# LOCALIZATION OF CEREBROSIDE - SULFOTRANSFERASE ACTIVITY IN THE GOLGI APPARATUS OF RAT KIDNEY

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Received April 16,1973

SUMMARY

A Golgi-rich fraction that contains both uridine diphosphogalactose: N-acetylglucosamine galactosyltransferase activity and 3'-phosphoadenosine-5'-phosphosulfate:cerebroside sulfotransferase activity has been isolated from rat kidney. Both activities are increased about 80-fold in the Golgi fraction compared to the homogenate. Little or no galactosyltransferase or sulfotransferase activity was found in purified nuclei, mitochondria, rough endoplasmic reticulum, plasma membranes and supernatant. The results indicate that both galactosyltransferase and sulfotransferase are localized in Golgi apparatus from rat kidney. This is the first evidence that Golgi apparatus functions to modify a lipid component of the cell.

Cerebroside-sulfotransferase is the enzyme responsible for the transfer of sulfate from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to cerebroside to form sulfatide. This activity has been shown to be present in microsomes prepared from rat (McKhann et al., 1965) and mouse (Sarlieve et al., 1971) kidneys and in smooth microsomes from rat brain (Farrell and McKhann, 1971). Since microsomes represent a heterogeneous collection of membranes derived from the endoplasmic reticulum, Golgi apparatus and plasma membranes of the cell, the exact cellular localization of cerebroside-sulfotransferase remains to be defined. This study is concerned with the subcellular localization of this enzyme in kidney. In addition to purified nuclei, mitochondria, plasma membranes and smooth and rough microsomes, we have isolated and characterized for the first time a Golgi-rich fraction from rat kidney. The cerebroside-sulfotransferase activity of these fractions has been determined. In addition, the distribution of uridine diphosphogalactose:N-acetylglucosamine, galactosyltransferase, an enzyme which has been shown to be a



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marker enzyme for Golgi apparatus in bovine (Fleischer et al., 1969) and rat (Morre et al., 1969); Fleischer and Fleischer, 1970) liver, was determined. The results indicate that both sulfotransferase and galactosyltransferase are localized in Golgi apparatus from rat kidney.

## MATERIALS AND METHODS

Male Holtzman rats, 200-250 gms, fed ad libitum were used throughout. The kidneys were fractionated into purified nuclei, mitochondria, rough and smooth microsomes and plasma membranes as described previously for rat liver (Fleischer and Fleischer, 1970). Golgi-rich fractions were prepared from kidney homogenates by a one-step zonal procedure slightly modified after that described previously for rat liver (Fleischer and Fleischer, 1970, and Van Golde et al., 1971).

Protein was determined by the Lowry procedure (Lowry et al., 1951) using crystalline bovine serum albumin as a standard. $Mg^{++}$-stimulated ATPase, rotenone-insensitive NADH-cytochrome c reductase and succinate-cytochrome c reductase activities were carried out as previously described (Van Golde et. al., 1971). Glucose-6-phosphatase was determined according to Swanson, 1955, except that incubations were made for five and ten minutes, and the phosphate released measured by the method of Chen et al., 1956. Galactosyltransferase was determined as described previously (Fleischer and Fleischer, 1970). Acid phosphatase was measured by the method of DeDuve et al., 1955, except that inorganic phosphate was measured by the method of Chen et al., 1956.

Cerebroside-sulfotransferase was measured essentially by the procedure of Farrell and McKhann, 1971, modified so that small amounts of enzyme (about 100 µg protein) could be used. Carrier PAPS was prepared from ATP and $Na_2SO_4$ by the method of Hodson and Schiff, 1969, using extracts of Chlorella pyrenoidosa as a source of sulfate-activating enzyme. $^{35}S$-PAPS (approximately 1 C/mmole) was purchased from New England Nuclear Corporation and diluted with carrier to a specific activity of approximately 1 mc/mmole. Mixed bo-

vine cerebrosides (Supelco Inc., Bellefonte, Penn.) were added to the assay as sulfate acceptor. In some experiments, psychosine or glucocerebrosides (Supelco) were used instead. Lipid acceptors were first suspended at 10 mg/ml in water containing 1% (w/v) Triton X-100 using a small Potter-Elvejhem homogenizer. For electron microscopy, fractions were fixed as obtained from the zonal rotor by mixing with an equal volume of 5% glutaraldehyde in 0.1 M cacodylate pH 7.4. After standing overnight in the refrigerator, the sample was centrifuged at 10,000 rpm for 5 - 10 min and the supernatant discarded. The pellet was washed with 0.25 M sucrose containing 0.1 M cacodylate pH 7.4 several times by decantation in the cold, treated with 1% osmium tetroxide, dehydrated, embedded, and sectioned as described previously (Fleischer and Fleischer, 1970).

