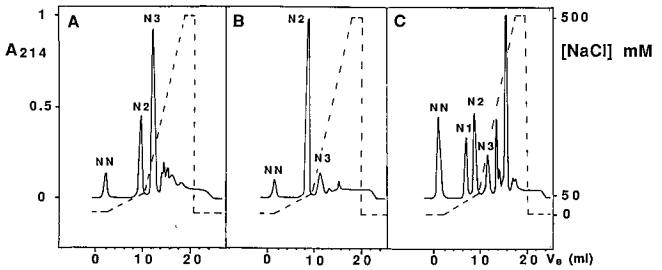


Fig. 2. Fractionation patterns at 214 nm of the carbohydrate-containing Bio-Gel P-100 fractions A (A), B (B) and C (C) derived from BeWo hCG-β on an FPLC HR 5/5 Mono Q column. The column was first eluted with 2 ml H_2O, followed by a linear concentration gradient (----) of 0–50 mM NaCl in 8 ml H_2O, and finally by a steeper gradient of 50–500 mM NaCl in 8 ml H_2O at a flow rate of 2 ml/min. The injection volume was 500 µl. Fractions were collected as indicated.

RESULTS

N-linked carbohydrate chains

Medium-pressure anion-exchange chromatography on Mono Q of Bio-Gel P-100 fraction A of PNGase-F digested BeWo hCG-β yields three carbohydrate-positive peaks, denoted NN, N2 and N3, respectively (Fig. 2A). Their elution positions correspond with those of neutral, disialylated diantennary and trisialylated triantennary reference oligosaccharides, respectively. Likewise, Bio-Gel P-100 fraction B is resolved in NN, N2 and N3, the only difference being that in this case N2 forms the main constituent (Fig. 2B), whereas fraction C leads, besides the fractions NN, N2 and N3, to an additional carbohydrate-containing peak, denoted N1, having the same retention volume as a monosialylated diantennary reference oligosaccharide (Fig. 2C). Additional peaks in the three Mono Q patterns represent residual salts and SDS, and do not contain carbohydrate. The corresponding fractions NN, N1, N2 and N3 from the three Mono Q separations were pooled, lyophilized and desalted on Bio-Gel P-2. Reappli-

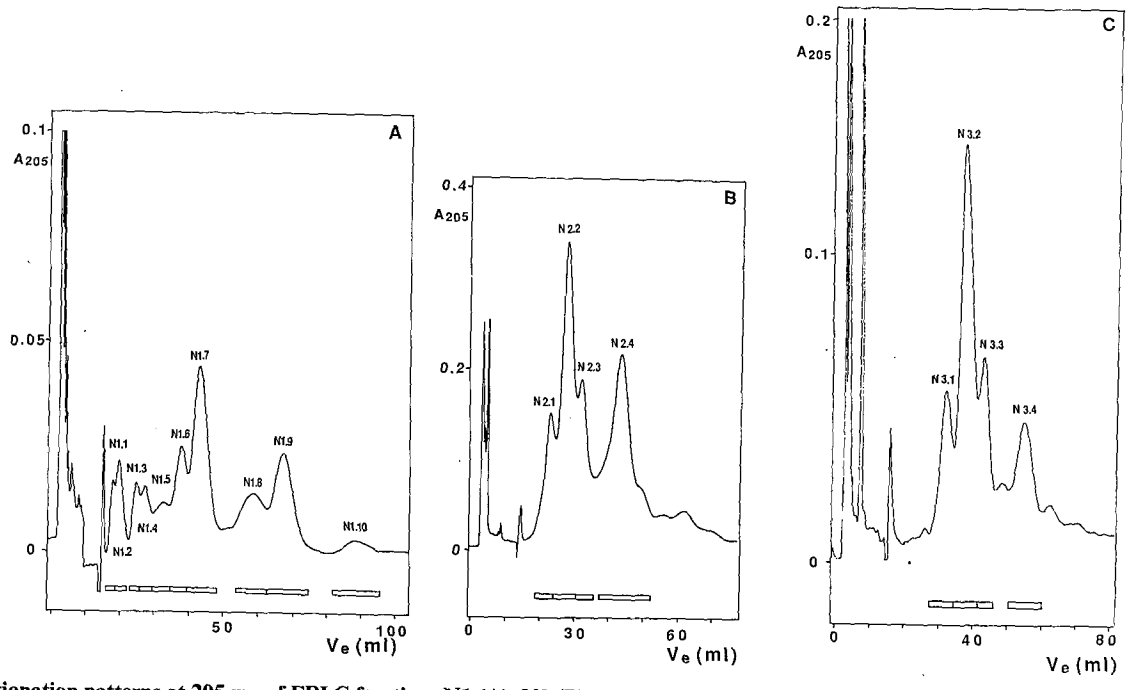


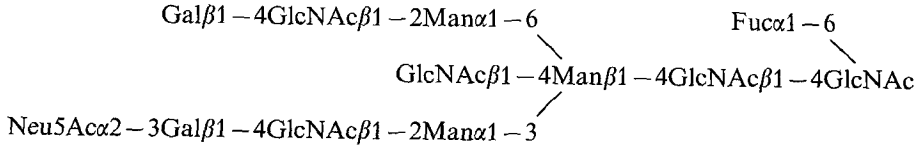
Fig. 3. Fractionation patterns at 205 nm of FPLC fractions N1 (A), N2 (B) and N3 (C) derived from BeWo hCG-β on an HPLC Lichrosorb-NH_2 column. The FPLC fractions were dissolved in 50 µl of a mixture of 30 mM K_2HPO_4/KH_2PO_4, pH 7.0, and acetonitrile (38:62, by vol.). The injection volume was 45 µl. The column was eluted isocratically with the same buffer at a flow rate of 2 ml/min at 25°C. Fractions were collected as indicated.

cation on Mono Q showed all fractions, except fraction NN, to be homogeneous in charge. Therefore, only NN was rechromatographed on Mono Q in a preparative way (not shown) and the resulting fractions NN, N1, N2 and N3 were desalted on Bio-Gel P-2. Fractions N1, N2 and N3 were pooled with the corresponding fractions from the preceding Mono Q separations. Summarizing, in this way, four final fractions were generated according to differences in charge, thereby maintaining the code system NN, N1, N2 and N3. Fraction NN was not investigated further because of the low amount of carbohydrate material in this fraction.

HPLC on Lichrosorb-NH_2 of fraction N1 yields ten subfractions N1.1 – N1.10 (Fig. 3A), fraction N2 four subfractions N2.1 – N2.4 (Fig. 3B), and fraction N3 four subfractions N3.1 – N3.4 (Fig. 3C). The HPLC fractions were investigated by 500-MHz or 600-MHz ^{1}H-NMR spectroscopy. In Table 1, the structures of the major N-linked oligosaccharides, occurring in BeWo hCG-β, are presented, together with their relative amounts. In the following, the ^{1}H-NMR data of the various compounds will be discussed in order of increasing complexity. Relevant ^{1}H-NMR data are compiled in Table 2. For the HPLC fractions not mentioned in Tables 1 and 2, either the amount of material was too low or they were too heterogenous mixtures for structure determination by NMR spectroscopy.

