GLYCOSAMINOGLYCAN-SYNTHESIZING ACTIVITY OF AN ISOLATED GOLGI PREPARATION FROM CULTURED MAST CELLS

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Summary

A Golgi fraction has been prepared from a population of cultured neoplastic mast cells previously shown to manufacture the glycosaminoglycan chondroitin 4-sulfate. This Golgi fraction was identified by electron microscopy and was also shown to have high galactosyl transferase activity, in common with Golgi fractions from liver and other tissues. When incubated with appropriate sugar nucleotide precursors together with oligosaccharide acceptors, the mast cell Golgi fraction demonstrated a significant enrichment in chondroitin-synthesizing activity over the homogenate and other membrane fractions. A similar incubation with endogenous acceptors gave a somewhat lower enrichment, demonstrating a probable rate-limiting effect of these acceptors. Products of both reactions were identified by chondroitinase ABC digestion. These results now give direct evidence indicating that the Golgi apparatus is the primary site for chondroitin biosynthesis in this cell type.

The Golgi apparatus has now been established as a major site for the synthesis of terminal carbohydrate portions of various glycoproteins (1,2). Several autoradiographic studies (3-5) have suggested that this organelle also has an important role in the biosynthesis of the polysaccharide (glycosaminoglycan) portion of proteoglycans, a special class of glycoprotein. Since this has not been established directly, the ability to isolate a Golgi fraction from a proteoglycan-forming tissue would be a significant aid in further characterizing the functional role of this organelle. Such a fraction could be used to demonstrate possible enrichment of glycosaminoglycan-forming enzymes.

Previous reports from this laboratory have concerned the use of cultured neoplastic mouse mast cells as model systems for the study of glycosaminoglycan biosynthesis (6,7). Such cells have been used to prepare microsomal

fractions capable of synthesizing the glycosaminoglycan portion of chondroitin 4-sulfate from sugar nucleotide precursors (7). The present study describes the preparation of a Golgi fraction from these cells and demonstrates that these fractions have a marked enrichment in chondroitin-synthesizing activity.

Experimental Procedure

The cells used in this study were originally derived from a solid Dunn-Potter P815 mouse mastocytoma and have been maintained in this laboratory for several years in continuous culture. Large volume spinner cultures (12 liters) of these cells were grown for 3-4 days as previously described (6), harvested by continuous flow centrifugation, and washed three times in phosphate-buffered saline to yield 5-10 ml of packed cells.

For subfractionation, 6 ml of the pooled cells were first suspended in an equal volume of homogenizing medium composed of 0.5 M sucrose in 0.1 M potassium phosphate buffer, pH 6.65, plus 0.005 M $MgCl_2$ (all subsequent sucrose solutions were made in this same buffer solution). The cells were then disrupted with 25-50 strokes of a tight-fitting glass Dounce homogenizer and the homogenate was fractionated by the following modification of the method of Leelavathi *et al.* (8) for preparation of Golgi fractions from rat liver. The homogenate was centrifuged for ten minutes at 2000 g and the yellowish, upper one-tenth of the resulting pellet was aspirated and combined with the entire 2000 g supernatant. This combined suspension was diluted by an equal volume of homogenization medium, and 4 ml portions were then layered over 8 ml of 1.7 M sucrose. The tubes were centrifuged for 60 minutes at 105,000 g (Beckman SW-41 rotor) to form a concentrated band ("membrane fraction") at the 0.5 M : 1.7 M interface. This membrane fraction was removed by aspiration, adjusted to 1.1 M in sucrose, and then diluted by the addition of two volumes of 1.1 M sucrose. Four ml of the membrane fraction was layered over 4 ml of 1.5 M sucrose, followed

by 4 ml of homogenizing medium. This gradient was then centrifuged at 105,000 g for 90 minutes (Beckman SW-41 rotor). The fluffy white band at the 0.5 M : 1.1 M interface (Golgi fraction) and the band at the 1.1 M : 1.5 M interface (mixed membrane fraction) were aspirated, suspended in homogenizing medium and pelleted by centrifugation at 105,000 g for thirty minutes.

For electron microscopy, pellets were fixed for two to four hours in glutaraldehyde, washed overnight in phosphate buffer, postfixed in osmium tetroxide, and then dehydrated and embedded in Spurr. Sections were examined and photographed on a Philips EM 300 electron microscope.

The protein contents of the homogenate and the various fractions were estimated by the Lowry procedure (9).

