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NORMAL GLYCOSAMINOGLYCAN PRODUCTION IN A GALACTOSYLATION-DEFECTIVE LECTIN-RESISTANT CHO CELL VARIANT

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Abstract

CHO cell variant clone 13 fails to incorporate galactose into either glycolipids or asn-linked oligosaccharides, even though intracellular UDP-galactose pools, galactosyltransferases, and galactose-acceptor macromolecules are normal. Clone 13 is now shown to produce normal amounts of chondroitin sulfate and heparan sulfate, the biosynthesis of which necessarily involves synthesis of a galactose-containing tetrasaccharide sequence. This is the first evidence of galactosyltransfer reactions which proceed normally in clone 13. Based on the known subcellular sites of glycosyltransfer reactions, it is proposed that a Golgi-specific defect involving compartmentalization may be responsible for the clone 13 trait.

Introduction

A variety of lectin-resistant variants have been isolated, many of which have well-defined surface carbohydrate alterations which affect the binding of lectins to the cell. Characterization of such variants has already led to major contributions to our understanding of how cell surface oligosaccharides are synthesized (1,2,3,4). One such variant is clone 13, which was selected from CHO cells for resistance to wheat germ agglutinin (5,6). Clone 13 is defective in its ability to incorporate galactose into either glycolipids or complex asn-linked oligosaccharides. The defect cannot be attributed to alterations in galactosyltransferase levels, UDP-galactose pools, or galactose-acceptor molecules, as all of these are normal in in vitro assays (5,6). Since the defect in clone 13 is already known to affect at least two distinct classes of galactose-containing glycoconjugates, it was of interest to determine whether other glycoconjugates are also affected. Glycosaminoglycans

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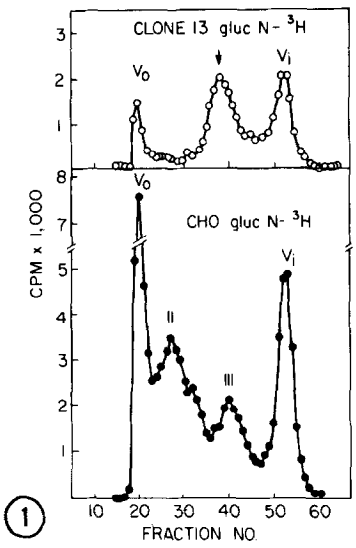


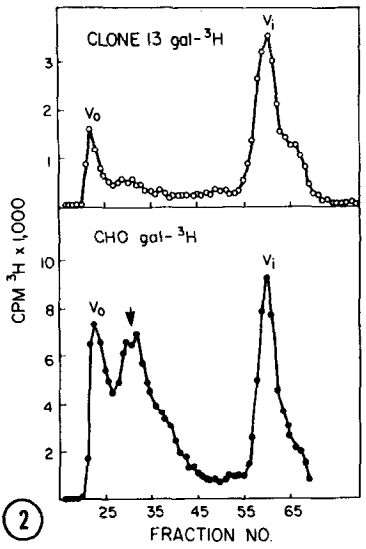


Figure 1

Bio-Gel P-10 elution profiles of [^{3}H]-glycopeptides prepared by Pronase digestion of [^{3}H]glucosamine-labeled cells. Upper panel, clone 13; lower panel, wild-type CHO cell glycopeptides.

Figure 2

Bio-Gel P-10 elution profiles of [^{3}H]-glycopeptides prepared by Pronase digestion of [^{3}H]galactose-labeled cells. Upper panel, clone 13; lower panel, wild-type CHO cell glycopeptides.

(GAGs)[1] were of particular interest since they are connected to serine residues of proteoglycan core proteins through a specific tetrasaccharide linkage sequence, glucuronosyl β1,3-galactosylβ1,3-galactosylβ1,4-xyloside, which is a biosynthetic prerequisite for assembly of the repeating disaccharide units of the GAGs (7). In this series of experiments, clone 13 is shown to produce normal amounts of the GAGs, chondroitin sulfate and heparan sulfate. This is the first indication of a class of galactosyltransfer reactions which proceed normally in clone 13, and provides a possible clue to the metabolic basis for the clone 13 trait.

