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DIFFERENT ASPARAGINE-LINKED SUGAR CHAINS ON THE TWO POLYPEPTIDE CHAINS OF HUMAN CHORIONIC GONADOTROPIN

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SUMMARY: Human chorionic gonadotropin (hCG) purified from placenta, like urinary hCG, is shown to have the sialylated forms of three neutral oligosaccharides: Galβ1→4GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc (N-1), Galβ1→4GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc (N-2) and Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc (N-3). Gel permeation chromatographic analysis of oligosaccharides released from α- and β-subunits of placental hCG has revealed that the α-subunit has one each of sialylated N-2 and N-3, while the β-subunit has one each of sialylated N-1 and N-2.

The structural complexities of the heterosaccharide prosthetic groups of glycoproteins suggest their participation in various phenomena that require cell specificity, such as homing mechanism (1), specific cellular adhesion (2) and differentiation process of multicellular organisms (3,4). Recent progress in studies of the clearance mechanism of various serum glycoproteins (5) and of the uptake of lysosomal enzymes by fibroblasts (6-8) have presented supporting evidence to the theory that sugar chains could serve as recognition sites which would be recognized by complementary protein structures on cell surface or of organelles. One of the biggest drawback to this theory is the phenomenon of microheterogeniety widely found in the carbohydrate moieties of glycoproteins.

Sugar chains of glycoproteins are synthesized by a concerted action of glycosyltransferases. Since no template as in the case of protein biosynthesis is included, the structures of the final sugar chains produced are determined by the specificity of each glycosyltransferase for a particular nucleotide sugar and for a particular glycose acceptor, and by its ability to synthesize a particular type of linkage. Therefore, microheterogeneity is now considered as

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an inherent characteristic of the carbohydrate moieties of glycoproteins (9). Although recent progress in studies of the biosynthesis of asparagine-linked sugar chains has revealed that the oligomannosyl core portion of these sugar chains are synthesized *en bloc* as a lipid bound intermediate and transfered to polypeptide chains (10-12), the processing pathway and outer chain elongation steps can produce partially completed sugar chains. Actually, the sugar chains of hen egg albumin (13-15) and bovine pancreatic ribonuclease (16), both of which have a single asparagine-linked sugar chain, were shown to be a mixture of a series of biosynthetic intermediates of asparagine-linked sugar chains. However, the experimental result reported in this paper indicates that the apparent phenomenon of microheterogeneity of sugar chains must in some cases (maybe in many cases) be interpreted with care, because the structures of sugar chains are distinct according to their location on polypeptide chain of glycoproteins with multiple carbohydrate attaching sites.

MATERIALS AND METHODS

Human chorionic gonadotropin (hCG, Lot. No.1279102) purified from human placenta and its α- (Lot. No.1329103) and β- (Lot. No.1439104) subunits were kindly supplied by Boehringer Mannheim Yamanouchi, Tokyo. All three samples were of more than 99% purity.

Hydrazinolysis of the glycoproteins was performed by the method previously reported (17) and the released oligosaccharides were reduced with NaB^3H_4 after *N*-acetylation.

Paper electrophoresis was carried out using pyridine-acetate buffer, pH 5.4 (pyridine:acetic acid:water=3:1:387). Bio-Gel P-4 (under 400 mesh) column chromatography was performed by using a column equipped with water jacket. During operation, the column was kept at 55°C by circulating warm water in the jacket. Differential refractometer R-403 (Waters Associates Inc., Framingham, Mass.) was used for monitoring standard glucose oligomers eluted from the column. The glucose oligomers were prepared by partial acid hydrolysis of dextran (18).

NaB^3H_4 (278 mCi/mmol) and sialidase from *Arthrobacter ureafaciens* (19) were purchased from New England Nuclear, Boston, Mass., and from Nakarai Chemicals, Ltd., Kyoto, respectively. Snail β-mannosidase (20), *Charonia lampas* α-fucosidase (21) and endo-β-*N*-acetylglucosaminidase D (22) were kindly supplied by Seikagaku Kogyo Company, Tokyo. α-Mannosidase (23), β-galactosidase (24) and β-*N*-acetylhexosaminidase (23) were purified from jack bean meal according to the cited references.