The ^{1}H-NMR spectrum of fraction N1.7 (not shown) indicates the presence of an $\alpha 2-3$-monosialylated, bisecting-GlcNAc-containing, fucosylated diantennary oligosaccharide.

The presence of the $\alpha 1-6$-fucosylated *N,N'*-diacetylchitobiose unit is recognized from the H-1 and NAc signals of GlcNAc-**1** and GlcNAc-**2**, as well as from the typical H-1, H-5 and methyl signals of L-fucose (Fuc; Table 2; see also Damm et al., 1987; Hård et al., 1990). The set of chemical shifts of the H-1 signals of Man-**4** and Man-**4'**, together with those of the H-2 signals of Man-**3**, Man-**4** and Man-**4'** reflects the substitution pattern of the branching point (Vliegenthart et al., 1983; Cahour et al., 1984). As compared to an $\alpha 2-3$-monosialylated diantennary reference compound (denoted N1.2 in Hård et al., 1990), upfield shifts are observed for Man-**4** H-1 ($\Delta\delta$ = −0.059 ppm) and Man-**3** H-2 ($\Delta\delta$ = −0.069 ppm), and downfield shifts for Man-**4'** H-1 ($\Delta\delta$ = 0.079 ppm), Man-**4** H-2 ($\Delta\delta$ = 0.062 ppm) and GlcNAc-**5** H-1 ($\Delta\delta$ = 0.011 ppm). The presence of the Neu5Ac$\alpha 2-3$Gal$\beta 1-4$GlcNAc$\beta 1-2$Man$\alpha 1-3$ antenna is deduced from the typical chemical shift values of Neu5Ac H-3a and H-3e, together with those of Man-**4** and Gal-**6** H-1 (Hård et al., 1990; Vliegenthart et al., 1983). In the *N*-acetyl region of the spectrum, the singlet at δ = 2.040 ppm is ascribed to GlcNAc-**5'**, and the signals at δ = 2.062 ppm and δ = 2.053 ppm are attributed to GlcNAc-**5** and GlcNAc-**9**, respectively (cf. N1.9). This means that extension of the reference compound with GlcNAc-**9** causes a downfield shift of the *N*-acetyl methyl protons of GlcNAc-**5** ($\Delta\delta$ = 0.015 ppm) and an upfield shift for those of GlcNAc-**5'** ($\Delta\delta$ = −0.007 ppm). The clearly visible multiplets at δ = 3.259 ppm and δ = 3.405 ppm are assigned to GlcNAc-**9** H-4 and H-5, respectively (Bouwstra, 1989).

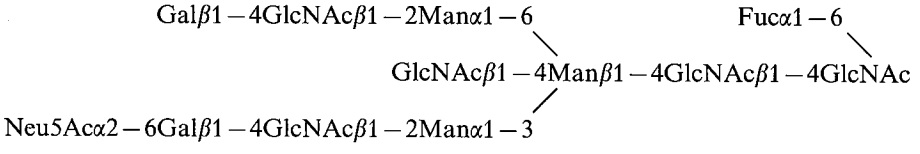


(N1.7)

Table 1. N-Linked carbohydrate chains occurring in BeWo hCG-β. The amounts were calculated on basis of FPLC and HPLC peak areas, corrected for the number of C=O groups, as described by Hård et al. (1990). The structure of the remaining 24% of the *N*-glycans has not been determined.

Code	Amount	Carbohydrate chain
	mol/100 mol	
N1.7	8	Neu5Acα2−3 | Neu5Acα2−6 | **Galβ1−4GlcNAcβ1−2Manα1−6** (**Fucα1−6**) **GlcNAcβ1−4Manβ1−4GlcNAcβ1−4GlcNAc** / **Galβ1−4GlcNAcβ1−2Manα1−3**
N1.9	5	
N2.1	6	Neu5Acα2−3 | Neu5Acα2−6 | **Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−6** (**Fucα1−6**) **Manβ1−4GlcNAcβ1−4GlcNAc** / **Galβ1−4GlcNAcβ1−2Manα1−3**
N2.3	11	
N2.2	19	Neu5Acα2−3 | Neu5Acα2−6 | **Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−6** (**Fucα1−6**) **GlcNAcβ1−4Manβ1−4GlcNAcβ1−4GlcNAc** / **Galβ1−4GlcNAcβ1−2Manα1−3**
N2.4.1	11	
N3.1	4	**Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−6** (**Fucα1−6**) **Manβ1−4GlcNAcβ1−4GlcNAc** / **Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−3** / **Neu5Ac′α2−3Galβ1−4GlcNAcβ1−4**
N3.2	12	**Neu5Ac′α2−3Galβ1−4GlcNAcβ1−2Manα1−6** (**Fucα1−6**) **GlcNAcβ1−4Manβ1−4GlcNAcβ1−4GlcNAc** / **Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−3** / **Neu5Ac*α2−3Galβ1−4GlcNAcβ1−4**

The ^{1}H-NMR spectrum of fraction N1.9 (not shown) proves the presence of an α2−6-sialylated analogue of N1.7.



(N1.9)

The identity of N1.9 is evident from comparison of its ^{1}H-NMR data with those of an α2−6-monosialylated, fucosylated diantennary reference compound (compound 1−3 in De Waard et al., 1991). Similar shift effects are observed for N1.9, as was discussed for N1.7, when compared to the non-bisected reference compound. Furthermore, the structural-reporter groups of N1.9, except those of the α1−6-fucosylated *N*,*N*′-diacetylchitobiose element and the NAc signals of GlcNAc-**5** and GlcNAc-**9**, fit those of a corresponding reference oligosaccharide alditol [compound IgM(GRA) in Cahour et al., 1984]. The unambiguous assignment of the *N*-acetyl methyl protons of an α2−6-monosialylated, bisecting-GlcNAc-containing, fucosylated diantennary glycopeptide has recently been performed using a combination of 2D NOE and 2D HOHAHA spectroscopy in ^1H_2O (Hård et al., 1991). It has to be noted that based on these data, a critical re-evaluation is necessary of the assignments of the GlcNAc-**9** and GlcNAc-**5** *N*-acetyl methyl signals reported in earlier papers on bisecting-GlcNAc-containing compounds.

The ^{1}H-NMR spectrum of fraction N2.1 (not shown) demonstrates the occurrence of an α2−3-disialylated, fucosylated diantennary carbohydrate chain.