[1] Abbreviations used: GAGs, glycosaminoglycans; HMW, high molecular weight; Δdi-4S, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose; HONO, nitrous acid.

Materials and Methods

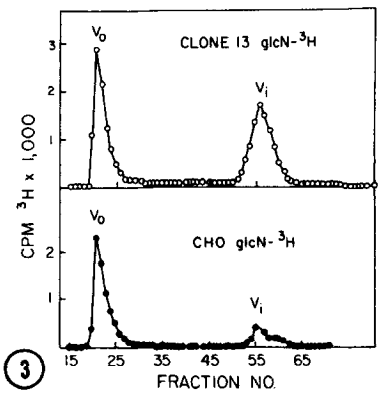
Cell culture conditions and the isolation of clone 13 from CHO cells have been described previously (5). Monolayer cultures in 150 mm Falcon dishes were labeled for 48 hr in 25 ml of complete growth medium plus 100 μC of [^{3}H] glucosamine or [^{3}H]galactose (New England Nuclear). The cells were rinsed three times with phosphate-buffered saline, then scraped into 4 ml of 50 mM Tris Cl, pH 8, containing 1 mg/ml Pronase (Calbiochem) and 2.5 mM $CaCl_2$. Pronase digestion proceeded at 37° in the presence of a few drops of toluene for 3 to 8 days, with fresh Pronase added daily. The digests were boiled and clarified by centrifugation prior to analysis by gel filtration through a 0.9 cm diameter x 100 cm high column of Bio-Gel P-10, 200-400 mesh (BioRad Laboratories), equilibrated with 0.15 M ammonium acetate, pH 6 or 0.1 M pyridine acetate, pH 5. Column fraction samplings were counted in Scintiverse (Fisher Scientific). Chondroitinase digestion was performed in 800 μl of 12.5 mM Tris Cl pH 8 containing 0.1 U chondroitinase ABC (Miles Laboratories), at room temperature for 24 hr. HONO degradation was performed as described by Shively and Conrad (8). Acid hydrolysis of presumed linkage sequence oligosaccharides was performed in 2 N H_2SO_4 for 4 hr at 100°. Descending paper chromatography was performed on Whatman 3MM paper in glacial acetic acid : n-butanol : 1 N NH_4OH (3:2:1), for analysis of chondroitinase digestion products, or pyridine : ethyl acetate : H_2O (1:3.6:1.15) (upper phase) for analysis of monosaccharides (5). Samples were mixed with nonradioactive authentic standards before application to the paper. Authentic 4,5-unsaturated disaccharides (Miles Laboratories) were visualized under UV light. Chromatograms were cut into 1-cm segments, soaked in 3 ml of water, mixed with 4 ml Scintiverse, and counted in a liquid scintillation counter.

Results and Discussion

Cells were metabolically labeled with [^{3}H]galactose[2] or [^{3}H] glucosamine[3] and subjected to extensive Pronase digestion. The resulting [^{3}H] glycopeptides were analyzed on a Bio-Gel P-10 column (Figs. 1 and 2). Labeled material eluting at the salt volume, V_i, was assumed to represent unincorporated metabolites. In the case of wild-type (lower panels, Figs. 1 and 2), high-molecular-weight (HMW) material eluting with the void volume (V_o) accounted for ∿33% of the incorporated label derived from either glucosamine or galactose. Peak II, labeled by both glucosamine (Fig. 1) and galactose (arrow, Fig. 2), probably represents complex glycopeptides, while Peak III, labeled

[2] UDP-[^{3}H]galactose derived from [^{3}H]galactose is in equilibrium with UDP-[^{3}H]-glucose via UDP-glucose-4'-epimerase. In wild-type cells, most of the [^{3}H]galactose supplied in complete medium is incorporated as galactose, but some is incorporated as glucose (e.g., glycolipids), or as other sugars metabolically derived from UDP-glucose (e.g., glucuronic acid, inositol, xylose). In clone 13, supplied [^{3}H]galactose is incorporated mainly as glucose and its derivatives (unpublished data).