RESULTS AND DISCUSSION

When the tritium labeled oligosaccharide fraction released from placental hCG by hydrazinolysis was analyzed by paper electrophoresis, a pattern shown in

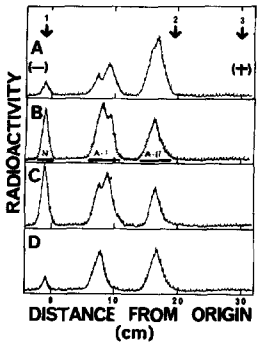

Fig. 1. Radioelectrophoretograms of the oligosaccharides liberated from hCG and its subunits. After reduction with NaB[^{3}H]$_4$, the oligosaccharide mixture was subjected to paper electrophoresis using pyridine-acetate buffer, pH 5.4, 80 V/cm for 90 min.

Arrows at the top of figure indicate the positions to which standards migrated: 1, lactitol; 2, 3'-sialyllactitol; 3, bromphenol blue. A, oligosaccharide released from urinary hCG by hydrazinolysis; B, those from placental hCG; C, those from the α-subunit of placental hCG; D, those from the β-subunit of placental hCG.

Fig. 1B was obtained. Peak N was a neutral oligosaccharide fraction and peaks A-I and A-II were mono- and disialyl-oligosaccharide fractions, respectively. This pattern was similar to that previously obtained from urinary hCG (17) (Fig. 1A). The only difference was that an almost negligible amount of neutral oligosaccharide fraction was detected in case of urinary hCG. This may indicate that desialylated hCG had been trapped by liver parenchymal cells while the hormone was circulating in the blood and that only the sialylated hCG was excreted through glomeruli. Since placenta is the place where hCG is produced, it is not surprising that both sialylated and nonsialylated hCG were included in the preparation. The oligosaccharide patterns obtained from α- and β-subunits of placental hCG are shown in Fig. 1C and D. Three oligosaccharide fractions N, A-I and A-II were also detected in both samples. The radioactivities of the three oligosaccharide fraction N (4.6×10^4 cpm), A-I (10.6×10^4 cpm) and A-II (8.0×10^4 cpm) obtained from 100 μg of placental hCG agreed with the calculated values (4.8×10^4 cpm, 10.2×10^4 cpm and 7.8×10^4 cpm, respectively) obtained by adding the values of each fraction from 34.8 μg of α-subunit (M.W. 16,000) and 65.2 μg of β-subunit (M.W. 30,000). Therefore, quantitative release of asparagine-linked sugar chains of three samples must have been obtained.

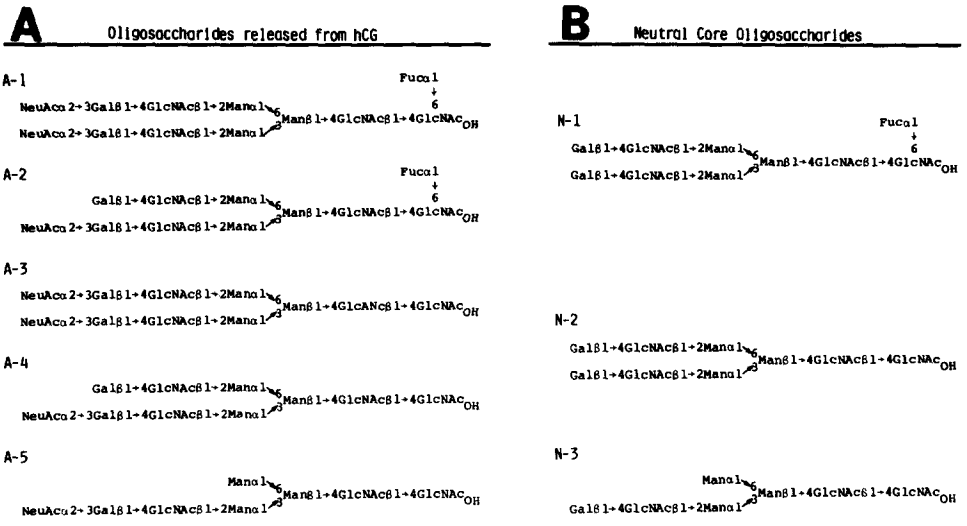

Fig. 2. Structures of asparagine-linked sugar chains of hCG (A) and their neutral core portions (B).

