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Carbohydrate Composition of Human Placental *N*-Acetylhexosaminidase A and B

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The carbohydrate composition of *N*-acetyl-β-D-hexosaminidases (EC 3.2.1.52) A, B and heat-converted B was determined by g.l.c. Similar quantities of mannose, *N*-acetyl-glucosamine and galactose are present in the A and B isoenzymes, whereas *N*-acetyl-neuraminic acid is found in significant amount in only the A isoenzyme. The heat-converted hexosaminidase B also contains only trace amounts of *N*-acetylneuraminic acid, but is about 1.5-fold richer in mannose and *N*-acetylglucosamine and nearly 2-fold richer in galactose than native hexosaminidase B. Since native and converted hexosaminidase B are thought to be composed of four identical protein chains, our results suggest that there may be variable glycosylation of these chains.

N-Acetyl-β-D-hexosaminidase (EC 3.2.1.52) exists in animal tissues in a variety of isoenzymic forms. These enzymes are capable of hydrolysing the β-glycosidic linkage between both *N*-acetylglucosamine or *N*-acetylgalactosamine and various aglycones (Wenger *et al.*, 1972). In human tissues two major isoenzymes, hexosaminidases A and B, have been identified and characterized (Robinson & Stirling, 1968). Genetic and immunological data as well as direct chemical analysis of these enzymes (Lalley *et al.*, 1964; Geiger *et al.*, 1975; Geiger & Arnon, 1976; Lee & Yoshida, 1976; O'Brien, 1978) suggest that hexosaminidase A is a tetramer composed of two α and two β polypeptide chains, whereas hexosaminidase B is composed of four presumably homogeneous β chains (Geiger & Arnon, 1976). Isoelectric focusing of separated hexosaminidases A and B reveals microheterogeneity of each form (Hayase *et al.*, 1973; Geiger *et al.*, 1978). In addition to these major forms, a group of minor isoenzymes including hexosaminidases S, I_1, I_2, P and C have also been found in human tissues and body fluids under normal or pathological conditions (Price & Dance, 1972; Stirling, 1972; Braidman *et al.*, 1974; Ikonne *et al.*, 1975), but these forms have not been as extensively characterized.

Recent studies on the uptake *in vivo* and *in vitro* of lysosomal hydrolases including hexosaminidase point to a key role of the carbohydrate moieties in the recognition of these proteins by specific receptors and their subsequent endocytosis (Neufeld *et al.*, 1977; Kaplan *et al.*, 1977; Stahl *et al.*, 1976*a*,*b*, 1978). The delineation of the carbohydrate composition of lysosomal enzymes would be essential before detailed studies of the structural sequences of the carbohydrate moieties of these proteins. The present paper represents the first report of the sugar composition of hexosaminidases A and B from human placenta, based on direct chemical analysis of the pure enzymes.

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Experimental

Materials

Sephadex G-50 was purchased from Pharmacia (Uppsala, Sweeden). DEAE-cellulose (DE-52) was a product of Reeve Angel (Clifton, NJ, U.S.A.) and 4-methylumbelliferyl β-D-*N*-acetylglucosaminide was supplied by Koch–Light Laboratories (Colnbrook, Bucks., U.K.). Diaflo ultrafiltration device and membranes were obtained from Amicon Corp. (Lexington, MA, U.S.A.). Tri-Sil was purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.) and ovalbumin from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Methods

Human hexosaminidases A and B. These were purified to apparent homogeneity from pooled placentae as described previously (Geiger & Arnon, 1976). The specific activities of the isoenzyme preparations used here were 180 and 240 units/mg of protein for hexosaminidases A and B respectively. One unit of enzymic activity is defined as the amount of enzyme that hydrolyses 1 μmol of the 4-methyl-

umbelliferyl β-D-N-acetylglucosaminide substrate (2mM concentration)/min in 40mM-sodium citrate buffer, pH4.4 at 37°C. Protein determinations were carried out as described by Lowry *et al.* (1951), with bovine serum albumin as the standard.

