LOCALIZATION OF GLYCOSYL TRANSFERASE ACTIVITIES IN A GOLGI APPARATUS-RICH FRACTION ISOLATED FROM RAT LIVER[1]

D. James Morre', L. Marcel Merlin and T. W. Keenan

Departments of Botany and Plant Pathology, Biology and Animal Sciences, Purdue University, Lafayette, Indiana 47907 and Departement des Radioéléments, Commissariat à l'Energie Atomique, 91-Saclay, France

Received October 2, 1969

Summary

A Golgi apparatus-rich fraction isolated from rat liver catalyzed the transfer of galactose from UDP-galactose to N-acetylglucosamine with the formation of N-acetylaminolactose as well as the transfer of glucosamine from UDP-N-acetylglucosamine to endogenous protein acceptors. Based on enzymatic and morphological criteria, Golgi apparatus fractions were estimated to contain at least 80% Golgi apparatus-derived material. Approximately half of the total glycosyl transferase activities of the original homogenates was recovered in the Golgi apparatus fraction. The glycosyl transferase activities of purified endoplasmic reticulum fractions were much lower than those of the Golgi apparatus. Plasma membrane fractions as well as the soluble supernatant fraction contained little or no activity.

Progress in Golgi apparatus isolation has continued for several years (3,8,13,14,15,16,17,18) beginning with the work of Kuff et al. (see 10) but the isolation of the Golgi apparatus in a form suitable for detailed and reproducible biochemical analyses has not been reported. This paper presents a relatively simple technique for isolation of intact Golgi apparatus from rat liver which is applicable to other cell types. In addition, we demonstrate the localization of glycosyl transferase activities within the isolated fraction. These activities serve as marker enzymes for the rat liver Golgi apparatus and provide direct verification of the role of the Golgi apparatus in the synthesis of complex polysaccharides as suggested from autoradiographic studies (11,19).

---

[1]This work was supported by grants from the National Science Foundation (GB 1084, 03044 and 7078). Purdue University AES Journal Paper Number 3823.

Methods and Materials

Livers (approximately 10 g each) were drained of blood, minced thoroughly with scalpels and transferred to 20 ml of chilled homogenization medium (37.5 mM Tris-maleate, pH 6.4; 0.5 M sucrose; 1% dextran (Sigma, ave mol wt 225,000), 5 mM $MgCl_2$ and 5 mM mercaptoethanol at 0 to 4°. Fixatives were not used. Homogenization was for 30 to 60 seconds using a Polytron 20 ST (Kinematica, Lucerne, Switzerland) homogenizer (14) operated at the slowest speed. The homogenate was squeezed through a single layer of Miracloth (Chicopee Mills, New York) and centrifuged for 30 min at 2,000 g (Spinco SW50). The friable upper 1/3 of the 2,000 g pellet was resuspended in a small volume (< 1.0 ml for a 5.4 ml gradient volume) of supernatant and then layered onto the following discontinuous sucrose gradient: 1.8 M, 1.6 M, 1.5 M and 1.25 M sucrose in a v/v ratio to sample of 0.25:0.5:1:1. All gradient solutions were prepared in 37.5 mM Tris-maleate, pH 6.4; 1% dextran and 5 mM $MgCl_2$. The gradient was centrifuged for 30 min at 150,000 g (SW50) to remove mitochondrial and endoplasmic reticulum contamination. The Golgi apparatus (density 1.12 to 1.14) collected at the 1.25 M sucrose-homogenate interface. Contaminating fractions occupied lower bands. The band containing Golgi apparatus was removed from the gradient, resuspended and pelleted at 2,000 g for 30 min. The yield was 4 to 7 mg Golgi apparatus protein per 10 g liver.

Endoplasmic reticulum was obtained from the 2,000 g supernatant by layering over a discontinuous sucrose gradient consisting of 2.0 M sucrose and 1.5 M sucrose in a v/v ratio to sample of 0.15:0.3. The gradients were centrifuged for 0.5 to 3 hours at 150,000 g and the band at the 1.5 to 2.0 M sucrose interface was removed and resuspended in distilled water. Plasma membrane fractions were obtained according to Emmelot et al. (4). Unless indicated otherwise, male rats, 200 to 250 g (50 days old) of the Wistar strain were fed a potato diet for 3 days to 2 weeks prior to sacrifice.