As already reported (17), the acidic peak corresponding to A-I obtained from urinary hCG was a mixture of A-2, A-4 and A-5 and that corresponding to A-II was a mixture of A-1 and A-3 listed in Fig. 2A. Probably because each fraction from placental hCG and its α- and β-subunits is also mixture of oligosaccharides, no clear cut difference was found between three samples by the study described so far. However, the following experiments revealed an interesting finding as to the distribution of asparagine-linked sugar chains in hCG molecule.

The radioactive oligosaccharide mixtures obtained from placental hCG and from its α- and β-subunits were converted to mixtures of neutral oligosaccharides by exhaustive sialidase digestion, and analyzed by Bio-Gel P-4 column chromatography. Three major oligosaccharide components (N-1, N-2 and N-3) were detected from the sample of placental hCG (Fig. 3A). The oligosaccharide pattern was almost the same as that obtained from urinary hCG, previously (17, not shown), indicating that the molar ratio of three neutral oligosaccharides, unlike the content of sialic acid, is rather constant and does not change from sample to sample. The structures of components N-1, N-2 and N-3 have previously been elucidated as shown in Fig. 2B by using urinary hCG (17). Sequential exo-

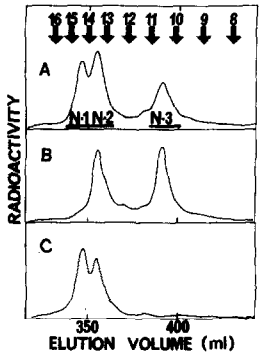

Fig. 3. Gel filtration patterns of the desialized oligosaccharide fractions. Radioactive neutral oligosaccharides were mounted on a Bio-Gel P-4 and eluted with distilled water. Effluent was fractionated 3.4 ml/tube, and the radioactivity in each tube was determined by liquid scintillation spectrometer. The black arrows indicate the eluting positions of glucose oligomers and *numbers* indicate the glucose units. A, oligosaccharide fraction obtained from placental hCG; B, that from the α-subunit of placental hCG; C, that from the β-subunit of placental hCG.

glycosidase digestion and endo-β-*N*-acetylglucosaminidase D digestion of each peak from placental hCG indicated that the three components N-1, N-2 and N-3 from this sample also have the structure as listed in Fig. 2B (data not shown).

Both α- and β-subunits of hCG contain two asparagine-linked sugar chains (25). Bio-Gel P-4 column chromatography of the desialized radioactive oligosaccharide fractions of two hCG subunits indicated that the α-subunit contains N-2 and N-3 in approximately equal amounts (Fig. 3B), while the β-subunit contains N-1 and N-2 (Fig. 3C). That the N-1, N-2 and N-3 released from the two subunits have the structures shown in Fig. 2B was also confirmed by sequential exoglycosidase digestion (data not shown).

Bahl analyzed the carbohydrate content of each subunit of hCG and found that the fucose residue is present only in the β-subunit(26). The complete absence of fucose in the α-subunit was also confirmed by Kennedy and Chaplin (27). Because the sum total of fucose-containing sugar chains (A-1 and A-2 in Fig. 2A) was approximately 25% (in mol) of the total oligosaccharides liberated from urinary hCG by hydrazinolysis, we have speculated that one of the two asparagine-linked sugar chains in the β-subunit may contain fucose (17). The data reported in this paper support all these previous estimation and has presented following two important subjects for future study.

The specific distribution of different sugar chains at four asparagine loci of hCG molecule cannot be explained by current knowledge of the biosynthetic mechanism of the asparagine-linked sugar chains. An unknown control mechanism such as steric effects coming from polypeptide moiety may also be included in the formation of asparagine-linked sugar chains of hCG, and this mechanism might also be working in the biosynthesis of other glycoproteins to guarantee the formation of sugar chains with fixed structure at the definite places on the polypeptide chain.

From the view point of the functional role of sugar chains in hCG, the strict distribution of different types of asparagine-linked sugar chain in hCG molecule is of particular interest. If the sugar chains in hCG were playing roles as signals, these four asparagine-linked sugar chains may be playing as different types of signal. Among them, N-3 might be interesting because it has an α-mannosyl residue as its non-reducing terminal and is not found to occur widely in other glycoproteins. Enzymatic removal of this α-mannosyl residue from intact hCG may reveal the functional role of the sugar chain of this glycohormone. An α-mannosidase suitable for this purpose must be found, because jack bean α-mannosidase, even though it has rather a wide aglycon specificity cannot remove the α-mannosyl residue from N-3 (28).

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