Conversion of hexosaminidase A into 'converted isoenzyme B'. This was carried out by controlled heating of pure enzyme preparations. Pure hexosaminidase A (0.5mg in 4ml) in 0.1M-sodium phosphate buffer, pH6.0, containing 0.1M-$(NH_4)_2SO_4$ was heated for 60min at 50°C. The enzyme was then cooled in an ice bath and chromatographed through a 10ml Sephadex G-50 column pre-equilibrated with 10mM-sodium phosphate, pH6.0. Fractions containing enzyme activity were pooled and chromatographed on a 2ml column of DE-52 DEAE-cellulose, which was pre-equilibrated with the same buffer. Column fractions containing enzyme activity that was not adsorbed on to the DEAE-cellulose were pooled, concentrated with an ultrafiltration device fitted with a UM-10 membrane and frozen. This enzyme sample, referred to as 'converted hexosaminidase B', has the same isoelectric-focusing profile, molecular weight, indistinguishable antigenic characteristics and the same heat-stability properties as the native hexosaminidase B (B. Geiger, unpublished work). The two preparations of converted hexosaminidase B analysed in the present study showed 80 and 95% conversion of hexosaminidase A into hexosaminidase B, based on enzymic-activity measurements.

Carbohydrate analyses. Samples of homogeneous preparations of native hexosaminidases A and B as well as heat-converted hexosaminidase B were dialysed against 0.1M-NH_4HCO_3 buffer, pH8.0, for 12h. The enzyme preparations containing 40–400μg of protein were mixed with 1–5μg of arabitol in screw-cap vials and freeze-dried. The enzyme samples and a standard monosaccharide mixture were methanolysed with 0.5ml of 1.5M-HCl in methanol for 16–20h at 80°C. Samples were re-N-acetylated and dried *in vacuo* over KOH as previously described (Etchison & Holland, 1974). Each sample was extracted with 3 × 1ml of diethyl ether and dried again over KOH/P_2O_5. The methyl glycosides were converted into the trimethylsilyl ethers by mixing the dry sample with 50μl of Tri-Sil and incubating at room temperature (23°C) for 15min and then for 3min at 75°C. The volatile solvent and reagents were evaporated with a stream of dry nitrogen and the samples dissolved in 10–25μl of carbon disulphide. Samples (2–5μl) of the derivatives containing the equivalent of 12–40μg of original enzyme protein were injected into the gas chromatograph and analysed as previously described, except that the oven temperature increase was programmed at 2°C/min (Alhadeff & Freeze, 1977). To determine the reliability of the analyses of very small amounts of carbohydrate, samples containing 20–200μg of ovalbumin (1–10μg of total carbohydrate) and 0.5–5.0μg of arabitol were similarly analysed by following identical sample preparations and derivative-preparation procedures. The results of these analyses in terms of μg of sugar/100μg of protein, ± S.D., were: mannose 3.0 ± 0.3; galactose 0.45 ± 0.09; N-acetylglucosamine 1.54 ± 0.30.

Results and Discussion

The carbohydrate compositions of hexosaminidases A and B are similar, but the content of N-acetylneuraminic acid is about 6-fold higher in hexosaminidase A than in hexosaminidase B. These results are in good agreement with the values reported previously, which showed 1.6 residues of N-acetylneuraminic acid/molecule of hexosaminidase A and undetectable amounts in hexosaminidase B (Geiger & Arnon, 1976). The glucose contents of hexos-

Table 1. *Carbohydrate composition of human placental hexosaminidases*

Samples of homogenous preparation of native hexosaminidases A, B and heat-converted B were prepared and subjected to g.l.c. as described under 'Methods'. Results are means ± S.D. of three or four analyses. Values in parentheses are expressed as μg of sugar/100μg of protein.