[3] [^{3}H]glucosamine is metabolically incorporated as N-acetylglucosamine, N-acetylgalactosamine, and sialic acid.



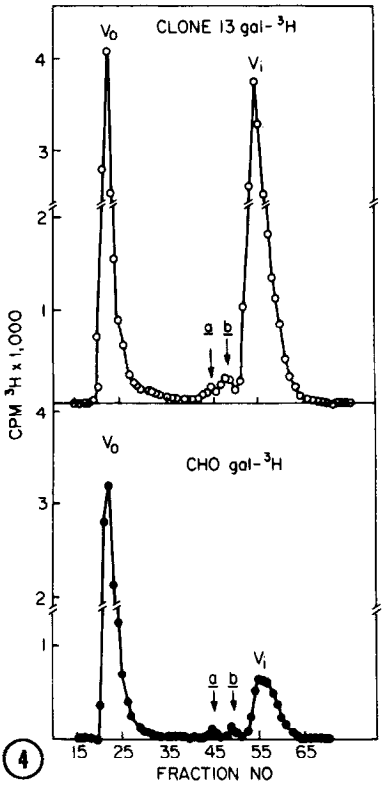


Figure 3

Bio-Gel P-10 elution profiles of [^{3}H]glucosamine-labeled HMW Pronase digest products (V_o from Figure 1), after treatment with chondroitinase ABC. Upper panel, clone 13; lower panel, wild-type.

Figure 4

Bio-Gel P-10 elution profiles of [^{3}H]galactose-labeled HMW Pronase digest products (V_o from Figure 2), after treatment with chondroitinase ABC. Upper panel, clone 13; lower panel, wild-type.

only with glucosamine, probably represents mannose-rich glycopeptides. In the case of clone 13 (upper panels, Figs. 1 and 2), HMW material accounted for ~22% of the incorporated glucosamine, and most of the incorporated galactose; the remaining incorporated glucosamine (arrow, Fig. 1) eluted in the same position as wild-type Peak III, and probably represents a mixture of mannose-rich glycopeptides and truncated complex glycopeptides lacking terminal sia-gal sequences (5). In the present studies, only the HMW material was analyzed further.

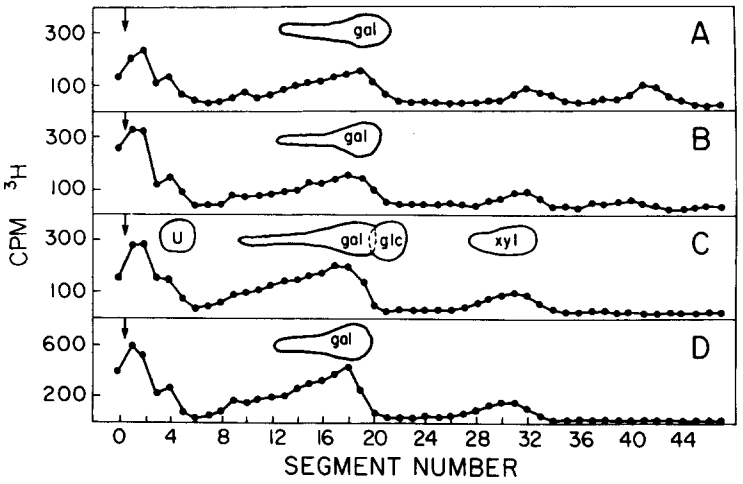


Figure 5

Paper chromatography of sugars released by acid hydrolysis from the "minor peaks" obtained after chondroitinase digestion of [^{3}H]galactose-labeled HMW material (peaks a and b from Figure 4). A, clone 13 peak b; B, clone 13 peak a; C, wild-type peak b; D, wild-type peak a. Arrows indicate origin positions. Radioactive sample lanes were cut into 1-cm segments for determining radioactivity. Positions of authentic standard monosaccharides, chromatographed on the same sheet in separate lanes between the radioactive samples, are indicated: U, glucuronic acid; gal, D(+)galactose; glc, D(+)-glucose; xyl, D(+)xylose.