Results

The yield and purity of the Golgi apparatus fraction was critically

dependent upon the homogenization procedure. With Polytron homogenization, large fragments of intact Golgi apparatus were obtained which sedimented at low centrifugal force. Other details of the procedure appeared less critical and the Mg^{++} and mercaptoethanol were included to preserve enzymatic activities

Initially, identification of isolated Golgi apparatus was based on their morphology which was so characteristic it served as a reliable marker (3,8,14). The degree of purity of the fractions suggested by electron microscope analyses of both thin sections and negatively stained preparations was verified by assays for enzyme activities known to be concentrated in other cell fractions (Table I). Mitochondrial contamination of the Golgi apparatus fractions was about 1% based on levels of succinic dehydrogenase.

TABLE I

5'-NUCLEOTIDASE AND GLUCOSE-6-PHOSPHATASE

Units of specific activity are μmoles inorganic phosphate/hr/mg protein ± standard deviation assayed after 10 or 15 minutes incubation at 37 ± 1° according to Emmelot et al. (4). Protein was estimated by the biuret method or by the method of Lowry et al. (12).

Fraction	Specific activity 5'-Nucleotidase	Glucose-6-phosphatase
Total homogenate	1.7 ± 0.1	2.6 ± 0.9
Endoplasmic reticulum	1.9 ± 0.2	12.1 ± 0.9
Golgi apparatus	3.2 ± 0.8	1.7 ± 0.4
Plasma membrane	54.0 ± 3.0	1.4 ± 0.9

A marker enzyme activity, UDP-galactose: N-acetylglucosamine galactosyltransferase (5), was concentrated in the Golgi apparatus fraction and was of much lower specific activity or absent from endoplasmic reticulum, plasma membrane and the microsomal supernatant (100,000 g, 3 hr) fractions (Table II). Evaluation of the activity for plasma membranes was complicated by a high level of nonspecific hydrolysis of UDP-galactose and the relatively long times required for preparation of this fraction. We recovered between 30

TABLE II

UDP-GALACTOSE: N-ACETYLGLUCOSAMINE GALACTOSYLTRANSFERASE

The reaction mixture contained 2 μmoles of Tris-HCl (pH 7.5), 1 μmole $MgCl_2$, 0.5 μmoles $MnCl_2$, 5 μmoles mercaptoethanol, 0.45 μmoles N-acetylglucosamine and 32.2 mμmoles (240 mc/mmole, Radiochemical Centre, Amersham, England, Radiochemical purity verified by paper chromatography as >95%) UDP-D-galactose-C^{14} (26,000 dpm) to which was added an aliquot of a suspension of cell fraction containing 0.15 to 0.65 mg protein in 0.5% Triton X-100 with a final volume of 0.2 ml. Incubations were at 37 ± 1° for 10 min. The reaction was stopped by placing the reaction mixture on an anion exchange column consisting of about 1 ml of packed BioRad AG_1-X_2 resin (Cl^- form) prepared in distilled water (20). N-acetylaminolactose and galactose were eluted from the columns with 3 x 0.4 ml washes with distilled water. The washes containing the radioactive products were collected in scintillator vials and radioactivity was determined by liquid scintillation spectrometry. To correct for nonspecific hydrolysis of UDP-galactose, a blank determination in the absence of N-acetylglucosamine (-HA) was subtracted. Dpm ± N-acetylglucosamine (± HA) and the accompanying specific activities are representative values from a single experiment. Average specific activities are from 3 to 8 determinations ± standard deviation. The unit of specific activity is mμmoles/hr/mg protein.