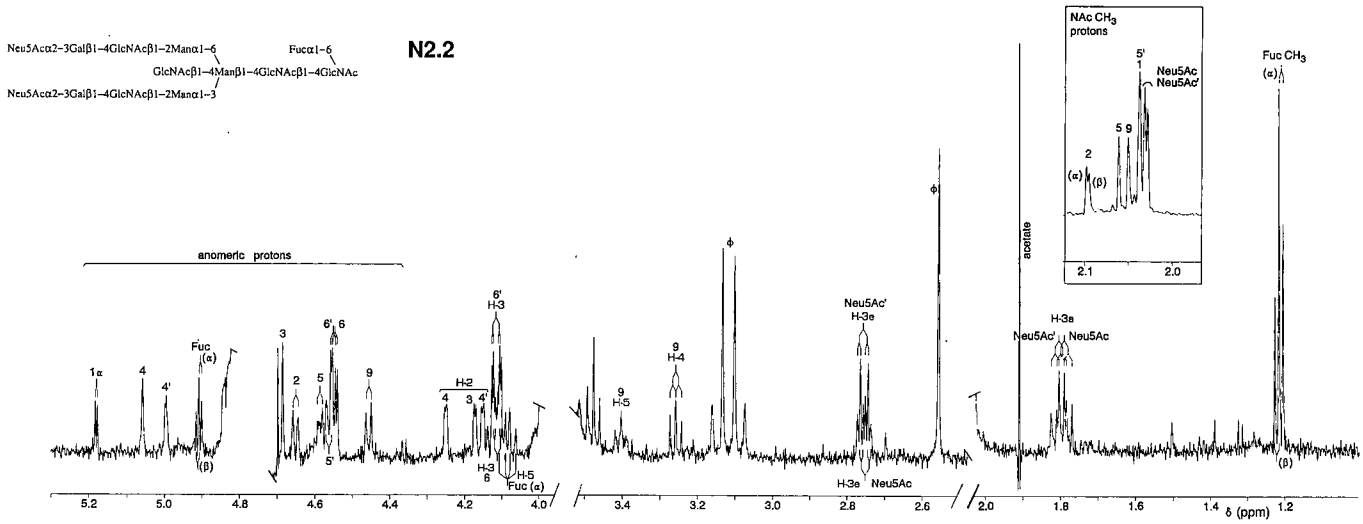


Fig. 4. Structural-reporter-group regions of the resolution-enhanced ^{1}H-NMR spectrum of fraction N2.2 obtained from BeWo hCG-β.

Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−6
Fucα1−6
Manβ1−4GlcNAcβ1−4GlcNAc (N2.1)
Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−3

The various structural-reporter-group signals of oligosaccharide N2.1 match those of an α2−3-disialylated, fucosylated diantennary reference compound (denoted N2.2 in Hård et al., 1990).

The ^{1}H-NMR spectrum of BeWo hCG-β fraction N2.2 (Fig. 4) indicates the presence of the following novel bisecting-GlcNAc-containing analogue of N2.1.

Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−6
Fucα1−6
GlcNAcβ1−4Manβ1−4GlcNAcβ1−4GlcNAc (N2.2)
Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−3

The extension of N2.1 with a bisecting GlcNAc-**9** residue is deduced from the characteristic combination of chemical shifts of the H-1 and H-2 atoms of Man-**3**, Man-**4** and Man-**4′**, together with the H-1, H-4, H-5 and NAc signals of GlcNAc-**9** (Bouwstra, 1989; Vliegenthart et al., 1983; Table 2). The presence of the GlcNAc-**9** residue in combination with two α2−3-linked Neu5Ac residues at Gal-**6** and Gal-**6′**, respectively, gives rise to two *N*-acetyl methyl singlets of the sialic acids at δ = 2.028 ppm and δ = 2.032 ppm, respectively. The assignments of the sialic acid H-3a and H-3e signals (Table 2) are based on a comparison of N2.2 with N2.3.

The ^{1}H-NMR spectrum of fraction N2.3 (not depicted) shows the presence of a disialylated, fucosylated diantennary oligosaccharide. The structural-reporter-group signals (Table 2) of N2.3 match those of an identical reference compound (denoted 2-1 in De Waard et al., 1991). In addition to compound N2.3, HPLC fraction N2.3 contains a high amount of compound N2.2, stemming from partial overlap with the preceding, larger fraction N2.2 (Fig. 3B).

The ^{1}H-NMR spectrum of HPLC fraction N2.4 (not depicted) shows considerable heterogeneity. Therefore, fraction N2.4 was further fractionated by HPAEC using PAD (Lee, 1990; Damm et al., 1989), yielding one major fraction, denoted N2.4.1, and two minor, later eluting, fractions (Fig. 5). Due to the low amount of material in the minor fractions, only the structure of the oligosaccharide in fraction N2.4.1 could be determined. From the ^{1}H-NMR spectrum of fraction N2.4.1 (Fig. 6A) the structure of the following novel bisecting-GlcNAc-containing analogue of compound N2.3 can be deduced.

Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−6
Fucα1−6
Manβ1−4GlcNAcβ1−4GlcNAc (N2.3)
Neu5Acα2−6Galβ1−4GlcNAcβ1−2Manα1−3

Neu5Acα2−3Galβ1−4GlcNAcβ1−2Manα1−6
Fucα1−6
GlcNAcβ1−4Manβ1−4GlcNAcβ1−4GlcNAc (N2.4.1)
Neu5Acα2−6Galβ1−4GlcNAcβ1−2Manα1−3

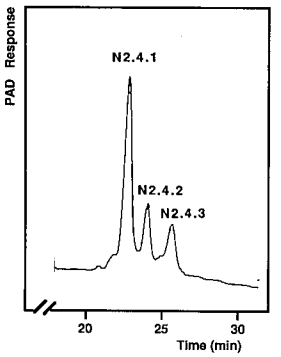


Fig. 5. Fractionation pattern of HPLC fraction N2.4 on a CarboPac PA1 column. Eluent A contained 0.1 M NaOH, and eluent B contained 0.1 M NaOH and 1 M sodium acetate. Elutions were carried out at a flow rate of 1 ml/min starting with 94% (by vol.) eluent A/6% (by vol.) eluent B for 0.4 min, then going to 87% (by vol.) eluent A/13% (by vol.) eluent B in 30.4 min, followed by a steeper gradient to 75% (by vol.) eluent A/25% (by vol.) eluent B in 35 min.

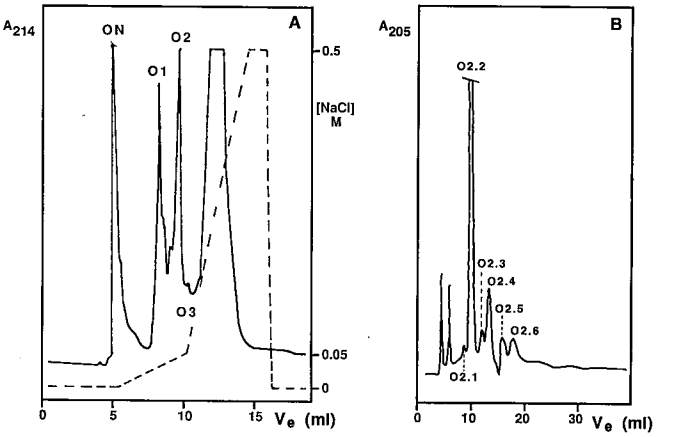


Fig. 7. Fractionation pattern at 214 nm of the β-elimination products derived from *N*-deglycosylated BeWo hCG-β on a FPLC Mono Q HR 5/5 anion-exchange column (A) and fractionation pattern at 205 nm of BeWo hCG-β FPLC fraction O2 on a Lichrosorb-NH_2 column (B).

