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THE O-LINKED OLIGOSACCHARIDE STRUCTURES ARE STRIKING DIFFERENT ON PREGNANCY AND CHORIOCARCINOMA HCG*

LAURENCE A. COLE

Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06510

ABSTRACT: hCG is a glycoprotein hormone which is detected in the serum and urine of pregnant women and of patients with hydatidiform mole and choriocarcinoma. The molecule contains 4 O-linked sugar chains. In an effort to identify cancer markers, the structures of these sugar units on the hCG produced in pregnancy and choriocarcinoma were compared. hCG molecules in patient urines were purified by immuno-affinity chromatography and gel filtration. β-elimination was used to cleave the O-linked sugar units, radioactive sodium borohydride to label them, and gel filtration on Bio-Gel P4 to size them and compare their elution volumes with those of standard oligosaccharides of known structure. A trisaccharide, NeuAcα2-3Galβ1-3GalNAc-, was found to be the principal unit attached to urinary hCG from pregnant women (10 samples). A hexasaccharide, NeuAcα2-3Galβ1-3(NeuAcα2-3Galβ1-4 GlcNAcβ1-6)GalNAc-, which accounted for just 6% (mean, range 0 - 14%) of the O-linked sugar units on pregnancy hCG, was the principal unit (mean 52% of total, range 50 - 56%) attached to the hCG from choriocarcinoma patient urines (3 samples). These results indicate that hexasaccharide-abundant hCG is an indicator of choriocarcinoma.

hCG is a glycoprotein hormone composed of 2 dissimilar subunits, α and β, joined non-covalently. Four O-linked sugar units are attached to hCG, all to serine residues at the COOH-terminal region of the β-subunit (1,2).

hCG is produced by the trophoblast in pregnancy and hydatidiform mole (product of androgenic conception), and by the neoplastic trophoblast in choriocarcinoma. While ultrasound can be used to differentiate normal pregnancy and mole, the interpretation of rising hCG levels in the mole patient in the months post-evacuation may be difficult. Early normal pregnancy, invasive mole and choriocarcinoma need to be considered. The general approach is to measure hCG levels weekly over a period ranging from 3 weeks to 3 months, and if levels persist, assume neoplasia and commence chemotherapy (3, 4). If specific structural characteristics could be identified on choriocarcinoma and/or invasive mole hCG, their recognition and management would be facilitated.

Several investigators have found distinct oligosaccharide structures on tumor-derived glycoproteins that are either absent or occur in trace amounts on those produced by normal cells (5-7). Others have found increased glycosyltransferase activities in tumor relative to normal tissues (8).

In an effort to identify markers of choriocarcinoma the structures of the O-linked sugar units on pregnancy and cancer hCG were examined.

MATERIALS AND METHODS

Urine samples from pregnant women were kindly provided by Dr. John Laferla of the University of Michigan. 100 ml samples were obtained from 10 women 7 to 15 weeks after their last menstrual period, and frozen within 15 min of collection. Frozen urine samples from 8 patients with choriocarcinoma were kindly provided by Dr. Roland Pattillo of the Medical College of Wisconsin and choriocarcinoma were kindly provided by Dr. Roland Pattillo of the Medical College of Wisconsin and Dr. Kenneth Bagshawe of Charing Cross Hospital Medical School, London. hCG was purified from 2 individual samples of choriocarcinoma urine, and from a pool generated from equal volumes of the 6 remaining samples. Upon thawing the pH of urine samples was adjusted to 7.0 with 10X PBS buffer, and 20 mM EDTA, 10 mM iodoacetate and 2 mM phenylmethylsulfonyl fluoride were added as preservatives. Urinary hCG was purified by immunoaffinity chromatography using rabbit anti-hCGβ antiserum-Sepharose (bound hormone eluted with 4 M guanidine hydrochloride, pH 4) and by gel filtration on Bio-Gel P100, according to procedures previously described (1). The purified samples contained between 50 and 760 μg hCG.

O-linked sugar units were released from hCG by β-elimination using the procedures previously described in detail (1). Briefly, samples were incubated with 0.1 M sodium hydroxide and 1.0 M sodium borohydride supplemented with 25 mCi NaB^3H_4 (to label released oligosaccharides) for 24 h at 45 C. After incubation mixtures were neutralized, sodium borate produced was extracted by multiple evaporations from methanol, and released sugar units separated from peptides by ion-exchange chromatography on columns of AG50X8.