The HMW material from each Pronase digest (V_o, Figs. 1 and 2) was treated with chondroitinase ABC, and the products were analyzed on the P-10 column (Figs. 3 and 4). Chondroitinase degraded ∿50% of the clone 13 HMW material, and ∿33% of the wild type HMW material, to small-molecular-weight products. In the case of galactose-labeled material, ∿9% of the label in the digest products was reproducibly resolved into two minor peaks, a and b, eluting just prior to the major small-molecular-weight peak at V_i (arrows, Fig. 4). These minor peaks were never observed in glucosamine-labeled material, and probably represent GAG linkage sequences attached to serine or small serine-containing peptides. Paper chromatographic analysis of sugars released from these presumed linkage sequences by acid hydrolysis revealed the presence of [^{3}H]galactose and [^{3}H]-xylose (Fig. 5). The major peaks eluting at V_i mainly consisted of Δ-di-4S disaccharides, characteristic digest products of chondroitin-4-sulfate (Fig. 6).

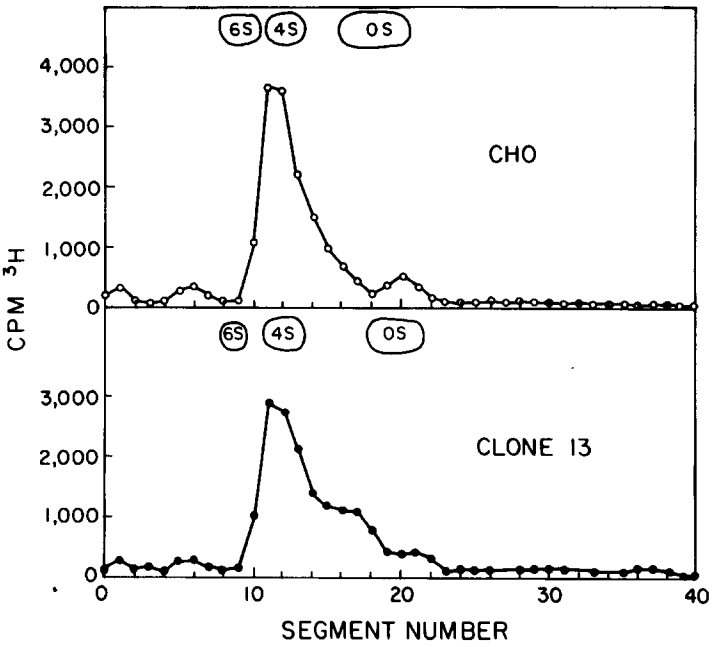


Figure 6

Paper chromatography of chondroitinase digestion products (V_i from Figure 4). The chromatogram was cut into 1-cm segments, and the radioactivity in each segment was determined. The positions of co-chromatographed authentic 4,5-unsaturated disaccharide standards are indicated: 6S, Δdi-6S; 4S, Δdi-4S; OS, Δdi-OS. Upper panel, wild-type; lower panel, clone 13.