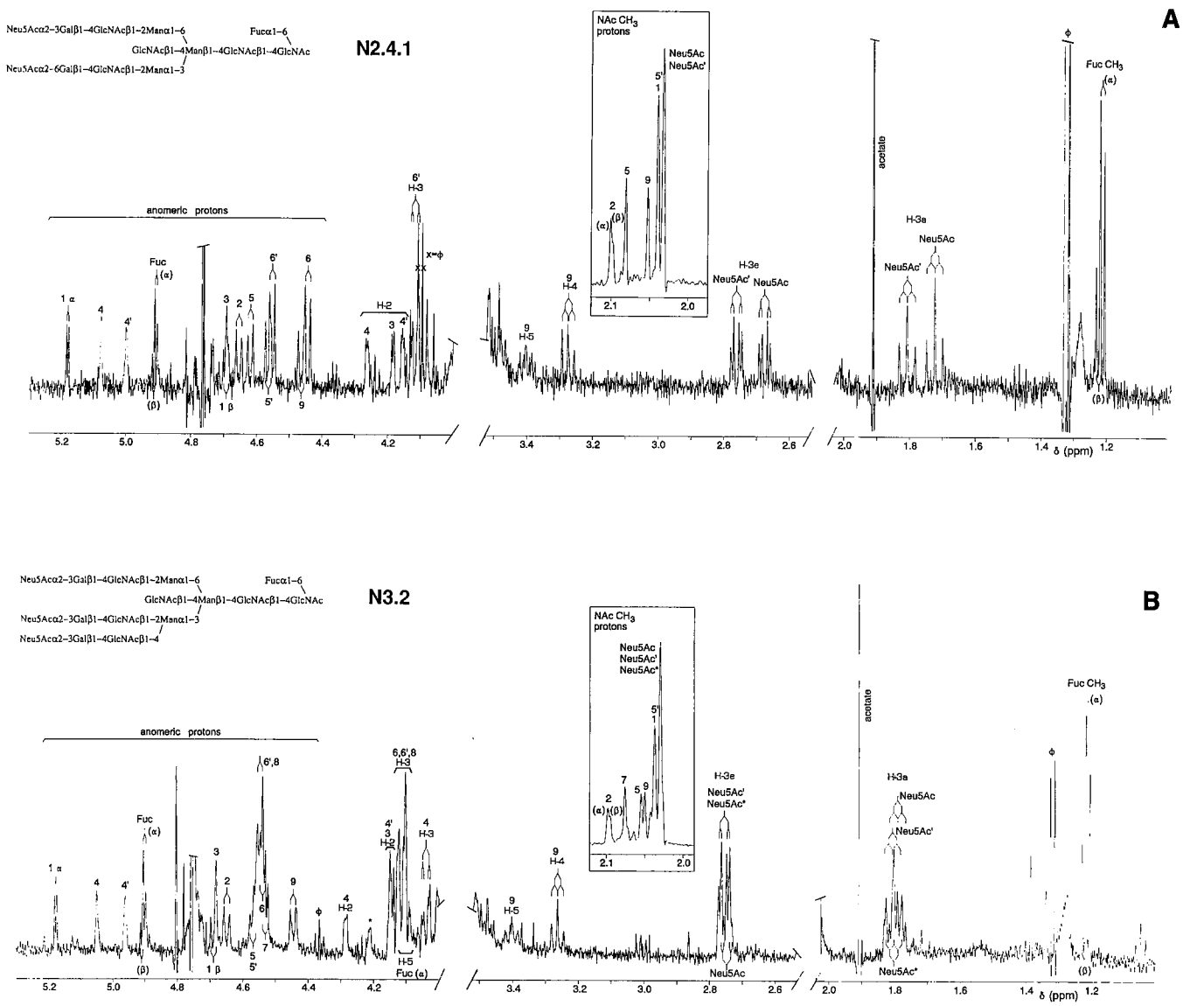
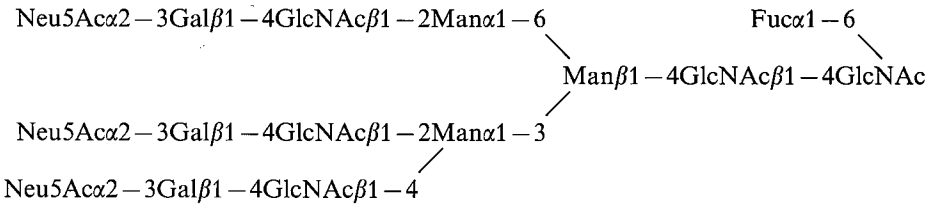


Fig. 6. Structural-reporter-group regions of the resolution-enhanced ^{1}H-NMR spectrum of (A) fraction N2.4.1 and (B) fraction N3.2 obtained from BeWo hCG-β.

The structure of N2.4.1 follows from a comparison of its ^{1}H-NMR data with those of oligosaccharide N2.3, considering the effects of the introduction of GlcNAc-**9**, manifest in the H-1 signals of Man-**3**, Man-**4**, Man-**4′**, GlcNAc-**5** and GlcNAc-**9**, the H-2 resonances of Man-**3**, Man-**4** and Man-**4′**, the H-4 and H-5 multiplets of GlcNAc-**9**, and the NAc singlets of GlcNAc-**5**, GlcNAc-**5′** and GlcNAc-**9**. In contrast to the shift effects observed for Neu5Ac NAc, when going from N2.1 to N2.2, the sialic acid NAc signals of N2.4.1 overlap.

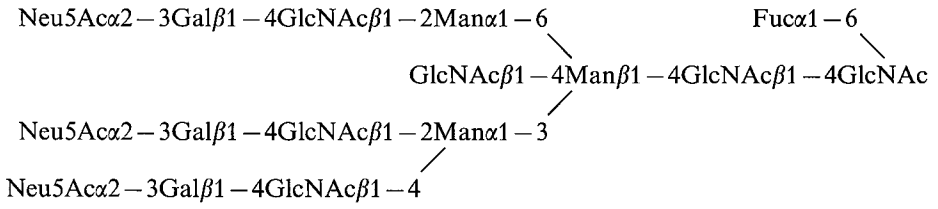
The ^{1}H-NMR spectrum of fraction N3.1 (not shown) indicates that it mainly (90%) contains an $\alpha 2-3$-trisialylated, fucosylated triantennary oligosaccharide.



(N3.1)

The ^{1}H-NMR parameters of N3.1 (Table 2) match those of an identical reference compound (denoted N3.2A in Hård et al., 1990). In addition to compound N3.1, a small amount ($\approx$10%) of oligosaccharide N3.2 (see below) is present in fraction N3.1, due to partial overlap of the two HPLC fractions (Fig. 3C).