Galactosyl transferase activities in the various fractions were assayed by a modification of the method of Wagner and Cynkin (10), employing ovalbumin as an exogenous acceptor of [^{14}C]galactose from UDP-[^{14}C]galactose (New England Nuclear). Paper chromatography [Ethanol : Ammonium acetate (pH 7.8) 5 : 2 for 18 hours] was used for separation of reaction products and components. The 5'-nucleotidase activity was assayed with [^{3}H]AMP (New England Nuclear) as the substrate, followed by paper chromatography as above.

The incubation mixtures for determination of chondroitin-synthesizing activities contained the following ingredients in a total volume of 0.025 ml: 0.05 M MES, pH 6.5: 0.01 M $MnCl_2$; 0.0005 M UDP-[^{14}C]glucuronic acid (New England Nuclear), 20 x 10^6dpm/μmole; 0.001 M UDP-N-acetylgalactosamine [prepared by the method of Maley (11)] and a pentasaccharide, 0.003 M (prepared from chondroitin 6-sulfate by testicular hyaluronidase followed by β-glucuronidase). The homogenate and various fractions were incubated at 37°C for 3 hours. Other incubations were conducted without the added pentasaccharide in order to measure the incorporation of glucuronic acid into endogenous material.

Following incubation, the total reaction mixtures were applied to Whatman #4 paper and chromatographed in butanol : acetic acid : 1 M ammonium hydroxide, 2 : 3 : 1 overnight. The origins (which contained all the added oligosaccharides and all the endogenous glycosaminoglycan) were counted directly on a planchet counter. This direct counting yields an efficiency of 10% of dpm.

Results and Discussion

Electron microscopy of the Golgi pellet demonstrated a relatively homogeneous fraction composed almost entirely of elements derived from the Golgi apparatus. The fraction was largely composed of stacks of curved, parallel Golgi cisternae analogous to the "dictyosome" described in other tissues (12,13). The small amount of visible contamination (by lysosomes

Table I

Galactosyl Transferase and 5'-Nucleotidase Activities of Cell Fractions

Fraction	Total mg protein	nMoles [^{14}C]galactose transferred per mg protein per hour	nMoles [^{3}H]adenosine formed per mg protein per hour
Homogenate	1950	1.7	11.3
2,000g supernatant*	1590	1.5	13.3
Membrane fraction	350	3.3	5.3
Golgi pellet	4	120.0	277.3
Mixed membrane pellet	4	2.0	185.3

*Composed of the 2,000g supernatant combined with the upper one-tenth of the 2,000g pellet.

Table II

Chondroitin-Synthesizing Activity of Cell Fractions

Fraction	Total mg protein	nmoles [^{14}C]glucuronic acid incorporated per mg protein per hour	
		endogenous acceptor	oligosaccharide acceptor
Homogenate	1950	0.07	2.2
2,000g supernatant	1590	0.17	4.5
Membrane fraction	350	0.18	8.8
Golgi pellet	4	1.1	90.0
Mixed membrane pellet	4	0.57	4.9

or plasma membrane) was visually estimated to compose no more than 10-15% by volume of the entire fraction. The mixed membrane fraction appeared to be composed of pieces of plasma membranes, some fragmented tubules of the Golgi apparatus, and free ribosomes.

Galactosyl transferase and 5'-nucleotidase assays of the homogenate and various fractions are shown in Table I. The galactosyl transferase activity of the Golgi fraction was found to be enriched approximately 70 times over the homogenate. The Golgi preparations from these neoplastic mast cells therefore demonstrated the same enrichment for galactosyl transferase activity that distinguishes Golgi in fractions derived from other tissues such as liver, pancreas, and kidney (8,14,15).

Incorporation of [^{14}C]glucuronic acid into oligosaccharides and into endogenous acceptors is shown in Table II. The enrichment of the Golgi fraction was approximately 40 times over the homogenate for incorporation into oligosaccharides. However, the incorporation into endogenous acceptors

in the Golgi fraction was not as high as that shown with exogenous acceptors, so it would appear that the endogenous substrate may be limiting.

Samples of the radioactive oligosaccharides were incubated with chondroitinase ABC in order to identify the products after glucuronic acid incorporation. Radioactivity was recovered as a mixture of saturated and unsaturated disaccharides, respectively representing terminal uronic acid additions or addition of uronic acid with subsequent sugars added.

Chondroitinase ABC degraded all of the labeled endogenous polysaccharide. Unsaturated disaccharide represented essentially all of the recovered radioactivity, indicating polymerization of sugars to form chondroitin on endogenous acceptors as described previously (7).

The preparation of Golgi fractions from proteoglycan-synthesizing tissues has not been previously described. Preparation of such a fraction from cultured mast cells has now been achieved. The marked enrichment for chondroitin-synthesizing activity by this preparation indicates that the Golgi apparatus is indeed the primary site for formation of the glycosaminoglycan portion of chondroitin sulfate in this cell.

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