		(Content mol/mol of glycoprotein)		
Sugar	Hexosaminidase ...	A*	B†	Converted B‡
Mannose		12.05 ± 0.73 (2.17)	14.2 ± 0.89 (2.37)	20.9 ± 1.17 (3.48)
Galactose		2.88 ± 0.97 (0.52)	2.10 ± 0.17 (0.35)	3.96 ± 0.40 (0.66)
Glucose		6.17 ± 2.00 (1.11)	1.92 ± 0.24 (0.32)	9.48 ± 0.35 (1.58)
N-Acetylglucosamine		6.48 ± 0.87 (1.46)	6.52 ± 0.58 (1.36)	9.60 ± 0.25 (2.00)
N-Acetylneuraminic acid		1.95 ± 0.60 (0.60)	0.31 ± 0.14 (0.09)	0.14 ± 0.12 (0.04)

* Average of three analyses in a single sample; mol.wt. 100000 (Geiger & Arnon, 1976).
† Average of four analyses of two samples; mol.wt. 108000 (Geiger & Arnon, 1976).
‡ Average of four analyses of two samples; mol.wt. 108000.

aminidases A and B are markedly different from each other, but these data must be interpreted cautiously, since the glucose may result from cellulose contamination and may not be covalently bound to the native oligosaccharides. The amounts of mannose and *N*-acetylglucosamine are nearly the same in both hexosaminidases A and B, whereas hexosaminidase A has about 1.5 times as much galactose as hexosaminidase B. Converted hexosaminidase B has a carbohydrate composition which appears to have about 1.5 times as much mannose and *N*-acetylglucosamine and nearly twice as much galactose as native hexosaminidase B. *N*-Acetylneuraminic acid is found in only trace quantities in converted hexosaminidase B. This result suggests that *N*-acetylneuraminic acid is probably bound only to the α subunit(s) of native hexosaminidase A.

Several observations suggest that the other component sugars are not equally distributed between the polypeptide chains. The carbohydrate composition of heat-converted hexosaminidase B is significantly different from that of native hexosaminidase B in spite of the fact that both have identical amino acid compositions. Furthermore, their molecular weights were shown to be identical on Sephadex G-200 as well as by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (B. Geiger, unpublished work). The increase in mannose, galactose and *N*-acetylglucosamine content of converted form B compared with native isoenzyme B can only result from an asymmetric distribution of these residues on the individual β chains. Variable glycosylation of apparently identical protein chains has been suggested for the β-D-glucuronidase of rat preputial glands (Tulsiani *et al.*, 1975) and demonstrated for bovine pancreatic ribonuclease (Plummer & Hirs, 1963). We do not know if the variable glycosylation of the β chain of hexosaminidase has physiological significance, but immediate glycosylation of the chains might exert a selective effect on the subsequent assembly of β chains into hexosaminidase A or hexosaminidase B. Alternatively, glycosylation of the assembled native enzyme may be influenced by the neighbouring α or β chains in hexosaminidase A or B respectively. Support for this comes from evidence suggesting that activity of human liver sialyltransferase may be influenced by the polypeptide backbone of the acceptor glycopeptides (Aguanno *et al.*, 1978). In contrast, the core sugars of ovalbumin (Kiely *et al.*, 1976) and immunoglobulin G (Beigman & Kuel, 1977) are added to these proteins while they are still associated with the ribosomes.

The relative molar proportions of mannose: galactose:*N*-acetylglucosamine found in hexosaminidases A, B and converted B (1.0:0.14–0.24: 0.50–0.60) resemble those reported for other glycosidases in different systems (Tulsiani *et al.*, 1975; Tomino *et al.*, 1975; Alhadeff *et al.*, 1978; Brot *et al.*, 1978; Himeno *et al.*, 1978), which have average molar proportions of mannose:galactose:*N*-acetylglucosamine of 1.0:0.2:0.5. These proportions are difficult to explain in terms of the typical oligosaccharide structures found in most serum glycoproteins (Kornfeld & Kornfeld, 1976), and may account for the specific recognition and subsequent endocytosis lysosomal hydrolases in several systems *in vitro* and *in vivo* (Neufeld *et al.*, 1977; Kaplan *et al.*, 1977; Stahl *et al.*, 1976*a*,*b*, 1978). Additional structural work is necessary before any speculation about the structure of these oligosaccharides, although analyses of some of the oligosaccharide chains of ovalbumin show proportions of mannose, galactose and *N*-acetylglucosamine (Yamashita *et al.*, 1978) similar to those found in these glycosidases.

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