The ^{1}H-NMR spectrum of fraction N3.2 (Fig. 6B) indicates the occurrence of the following novel bisecting-GlcNAc-containing analogue of N3.1.



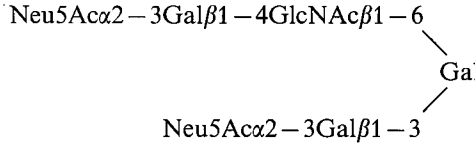
(N3.2)

The presence of a bisecting GlcNAc-**9** and the triantennary character of N3.2 can be inferred from the chemical shifts of the H-1 signals of Man-**3**, Man-**4**, Man-**4′** and GlcNAc-**9**, and the H-2 signals of Man-**3**, Man-**4** and Man-**4′**. As compared to N3.1, upfield shifts are observed for Man-**4** H-1 ($\Delta\delta$ = -0.061 ppm) and Man-**3** H-2 ($\Delta\delta$ = -0.07 ppm), and downfield shifts for Man-**4′** H-1 ($\Delta\delta$ = 0.059 ppm) and H-2 ($\Delta\delta$ = 0.078 ppm), resembling the effects observed for the diantennary compounds when going from non-bisected to bisected carbohydrate chains (Table 2). The assignment of the doublet at δ = 4.447 ppm to GlcNAc-**9** H-1 was confirmed by a 2D HOHAHA experiment, showing that the well-resolved H-4 (δ = 3.264 ppm) and H-5 (δ = 3.404 ppm) multiplets of GlcNAc-**9** are connected to the corresponding GlcNAc-**9** H-1 (δ = 4.447 ppm), H-2 (δ = 3.69 ppm), H-3 (δ = 3.567 ppm), H-6 (δ = 3.955 ppm) and H-6′ (δ = 3.69 ppm) resonances. In addition to compound N3.2 this fraction contains minor amounts (< 10%) of compounds N3.1 and N3.3, originating from the neighbouring, partially overlapping, HPLC fractions.

O-linked carbohydrate chains

Medium-pressure anion-exchange chromatography on Mono Q of the mixture of chemically released O-linked oligosaccharides obtained from BeWo hCG-β gives rise to four carbohydrate-positive peaks, denoted ON, O1, O2 and O3 (Fig. 7A). HPLC on Lichrosorb-NH_2 gives rise to six subfractions for ON and four subfractions for O1 (not shown). Due to the low amount of material, for none of these subfractions, structural elucidation could be carried out. Mono Q fraction O2 was subfractionated by HPLC into six subfractions, denoted O2.1 – O2.6 (Fig. 7B); only the amounts of material in fractions O2.2, O2.3 and O2.4 were sufficient for structure determination by ^{1}H-NMR spectroscopy. Mono Q fraction O3 was directly analyzed by ^{1}H-NMR spectroscopy. In Table 3, relevant ^{1}H-NMR parameters are compiled for oligosaccharides O2.2, O2.3, O2.4 and O3.

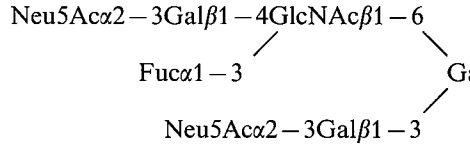
The ^{1}H-NMR spectrum of fraction O2.2 (Fig. 8A) indicates the presence of the following disialylated hexasaccharide alditol.



(O2.2)

The various structural-reporter-group signals of O2.2 match those previously obtained for an identical oligosaccharide alditol structure (cf. compound IV in Korrel et al., 1984).

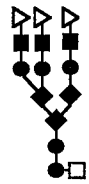
The structural-reporter-group regions of the ^{1}H-NMR spectrum of fraction O2.3 are shown in Fig. 8B and indicate the occurrence of the following novel compound.



(O2.3)

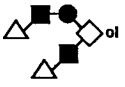
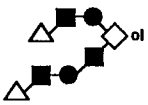
The core structure GlcNAcβ1 – 6(Galβ1 – 3)GalNAc-ol is deduced from the GalNAc-ol H-2, H-3, H-5 and NAc signals. The lower branch, Neu5Acα2 – 3Galβ1 –, is inferred from the typical combination of chemical shifts for Neu5Ac^{3,3} H-3a, H-3e and NAc, as well as Gal^3 H-1 and H-3 (Table 3). The presence of fucose in the upper branch Neu5Acα2 – 3Galβ1 – 4(Fucα1 – 3)GlcNAcβ1 – is evident from the α-anomeric signal at δ = 5.100 ppm ($^3J_{1,2}$ = 3.5 Hz), together with the methyl doublet at δ = 1.166 ppm, and the H-5 signal at δ = 4.798 ppm (measured at 40 °C; Lamblin et al., 1984). The structure of the upper branch is further deduced from the GlcNAc^6 H-1 and NAc (cf. compound A-6 in Lamblin et al., 1984), the Gal^{4,6} H-1, and the Neu5Ac^{3,4,6} H-3a, H-3e and

Table 2. ^{1}H Chemical shifts of structural-reporter-group protons of the constituent monosaccharides of the N-linked oligosaccharides derived from BeWo hCG-β. Chemical shifts are given at 27°C and were measured in ^2H_2O relative to internal acetone (δ = 2.225 ppm; Vliegenthart et al., 1983). Compounds are represented using the following symbols: (□) Fuc; (■) Gal; (●) GlcNAc; (◆) Man; (△) Neu5Acα2–3; (○) Neu5Acα2–6. For numbering of the monosaccharide residues, see Table 1. n.d., not determined. α and β stand for the anomeric configuration of GlcNAc-**1**.