## RESULTS

A Golgi-rich fraction from rat kidney, has been isolated using procedures developed for the isolation of Golgi-rich fractions from rat liver (Fleischer and Fleischer, 1970). The fraction is rich in morphologically identifiable Golgi apparatus (Fig. 1). About 70% of the material consists of stacked cisternae with attached tubules, a structure which is typical of Golgi apparatus. The major contaminant appears to be large smooth-surfaced membranes probably derived from plasma membranes. The fraction is enzymatically unique compared with other purified cell fractions (Table 1). The Golgi-rich fraction from kidney is enriched with cerebroside sulfotransferase and with galactosyltransferase activities to about the same extent as compared to the homogenate. These activities appear to be localized in the Golgi apparatus, since, of the other fractions, only smooth microsomes show a significant level of both of these activities. The activity found in smooth microsomes probably reflects the presence of Golgi-derived membranes in this fraction equivalent to about 8% of the protein. The Golgi fraction from rat kidney can be distinguished from endoplasmic reticulum by its low level of glu-

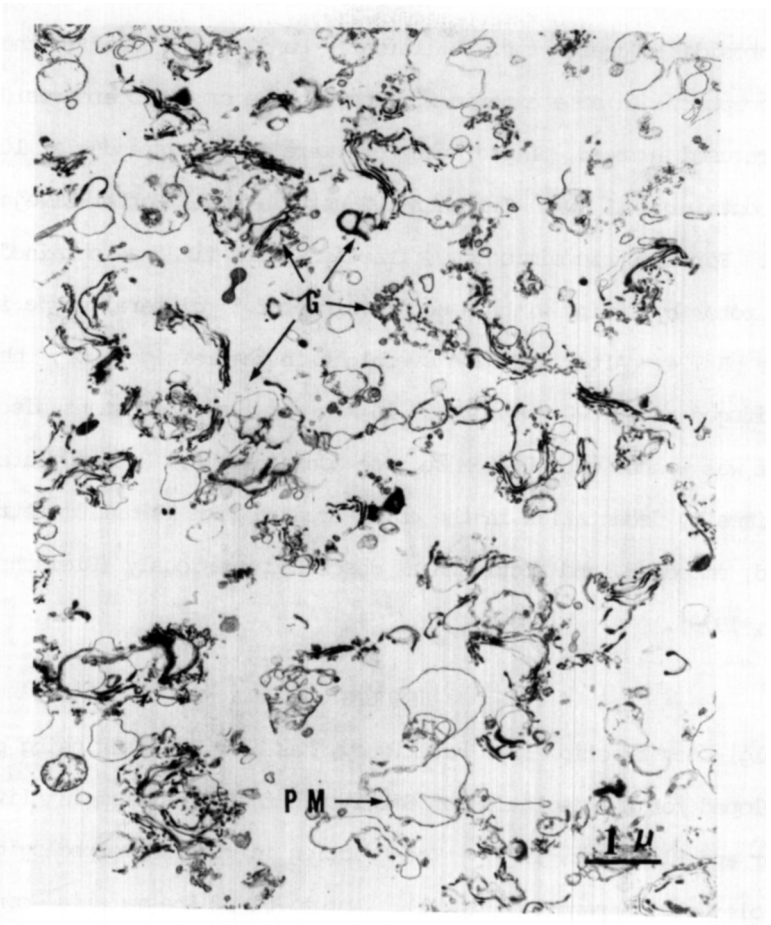


Fig. 1. Electron micrograph of a Golgi-rich fraction from rat kidney. An average of about 70% morphologically identifiable Golgi apparatus (G) was observed in a number of fields. The main contaminant appears to be plasma membranes (PM). Optical magnification x 12,500. The bar equals 1 μ.

cose-6-phosphatase and from plasma membrane by its low level of ATP'ase. Isolated rat liver Golgi apparatus, although very active in galactosyltransferase activity, (374 nmoles/hr/mg protein), showed no detectable sulfotransferase activity.

The sulfotransferase of the Golgi fraction appears to be specific for galactosyl ceramides as compared to glucosylceramide (Table II). Psychosine (galactosyl sphingosine) can also serve as an acceptor of sulfate from 3'-phosphoadenosine-5'-phosphosulfate.