Gel filtration on 1 x 110 cm columns of Bio-Gel P4 was carried out by the procedures previously described (1). The preparation and structure of oligosaccharitol standards (from hCG batch CR123, NIADDKD) is described elsewhere (1). Standard 1, NeuAcα2-3Galβ1-3(NeuAcα2-3Galβ1-4GlcNAcβ1-6)GalNAc-ol; 2, NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc-ol; 3, NeuAcα2-3Galβ1-3 GalNAc-ol; 4, NeuAcα2-6 GalNAc-ol; 5, Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAc-ol; 6, Galβ1,3GalNAc-ol.

RESULTS AND DISCUSSION

hCG was isolated from the urine of 10 women with normal pregnant women. hCG was also purified from urines of 2 patients, and from a pool of urine from 6 patients, with choriocarcinoma. The O-linked sugar units were released from the 10 pregnancy and 3 choriocarcinoma hCG preparations as oligosaccharitols (product of reduction of attachment sugar) by alkaline - borohydride β-elimination. The structures of oligosaccharitols were examined by gel filtration on columns of Bio-Gel P4. Fig. 1 shows the elution volumes of the oligosaccharitols from a preparation of hCG from a

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Abbreviations: GalNAc-ol, N-acetylgalactosaminitol; GalNAc, N-acetylgalactosamine; Gal, galactose; GlcNAc, N-acetylglucosamine; NeuAc, N-acetylneuraminic acid.

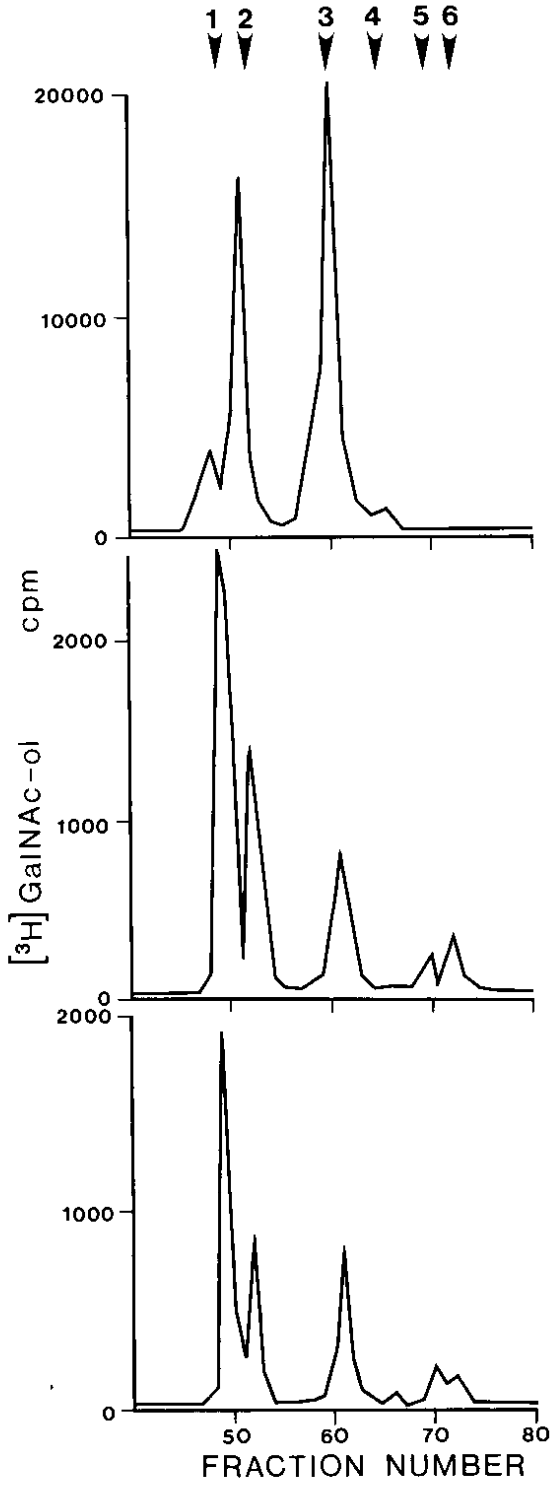

Fig. 1. Gel filtration on Bio-Gel P4 of the oligosaccharitols released by the β-elimination of pregnancy urine hCG (upper panel), choriocarcinoma urine hCG (middle panel) and hCG from pooled choriocarcinoma urine (lower panel). Arrows are the elution volumes of oligosaccharitol standards: 1, NeuAcα2-3Galβ1-3(NeuAcα2-3Galβ1-4GlcNAcβ1-6)GalNAc-ol; 2, NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc-ol; 3, NeuAcα2-3 Galβ1-3 GalNAc-ol; 4, NeuAcα2-6 GalNAc-ol; 5, Galβ1-3 (Galβ1-4GlcNAcβ1-6)GalNAc-ol; 6, Galβ1,3GalNAc-ol.