Reporter group	Residue	Chemical shift in							
		 N1.7	 N1.9	 N2.1	 N2.2	 N2.3	 N2.4.1	 N3.1	 N3.2
		ppm							
H-1	GlcNAc-**1**α	5.180	5.180	5.181	5.180	5.181	5.181	5.181	5.181
	GlcNAc-**1**β	n.d.	n.d.	4.693	n.d.	n.d.	4.693	n.d.	4.691
	GlcNAc-**2**	4.658	4.658	4.664	4.652	n.d.	4.653	n.d.	4.650
	Man-**3**	n.d.	n.d.	n.d.	4.685	n.d.	4.691	n.d.	4.684
	Man-**4**	5.059	5.076	5.116	5.058	5.135	5.075	5.114	5.053
	Man**4'**	5.007	5.008	4.921	4.995	4.924	4.996	4.905	4.964
	GlcNAc-**5**	4.584	4.617	4.573	4.586	4.606	4.619	4.560	4.57[a]
	GlcNAc-**5'**	4.577	4.578	4.573	4.563	4.574	4.564	4.571	4.566
	Gal-**6**	4.547	4.444	4.544	4.546	4.444	4.443	4.542[b]	4.54[a,c]
	Gal-**6'**	4.477	4.477	4.549	4.551	4.549	4.552	4.548[b]	4.55[a,c]
	GlcNAc-**7**	–	–	–	–	–	–	4.542[b]	4.534
	Gal-**8**	–	–	–	–	–	–	4.548[b]	4.55[a,c]
	GlcNAc-**9**	4.460	4.465	–	4.456	–	4.463	–	4.447
H-2	Man-**3**	4.177	4.185	4.249	4.172	4.255	4.183	4.211	4.14[a]
	Man-**4**	4.252	4.260	4.188	4.249	4.195	4.262	4.211	4.289
	Man-**4'**	4.141	4.139	4.11[a]	4.149	4.11[a]	4.154	4.10[a]	4.15[a,f]
H-3	Gal-**6**	4.115	n.d.	4.113	4.112	n.d.	n.d.	4.117	4.12[a,f]
	Gal-**6'**	n.d.	n.d.	4.116	4.116	4.117	4.118	4.117	4.12[a,f]
	Gal-**8**	–	–	–	–	–	–	4.117	4.12[a,f]
H-3a	Neu5Ac	1.790	1.721	1.797	1.790	1.718	1.722	1.802	1.793
	Neu5Ac'	–	–	1.802	1.805	1.804	1.806	1.802	1.809[d]
	Neu5Ac*	–	–	–	–	–	–	1.802	1.804[d]
H-3e	Neu5Ac	2.752	2.672	2.758	2.751	2.669	2.673	2.757	2.749
	Neu5Ac'	–	–	2.758	2.759	2.759	2.760	2.757	2.758
	Neu5Ac*	–	–	–	–	–	–	2.757	2.758
H-4	GlcNAc-**9**	3.259	n.d.	–	3.259	–	3.274	–	3.264
H-5	GlcNAc-**9**	3.405	n.d.	–	3.404	–	3.401	–	3.404
NAc	GlcNAc-**1**	2.038	2.038	2.039	2.037	2.038	2.038	2.039	2.038
	GlcNAc-**2**α	2.098	2.098	2.097	2.098	2.097	2.099	2.096	2.099
	GlcNAc-**2**β	2.093	2.092	2.093	2.095	2.097	2.096	2.096	2.096
	GlcNAc-**5**	2.062	2.080	2.048	2.061	2.069	2.080	2.044	2.056[e]
	GlcNAc-**5'**	2.040	2.042	2.044	2.037	2.044	2.038	2.044	2.038
	GlcNAc-**7**	–	–	–	–	–	–	2.074	2.077
	GlcNAc-**9**	2.053	2.054	–	2.050	–	2.051	–	2.051[e]
	Neu5Ac	2.029	2.030	2.032[g]	2.028/2.032	2.032[g]	2.031[g]	2.031[h]	2.031[h]
H-1	Fucα	4.897	4.898	4.893	4.904	4.894	4.905	4.892	4.904
	Fucβ	4.905	4.907	4.900	4.911	4.901	4.912	4.901	4.912
H-5	Fucα	4.101	4.098	4.097	4.084	4.10[a]	4.086	4.097	4.10[a]
	Fucβ	n.d.	4.133	4.133	4.132	n.d.	n.d.	n.d.	n.d.
CH_3	Fucα	1.207	1.208	1.211	1.211	1.211	1.212	1.211	1.212
	Fucβ	1.219	1.218	1.222	1.222	1.222	1.224	1.223	1.224

[a] Some values are given with only two decimals because of spectral overlap or virtual couplings.
[b–e] Assignments may have to be interchanged.
[f] Obtained from 2D HOHAHA spectrum.
[g] Signal belonging to two Neu5Ac residues.
[h] Signal belonging to three Neu5Ac residues.

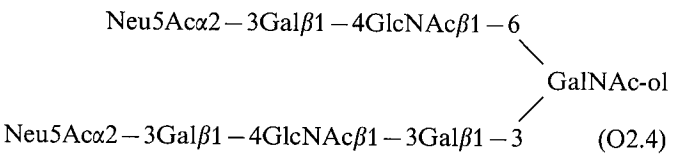
Table 3. ^{1}H Chemical shifts of structural-reporter-group protons of the constituent monosaccharides of the O-linked oligosaccharides derived from BeWo hCG-β. Chemical shifts, measured at 500 MHz (O2.3 at 600 MHz) in ^2H_2O at 27°C, are given relative to internal acetone (δ = 2.225 ppm; Vliegenthart et al., 1983). Compounds are represented using the following symbols: (□) Fuc; (■) Gal; (◇ol) GalNAc-ol; (●) GlcNAc; (△) Neu5Acα2–3; (○) Neu5Acα2–6. Superscript numbers at the name of a sugar residue indicate the linkage positions of the residue itself and of the adjacent residues. n.d., not determined.

Residue	Reporter group	Chemical shift in			
		O2.2 	O2.3 	O2.4 	O3 
		ppm			
GalNAc-ol	H-2	4.388	4.388	4.383	4.378
	H-3	4.067	4.067	4.047	4.068
	H-4	3.440	3.427	3.453	3.524
	H-5	4.266	4.255	4.263	4.238
	NAc	2.065	2.065	2.066	2.042
Gal^3	H-1	4.530	4.531	4.447	4.542
	H-3	4.114	4.114	n.d.	4.117
	H-4	3.928	n.d.	4.125	n.d.
Neu5Ac^{3,3}	H-3a	1.799	1.800	–	1.800
	H-3e	2.775	2.774	–	2.775
	NAc	2.033	2.032	–	2.032
GlcNAc^6	H-1	4.553	4.556	4.548	–
	H-6	4.005	n.d.	4.006	–
	NAc	2.061	2.053	2.055	–
Gal^{4,6}	H-1	4.547	4.515	4.548	–
	H-3	4.114	4.067	4.115	–
	H-4	3.957	n.d.	n.d.	–
Neu5Ac^{3,4,6}	H-3a	1.799	1.800	1.799	–
	H-3e	2.756	2.76	2.757	–
	NAc	2.031	2.032	2.031	–
Fuc	H-1	–	5.100	–	–
	H-5	–	4.798[a]	–	–
	CH_3	–	1.166	–	–
GlcNAc^3	H-1	–	–	4.679	–
	NAc	–	–	2.033[b]	–
Gal^{4,3}	H-1	–	–	4.559	–
	H-3	–	–	4.115	–
Neu5Ac^{3,4,3}	H-3a	–	–	1.799	–
	H-3e	–	–	2.757	–
	NAc	–	–	2.031[b]	–
Neu5Ac^6	H-3a	–	–	–	1.691
	H-3e	–	–	–	2.725
	NAc	–	–	–	2.032

[a] Measured at 40°C.
[b] Assignments may have to be interchanged.

NAc signals. The upfield shift of Gal^{4,6} H-1 ($\Delta\delta$ = −0.032 ppm), when O2.3 is compared with O2.2, is similar to the effect observed previously for Gal^{4,3} H-1 ($\Delta\delta$ = −0.031 ppm), when going from Neu5Acα2–3Galβ1–4GlcNAcβ1–3(Neu5Acα2–6)GalNAc-ol to Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAcβ1–3(Neu5Acα2–6)GalNAc-ol (see compounds F II-2-4 and F II-2-7 in Capon et al., 1989).