Table I

Distribution of Sulfotransferase and Other Marker Enzymes in

Purified Kidney Subcellular Fractions

| Fraction | Cerebroside Sulfotransferase*† | Galactosyl Transferase*† | Glucose-6 P'ase† | ATPase** | Succinate Cyt. c red. | Acid P'ase |
|---|---|---|---|---|---|---|
| Homogenate | 0.2 | 7.2 | 0.06 | 0.15 | 0.19 | 0.03 |
| Nuclei | 0.0 | 3.9 | 0.13 | 0.09 | 0.04 | - |
| Mitochondria | 0.0 | 1.9 | 0.02 | 0.59 | 0.90 | 0.03 |
| Rough Microsomes | 0.2 | 3.6 | 0.18 | 0.27 | 0.01 | 0.01 |
| Smooth Microsomes | 1.3 | 38 | 0.31 | 1.0 | 0.01 | - |
| Golgi Apparatus | 14.0 | 650 | 0.05 | 0.28 | 0.01 | 0.03 |
| Plasma Membrane | 0.0 | 1.2 | 0.04 | 1.1 | 0.09 | 0.01 |
| Supernatant‡ | 0.0 | 0.0 | - | - | - | - |

*Activities expressed as nmoles/hr/mg protein, all others in μmoles/min/mg protein

**90% of this activity is sensitive to 1 mM oligomycin when mitochondria are used. The activity in the other fractions are < 10% sensitive.

†At 37°, all others at 32°

‡Supernatant after removal of microsomal fraction at 105,000 x g for 1 hour

Table II

Substrate Specificity of the Sulfotransferase of Rat Kidney Golgi

| Substrate added | Specific Activity |
|---|---|
| none | 0.3 |
| galactosyl cerebrosides | 15.7 |
| glucosyl cerebroside | 0.0 |
| psychosine | 4.7 |

Specific activity is expressed as nmoles sulfate transferred to lipid fraction per hour per mg protein.

## DISCUSSION

Galactosyl ceramide (cerebroside) is generally considered to be the *in vivo* precursor of sulfated galactosyl ceramide (sulfatide) (McKhann and Ho, 1965; Stoffyn, Stoffyn and Hauser, 1971) although the *in vitro* sulfation of psychosine (galactosyl sphingosine) has also been demonstrated (Cumar *et al.*, 1968; Nussbaum and Mandel, 1972). Cerebroside-sulfotransferase from rat brain can utilize both substrates, *in vitro* (Farrell and McKhann, 1971). This appears to be also true of the enzyme in rat kidney Golgi. Glucosyl cerebrosides, however, do not act as acceptors for the sulfotransferase from either source.

Evidence that cerebroside-sulfotransferase is the enzyme responsible for the biosynthesis of sulfatide rests on the close correlation of the level of this enzyme in a tissue with the level of sulfatide found in that tissue. Thus, in developing rat brain, the appearance of cerebroside-sulfotransferase precedes somewhat the period of onset of myelination, when sulfatide accumulates in brain (McKhann and Ho, 1967). Liver, which contains little or no sulfatides (Kwiterovich *et al.*, 1970) also shows little or no sulfotransferase

activity (Nussbaum and Mandel, 1972), whereas kidney shows both a high sulfatide and high sulfotransferase activity. The sulfatide formed in vitro from added galactosyl ceramide by the enzyme from kidney microsomes has the same structure as natural sulfatide (Stoffyn et al., 1971). In bovine kidney, the highest concentration of sulfatide is found in the medulla, but sulfatide is also present in the cortex (Karlsson et al., 1968). As expected from the low sulfatide content of liver, cerebroside-sulfotransferase is not detectable in liver Golgi. Since kidney is a very heterogeneous population of cell types, it may be that not all cell types in kidney contain this enzymatic activity.

In the present studies we have shown that the Golgi apparatus of kidney, like that of liver, is the locus of galactosyltransferase activity. This enzyme is involved in the modification of glycoproteins during secretion. Our results indicate, in addition, that the Golgi apparatus of rat kidney is capable of modifying lipids such as cerebrosides to form sulfatide. This is the first direct evidence that Golgi functions in mammalian cells not only to transport and modify secretory products, but also to modify a lipid which ultimately becomes a component of the membranes of the cell.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Akitsugu Saito for the excellent electron microscopy and Mrs. Ikuko Ishii for able technical assistance in this work. The advice and encouragement of Dr. Sidney Fleischer in the course of this work is also gratefully acknowledged. We also wish to thank Dr. Jerome Schiff for his gift of $^{35}$S-PAPS and for a starting culture of Chlorella pyrenoidosa. Supported in part by USPHS, NIH Grant AM14632 and an USPHS International Fellowship to F.Z.

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