Cell Fraction	dpm +HA	dpm -HA	Sp. Act.	Average Sp. Act.
Total homogenate	405	233	2.4	2.4 ± 0.8
Golgi apparatus	5218	272	218.0	228.0 ± 11.0
Endoplasmic reticulum	278	198	3.0	3.6 ± 0.9
Plasma membrane	1934	1871	1.4	1.1 ± 1.0
Microsomal supernatant	180	171	0.1	0.2 ± 0.1
None	166		- -	- - - -

and 70% of the galactosyl transferase in the Golgi apparatus fraction with this fraction having an average specific activity nearly 100 times that of the total homogenate. Preparations from 10 different animals showed a similar distribution of enzyme activity with only slight differences comparing males with virgin females. When the enzymatic transfer of glucosamine from UDP-N- acetylglucosamine to endogenous protein acceptors was measured (Table III), the results were similar to those for the galactosyl transferase in the presence of exogenous acceptor (Table II).

Discussion

The rat liver Golgi apparatus *in situ* is composed of heterogeneous elements

TABLE III

ENZYMATIC TRANSFER OF GLUCOSAMINE-C^{14} FROM UDP-N-ACETYLGLUCOSAMINE- C^{14} TO ENDOGENOUS PROTEIN ACCEPTORS

Transfer of glucosamine-C^{14} from UDP-N-acetylglucosamine-C^{14} to protein was measured according to Wagner and Cynkin (23,24). The incubation mixture contained 80 µliters of suspended cell fraction (0.3 to 0.5 mg protein), 0.8 mµmole (15,000 cpm) UDP-N-acetylglucosamine-C^{14}, 0.5 µmole $MgCl_2$, 0.5 µmole EDTA and 4.0 µmoles Tris-maleate buffer (pH 7.1) in a final volume of 100 µliters. Incubations were at 37° for 15 minutes and terminated by the addition of 3 ml of 5% trichloroacetic acid. Washed precipitates were prepared for determination of radioactivity by liquid scintillation spectrometry as described previously (23,24). Animals were 200 to 250 g males of the Holtzman strain fed a Purina Laboratory Chow diet.*

Cell Fraction	Specific activity µµmoles/hr/mg protein	Percent of total activity
Total homogenate	7 to 9	100
Golgi apparatus	500 to 900	40 to 50
Endoplasmic reticulum	2	
Plasma membrane	0	
Microsomal supernatant	0	

*We wish to thank R. R. Wagner of Tufts University School of Medicine for conducting these analyses for us at Purdue University and R. W. Cheetham and W. N. Yunghans for supplying the endoplasmic reticulum and plasma membrane fractions.

i.e., plate-like regions of various types corresponding to the stacked cisternae seen in cross section, several kinds of tubules, intercisternal regions, secretory vesicles and the cisternal contents. All of these elements have been identified in the isolated preparations and the isolated Golgi apparatus appear to be representative of the Golgi apparatus of the intact cell (Morre', et al., to be published.) Since the differential centrifugation step selects for larger fragments, some fractionation is likely. Perhaps as much as 50% of the Golgi apparatus are lost in this manner (Tables II and III).

The galactosyl transferase which catalyzes the incorporation of galactose from UDP-galactose to glucose or N-acetylglucosamine with the formation of the corresponding disaccharide (lactose or N-acetylaminolactose), utilizes a defined acceptor of low molecular weight (20) and with the relatively simple assay system provides a useful marker enzyme for the rat liver Golgi apparatus.

The presence of this activity and its concentration in the hepatocyte Golgi apparatus supports the growing contention that milk lactose may be synthesized within the Golgi apparatus of lactating mammary cells (1,2). The enzyme which catalyzes the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to an endogenous protein acceptor (23,24) is also concentrated in the Golgi apparatus fraction. With both transferases (exogenous or endogenous acceptor) we recover on the average between 40 and 50% of the activity in the Golgi apparatus fraction (Tables II and III).

The N-acetylglucosamine and galactosyl transferases appear as only two of a number of similar glycosyl transferase activities associated with the Golgi apparatus fraction from rat liver. Schachter et al. (21,22, personal communication), have localized other sugar transferases in a Golgi apparatus-rich fraction when assayed in the presence of suitable mucopolysaccharide acceptors. Again, the isolated Golgi apparatus fraction exhibits the highest specific activity when compared to other cell fractions and the activity of the Golgi apparatus fraction accounts for approximately 40% of the total activity of the homogenates. These results plus the results reported here and those of Wagner and Cynkin (23,24) show for the first time a group of enzyme markers in the smooth membrane fraction of rat liver that are low or absent from the rough endoplasmic reticulum and plasma membrane (see also Hagopian et al. (7) for HeLa cells). The present report confirms their localization within the smooth membrane fraction and provides evidence consistent with localization in the Golgi apparatus.