The structural-reporter-group regions of the ^{1}H-NMR spectrum of fraction O2.4 are shown in Fig. 8C, and indicate the presence of the following novel oligosaccharide alditol.



(O2.4)

The GlcNAcβ1–6(Galβ1–3)GalNAc-ol core structure can be deduced from the typical GalNAc-ol H-2, H-5 and NAc reporters (Table 3, see also the corresponding asialo compound denoted 21a in Klein et al., 1988). The upper branch Neu5Acα2–3Galβ1–4GlcNAcβ1–6GalNAc-ol is deduced from the set of chemical shifts of the Neu5Ac^{3,4,6}, Gal^{4,6} and GlcNAc^6 structural-reporter-group signals (cf. compound O2.2, Table 3). Compared to the corresponding signals in O2.2, the NAc singlet of GlcNAc^6 has shifted slightly upfield ($\Delta\delta$ = −0.006 ppm) in compound O2.4. For the lower branch the presence of the α2–3-sialylated *N*-acetyllactosamine unit, β1–3-linked to Gal^3, is inferred from the H-1 and H-4 resonances of Gal^3, the H-1 and NAc signals of GlcNAc^3, the H-1 and H-3 signals of Gal^{4,3}, and from the H-3a, H-3e and NAc reporters of the Neu5Ac^{3,4,3} residue (Table 3, see also compound 6a$_2$ in Van Halbeek et al., 1988).

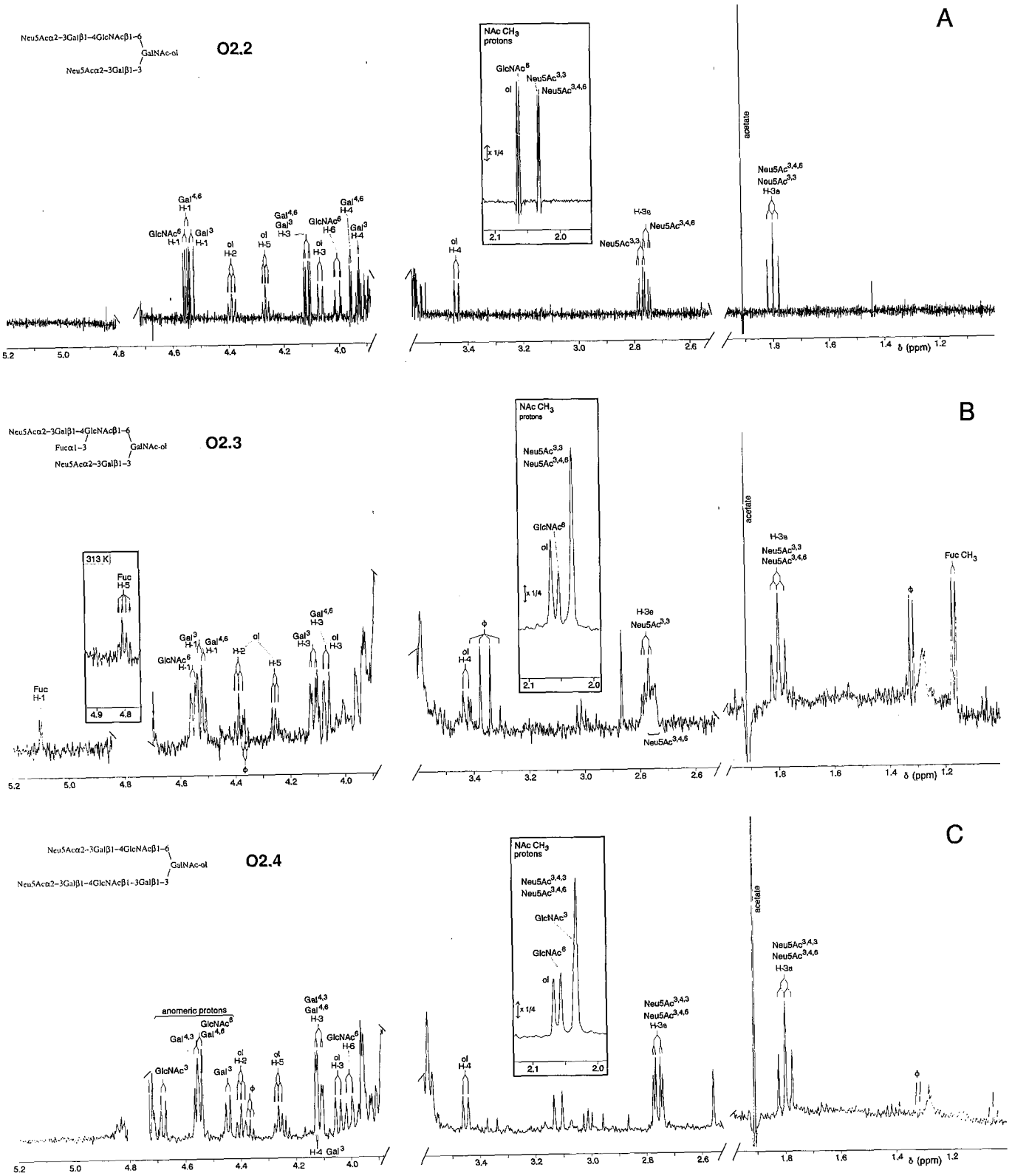
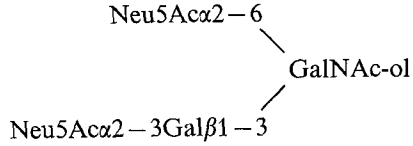


Fig. 8. Structural-reporter-group regions of the resolution-enhanced ^{1}H-NMR spectra of the O-linked oligosaccharides O2.2 (A), O2.3 (B) and O2.4 (C) obtained from BeWo hCG-β.

The ^{1}H-NMR spectrum (not shown) of Mono Q fraction O3 indicates the presence of two oligosaccharide alditols in a molar ratio of approximately 2:1. The minor component is compound O2.2, the most abundant carbohydrate in the overlapping preceding fraction O2. The major oligosaccharide in fraction O3 has the following structure.



(O3)

Table 4. O-Linked carbohydrate chains occurring in BeWo hCG-β. Absolute relative amounts cannot be given because of the large amounts of non-carbohydrate material in the FPLC fractions.

Carbohydrate chain	Code	Relative amount
Neu5Acα2–3Galβ1–4GlcNAcβ1–6 GalNAc-ol Neu5Acα2–3Galβ1–3	O2.2	100
Neu5Acα2–3Galβ1–4GlcNAcβ1–6 Fucα1–3 GalNAc-ol Neu5Acα2–3Galβ1–3	O2.3	20
Neu5Acα2–3Galβ1–4GlcNAcβ1–6 GalNAc-ol Neu5Acα2–3Galβ1–4GlcNAcβ1–3Galβ1–3	O2.4	40
Neu5Acα2–6 GalNAc-ol Neu5Acα2–3Galβ1–3	O3	45

The structural-reporter groups of O3 match those of an identical oligosaccharide alditol structure (cf. compound O3 in Damm et al., 1987; see Table 3). In Table 4, the structures of the major O-linked oligosaccharides of BeWo hCG-β are given together with their relative amounts.