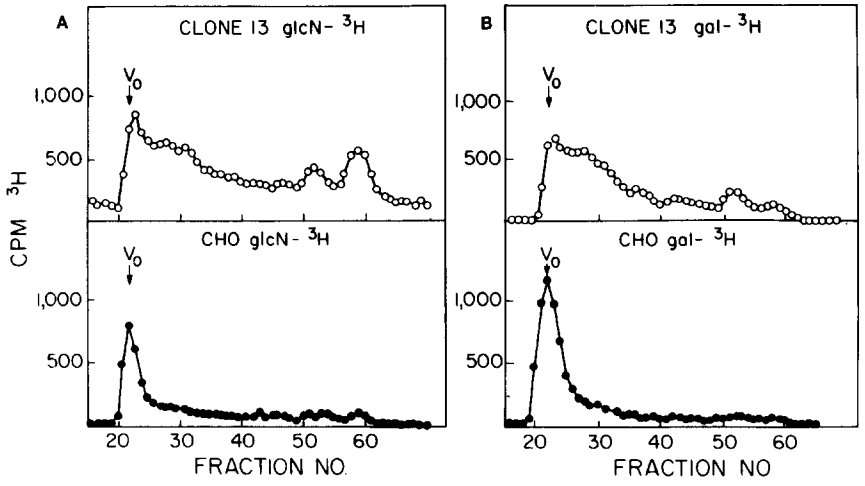


Figure 7

Bio-Gel P-10 elution profiles of chondroitinase-resistant HMW material (V_o from Figures 3 and 4), after treatment with HONO. A, [^{3}H]glucosamine-labeled material; B, [^{3}H]galactose-labeled material. Upper panels, clone 13; lower panels, wild-type.

The chondroitinase-resistant HMW material (V_o, Figs. 3 and 4) was next treated with HONO, and the reaction mixture was again analyzed on the P-10 column (Fig. 7). HONO cleaves heparan sulfate at glucosamine residues bearing free or N-sulfated amino groups (8). Since heparan sulfate also contains N-acetylated residues which are resistant to HONO, and since the variously-substituted glucosamine residues are more-or-less randomly distributed along the chain, the degradation products are a heterogeneous mixture of oligosaccharides with a broad size range. In the case of wild type, only ∿50% of the chondroitinase-resistant HMW material is susceptible to HONO, while essentially all of the clone 13 chondroitinase-resistant HMW material is susceptible.

From this data, it can be calculated that, for both clone 13 and wild-type CHO cells, heparan sulfate and chondroitin sulfate each account for ∿11% of the total incorporated label derived from glucosamine. We therefore conclude that the biosynthesis of these GAGs is unimpaired in clone 13 cells.

Substantial amounts of wild-type HMW material persists even after chondroitinase digestion and HONO treatment (V_o, Fig. 7). Since no appreciable amount of this material is found in clone 13, its biosynthesis is probably affected by the clone 13 defect. Preliminary results indicate that the material contains less than 10% sialic acid, and very little can be converted to smaller molecular size by mild alkali. Chromatography on DEAE-Sephacel resolves the material into at least three peaks. Although the material(s) have not yet been identified, it is likely that at least a portion consists of the asn-linked "erythroglycan"-like polysaccharide (9) which has recently been described for CHO cells (10).

The galactosyltransfer reactions involved in the assembly of glycolipids and complex asn-linked oligosaccharides occur in the Golgi apparatus (11). These reactions are known to be defective in clone 13 (5). In contrast, the galactosyltransfer reactions involved in the assembly of the linkage sequence of GAGs, which proceed normally in clone 13, take place in the rough endoplasmic reticulum (12,13). We therefore propose that there exists a Golgi-

specific compartmentalization problem of some kind which accounts for the clone 13 trait.

For example, a specific mechanism has been described for transporting UDP-galactose from the cytosol (where it is synthesized) into the Golgi lumen (15). In addition, a further specific mechanism has been described for removing the inhibitory product of the galactosyltransfer reaction, UDP, by the action of a luminally-oriented nucleotide diphosphatase (16,17), and subsequent transport of UMP out of the Golgi apparatus. A defect in these mechanisms could account for the clone 13 trait. Alternatively, the clone 13 defect may be due to improper insertion of the galactosyltranferases in the Golgi membrane, or there may be a defect in the mechanism which transports nascent glycoproteins through the Golgi apparatus.

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