Bio-Gel P4. Fig. 1 shows the elution volumes of the oligosaccharitols from a preparation of hCG from a pregnant women (upper panel), from urinary hCG from an individual with choriocarcinoma (middle panel), and from choriocarcinoma pooled urine hCG (lower panel). The elution positions of the peaks were compared to those of standard oligosaccharitols (indicated by numbered arrows) of established structure. Of the sugar units from the urinary hCG from a pregnant women (Fig. 1, upper panel), 8% eluted in the position of the hexasaccharide (standard 1), 38% in the position of the tetrasaccharide (standard 2), 51% in the position of the trisaccharide (standard 3) and 3% in the position of the disaccharide (standard 4). Very similar results were obtained with the other 9 pregnancy urine preparations, the variation in the proportion of any peak not exceeding 8% of that of the sample shown. Whereas the trisaccharide was the principal sugar unit from the 10 hCG samples from urine of pregnant women, the hexasaccharide predominated in those from choriocarcinoma patient urines. Of the sugar units in the first choriocarcinoma urine hCG (Fig. 1, middle panel), 50% eluted in the position of the hexasaccharide (standard 1), 20% in the position of the tetrasaccharide (standard 2), 20% of the trisaccharide (standard 3), and none in the position of disaccharide (standard 4). Of this preparation, 4% eluted in the position of NeuAc-free tetrasaccharide (standard 5) and 6% with the NeuAc-free disaccharide (standard 6). The results were very similar with the second individual choriocarcinoma sample (elution pattern not shown), 56, 13, 23, 4 and 4% in the position of standards 1, 2, 3, 5 and 6, respectively, and with the sample from pooled choriocarcinoma urine (Fig. 1, lower panel), 50, 19, 24, 1, 4 and 3% in the position of standards 1, 2, 3, 5 and 6 respectively. A clear and consistent difference is apparent, most notably in hexasaccharide content, in the sugar units O-linked to pregnancy and choriocarcinoma hCG. Table 1 shows the hexasaccharide content of all preparations. A very significant difference ($P<0.0005$) was found in the hexasaccharide content of pregnancy and choriocarcinoma hCG. This demonstration of a consistent and statistically significant difference in the sugar structure on a glycoprotein in cancer patients vs. normal subjects indicates that a defined change (tri- to hexasaccharide structure) in hCG O-glycosylation accompanies carcinogenesis.

Interestingly, the principal difference in the O-linked sugar units on urinary hCG from

Table 1. Proportion of sugar units eluting in position of hexasaccharide (standard 1)

Sample	Proportion mean ± S.D.	(range)
hCG from pregnant women (10 samples)	6.0 ± 5.8%	(0 - 14%)
hCG from choriocarcinoma patient urine (3 samples)	52.0 ± 3.4%[a]	(50 - 56%)

[a] $P<0.0005$ vs. urinary hCG from pregnant women in 2-sided Student's t test

choriocarcinoma patients and pregnant women resides in the content of hexasaccharide, a structure to the best of our knowledge only previously identified on cancer glycoconjugates (7, 9, 10). The increased content of hexasaccharide could be the result of altered activity in the cancer cell of the enzyme which adds the GlcNAc to Galβ1-3GalNAc- (the substrate), β6-N-acetylglucosaminyltransferase (11), initiating hexasaccharide rather than trisaccharide formation. Alternatively, since NeuAcα2-3Galβ1-2GalNAc-, the trisaccharide is not a substrate of β6-N-acetylglucosaminyltransferase, the increased hexasaccharide content could be the outcome of diminished α3-sialyltransferase activity (the enzyme that competes with the β6-N-acetylglucosaminyltransferase substrate, converting it to the trisaccharide).

We are currently developing a hexasaccharide -specific monoclonal antibody for use in the assay of choriocarcinoma hCG. This antibody could be labeled and used together with an hCG-specific capture antibody in a 2-site immunoradiometric assay for choriocarcinoma screening and for the post-mole management of trophoblast disease. Our studies have been restricted to studying hCG from the extremes of normal pregnancy and choriocarcinoma. Further research is needed to examine sugar structures on invasive and benign mole hCG.

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