DISCUSSION

This study presents the structures of the major N- and O-linked carbohydrate chains of the β subunit of hCG isolated from the culture fluid of the human choriocarcinoma cell line BeWo. In recent years, the carbohydrate chains of hCG stemming from patients suffering from various trophoblastic diseases have obtained considerable attention (Endo et al., 1987, 1988a, b; Mizuochi et al., 1983, 1985; Nishimura et al., 1985; Kobata, 1988). These investigations have shown that the *N*-glycosylation pattern in precancerous invasive mole urinary hCG and choriocarcinoma urinary hCG has shifted towards higher levels of triantennary oligosaccharides and higher levels of α1–6-fucosylation of the asparagine-linked GlcNAc. In addition, hCG from choriocarcinomas possesses (α1–6-fucosylated) diantennary structures having a 2,4-branched Man-**4** and a terminal Man-**4′** residue (Kobata, 1988). In contrast to normal urinary hCG, a large variation in the sialylation level occurs in the oligosaccharides of hCG isolated from the urine of choriocarcinoma patients.

In BeWo hCG-β an increased relative amount of triantennary, namely 26% of the total amount of N-linked carbohydrate chains vs 2% for normal urinary hCG-β (Damm et al., 1988), and α1–6-fucosylated, namely 95% vs 42% in normal urinary hCG-β (Damm et al., 1988), N-linked chains are detected. The triantennary oligosaccharides recently reported for normal urinary hCG-β, in which they form 5% of the total N-linked glycans (Damm et al., 1988), were not found in BeWo hCG-β. The N-linked chains in BeWo hCG-β are nearly completely sialylated, as they are in normal urinary hCG-β. However, in contrast to normal hCG-β, a considerable amount of these Neu5Ac residues occur in the α2–6-linkage, namely 21% of the total amount of Neu5Ac. It is not clear whether the occurrence of high amounts of α2–6-linked Neu5Ac is unique for BeWo hCG-β, because in some of the earlier studies concerning other choriocarcinoma hCG species, the Neu5Ac-Gal linkage type was not determined (Mizuochi et al., 1985). In this context, it has to be noted that only the presence of sialic acid, but not the type of linkage, seems to be important for the biological properties of hCG (Amano et al., 1989). Furthermore, 76% of the N-linked carbohydrate chains of BeWo hCG-β contain a bisecting GlcNAc residue. The presence of a bisecting GlcNAc in hCG has never been reported, neither for other choriocarcinoma nor for normal urinary hCG. In the case of γ-glutamyltranspeptidase, it has been shown that this glycoprotein from rat AH-66 hepatoma cells have N-linked bisecting-GlcNAc-containing carbohydrate chains, whereas that of normal rat liver cells is devoid of these type of *N*-glycans (Yamashita et al., 1983). A bisecting GlcNAc seems to have a profound influence on the conformation of *N*-glycans (Paulsen et al., 1986), but the biological significance still remains to be established.

The most abundant O-linked carbohydrate chains in hCG-β purified from the urine of pregnant women are the trisaccharide Neu5Acα2–3Galβ1–3GalNAc-ol, the tetrasaccharide Neu5Acα2–3Galβ1–3(Neu5Acα2–6)GalNAc-ol and the hexasaccharide Neu5Acα2–3Galβ1–4GlcNAcβ1–6(Neu5Acα2–3Galβ1–3)GalNAc-ol (Kessler et al., 1979b; Damm et al., 1987). Partially sialylated and asialo forms of these oligosaccharides (Amano et al., 1988), and the disaccharide Neu5Acα2–6GalNAc-ol have also been reported (Cole et al.,

1985). In hCG isolated from the urine of choriocarcinoma patients, the amount of the hexasaccharide is markedly increased, and significant changes in the amount are also observed for the other *O*-glycans (Amano et al., 1988; Cole, 1987). In the present study, the hexasaccharide was found as the main *O*-glycan and the tetrasaccharide as a minor constituent. Main findings are two novel O-linked carbohydrate chains, namely the heptasaccharide O2.3 and the octasaccharide O2.4 (see Table 4). It should be noted that the structural element Neu5Acα2 – 3Galβ1 – 4(Fucα1 – 3)GlcNAcβ1 –, which occurs in the heptasaccharide, is the sialylated Lewis x (Le^x or stage-specific embryonic antigen 1) determinant (Gooi et al., 1981). This structural element has previously been found in a ganglioside from human kidney (Rauvala, 1976), in rat-brain glycoproteins (Krusius and Finne, 1978) and in *O*-glycans derived from bronchial mucus glycoproteins of patients suffering from cystic fibrosis (Lamblin et al., 1984). A similar oligosaccharide alditol as the heptasaccharide, but having a non-sialylated Le^x epitope, has been isolated from cystic fibrosis mucus (compound b-8.1 in Breg et al., 1987) and from the hinge region of human secretory immunoglobulin A (compound A-2 in Pierce-Crétel et al., 1989). Recently, the sialylated Le^x epitope has received considerable attention, since it seems to be the critical determinant in the adhesion of leukocytes to endothelium (Springer and Lasky, 1991). Le^x-Le^x, i.e. carbohydrate-carbohydrate, interaction has been shown to modulate cell adhesion at the morula stage of mouse embryogenesis and during F9 teratocarcinoma cell aggregation (Hakomori, 1991). The structural element Neu5Acα2 – 3Galβ1 – 4GlcNAcβ1 – 3Galβ1 – 3GalNAc-ol, which occurs in *O*-glycan O2.3, has previously been identified in an oligosaccharide alditol isolated from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis (denoted $6a_2$ in Van Halbeek et al., 1988).

Lately, the structure of the O-linked oligosaccharides of the human transferrin receptor from BeWo cells has been determined (Do et al., 1990). Two neutral glycans, namely GalNAc and Gal-GalNAc, were found. The BeWo cell line is positive for blood group A antigens (Do et al., 1990), but these determinants reside neither in the *O*-glycans of the transferrin receptor nor in the O- or N-linked carbohydrate chains of hCG-β.

In conclusion, the present report shows that the carbohydrates of BeWo hCG-β differ from those of normal urinary hCG-β. These findings stress the care that has to be taken when evaluating the suitability of different *in vitro* systems for the production of (clinically) important glycoproteins. On one hand, non-human cell lines can introduce immunologically problematic carbohydrates (Hård et al., 1989; Hokke et al., 1990), and on the other hand, transformed human cells often synthesize cancer-associated oligosaccharide epitopes (Rademacher et al., 1988; Kamerling et al., 1990).

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