Sugar transferases may be involved in either product or membrane transformations or both although the precise roles remain to be elucidated. One possibility is that certain of the secretory products being exported require additions of sugars as part of the secretory or assembly process. For example, Jones et al. (9) have suggested that the lipoproteins secreted by liver may not be pure lipoproteins but rather glycolipoproteins. A second possibility is that the production of plasma membrane-like secretory vesicles by the Golgi

apparatus (6) involves additions of sugars to the membrane.

Acknowledgements

A portion of this study was conducted in the laboratory of Prof. E. L. Benedetti, Institute de Biologie Moléculaire de la Faculté des Sciences de Paris during a sabbatical leave of absence from Purdue University by the senior author. We thank H. Schachter, University of Toronto for helpful suggestions concerning the galactosyl transferase assay procedure and Prof. Y. Cohen for the use of facilities and encouragement.

References

1. Brew, K., Nature 222, 671 (1969).
2. Coffey, R. G., and Reithel, F. J., Biochem. J. 109, 169, 177 (1968).
3. Cunningham, W. P., Morre', D. J., and Mollenhauer, H. H., J. Cell Biol. 28, 169 (1966).
4. Emmelot, P., Bos, C. J., Benedetti, E. L., and Rümke, P. H., Biochim. Biophys. Acta 90, 126 (1964).
5. Fleischer, B., Fed. Proc. 28, 404 (1969).
6. Grove, S. N., Bracker, C. E., and Morre', D. J., Science 161, 171 (1968).
7. Hagopian, A., Bosmann, H. B., and Eylar, E. H., Arch. Biochem. Biophys., 128, 387 (1968).
8. Hamilton, R. L., Morre', D. J., Mahley, R., and LeQuire, V. S., J. Cell Biol. 35, 53A (1967).
9. Jones, A. L., Ruderman, N. B., and Herrera, M. G., J. Lipid Res. 8, 429 (1967)
10. Kuff, E. L., and Dalton, A. J., in "Subcellular Particles" (Hayashi, T., ed.), 114. Academic Press, New York (1959).
11. Lane, N., Caro, L., Otero-Vilardebo, L. R. and Godman, G. C., J. Cell Biol. 21, 339 (1964).
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).
13. Mahley, R. W., Hamilton, R. L., Morre', D. J. and LeQuire, V. S., Fed. Proc. 27, 666 (1968).
14. Mollenhauer, H. H., Morre', D. J., and Bergman, L., Anat. Record 158, 313 (1967).
15. Morre', D. J., Cheetham, R. and Yunghans, W., J. Cell Biol. 39, 96A (1968).
16. Morre', D. J., and Mollenhauer, H. H., J. Cell Biol. 23, 295 (1964).
17. Morre', D. J., Mollenhauer, H. H. and Chambers, J. E., Exptl. Cell Res. 38, 672 (1965).
18. Morre', D. J., Mollenhauer, H. H., Hamilton, R. L., Mahley, R. W. and Cunningham, W. P., J. Cell Biol. 37, 157A (1968).
19. Neutra, M. and LeBlond, C. P., J. Cell Biol. 30, 119, 137 (1966).
20. Palmiter, R. D., Biochim. Biophys. Acta 178, 35 (1969).
21. Schachter, H., McGuire, E. J., and Roseman, S., Proc. Canadian Fed. Biol. Soc. 10, 78 (1967).
22. Schachter, H., Jabbal, I., and Roseman, S., Proc. Canadian Fed. Biol. Soc., 12, 77 (1969).
23. Wagner, R. R., and Cynkin, M. A., Arch. Biochem. Biophys. 129, 242 (1969).
24. Wagner, R. R., and Cynkin, M. A., Biochem. Biophys. Res. Comm., 35, 139 (1969).