Immunochemical Identification of Very Low Density Serum Lipoproteins in Golgi Apparatus from Rat Liver[1]

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In mammalian systems, lipids are carried in the circulation as complex lipoproteins with the liver being the principal site of fatty acid absorption and conversion to lipoprotein triglyceride. The lipoproteins facilitate transport from liver to extrahepatic tissue where the triglycerides are utilized. Alternatively, they may accumulate in the circulation and contribute to cardiovascular disease such as atherosclerosis.

Of the various lipoprotein classes found in plasma (10), very low density lipoproteins (VLDL) have a density of less than one, a diameter of 300 to 800Å and a triglyceride content of 50% or more. This form of lipoprotein is a major vehicle for triglyceride transport and has been consistently implicated in the formation of pathological lipid deposits in blood vessels (2). When livers are perfused *in situ* with free fatty acids (FFA), the major portion of the esterified fatty acids released appear as triglycerides in lipoproteins of the VLDL fraction (3).

Studies on the sites of lipoprotein production generally have implicated the Golgi apparatus in the assembly and secretion of lipoproteins (1, 3, 5, 6, 11). Osmiophilic particles having diameters near those of VLDL were observed in electron micrographs of rat livers perfused with FFA, whereas these particles were much less prevalent in micrographs of non-perfused livers (3). This report introduces immunochemical evidence in support of the hypothesis that the Golgi apparatus is a site of VLDL assembly and secretion.

Materials and Methods

The first step in immunochemically linking VLDL with the Golgi apparatus was to isolate VLDL from rat serum by a flotation centrifugation method adapted from the procedure of Lindgren et al. (8). The animals, 200 to 250 g male rats (Holtzman Company, Madison, Wisconsin), were fasted for 24 hours before bleeding to reduce the numbers of chylomicra in the plasma. VLDL was isolated from plasma as a floating fraction after prolonged centrifugation (20 hours at 35,000 rpm, Spinco # 40 rotor) through saline solution (density < 1.006). The isolated VLDL was then used as an antigen to stimulate the synthesis of VLDL antibody in rabbits. For this purpose, VLDL suspended in Freund's complete adjuvant was injected intramuscularly into white male rabbits. Three weeks after the first injection, the rabbits were

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challenged with an intravenous injection of freshly prepared VLDL without adjuvant. After an additional 2 weeks, the rabbits were bled, an antiserum fraction containing the VLDL antibody was separated and a titer determination was made by serial dilution using the precipitin interface ring test. The precipitin interface ring test was also used to detect VLDL in cell fractions of rat liver prepared by low shear homogenization (Polytron) and differential and sucrose density gradient centrifugation (9).

All fractions were tested for a precipitin reaction with VLDL antiserum after their resuspension in distilled water alone or after subjection to a series of 3 freezing and thawing cycles to release VLDL from membrane-bounded compartments.

Results

Rat serum and purified VLDL subjected to freezing and thawing gave a normal precipitin reaction with the VLDL antiserum (Table 1) showing that immunochemical reactivity was not altered by the freeze-thaw cycles. Of the several fractions tested using the precipitin interface ring test, VLDL was conclusively demonstrated only in purified Golgi apparatus which had been frozen and thawed to disrupt secretory vesicles. Double diffusion analysis in Ouchterlony plates against the antiserum gave a precipitin line similar to native VLDL with purified Golgi apparatus fractions. The total homogenate, a mitochondria-microbody-lysosome fraction, purified endoplasmic reticulum and a supernatant fraction were immunochemically inactive both before and after the freeze-thaw cycles. Both the frozen and thawed crude Golgi apparatus fraction and the treated purified Golgi apparatus fraction were marginally reactive which suggested small quantities of immunochemically accessible VLDL.

TABLE 1.

Immunochemical Evidence for Distribution of Very Low Density Lipoproteins Among Rat Liver and Plasma Fractions

Source of Antigen	Positive Prescription Test with VLDL Antibody Showing Presence of VLDL Antigen	
	Untreated	Frozen-Thawed
PLASMA FRACTIONS		
Serum	+	+
Purified VLDL	+	+
LIVER FRACTIONS		
Total Homogenate	—	—
Microsomal Supernatant	—	—
Mitochrondria-Microbody-Lysosome	—	—
Endoplasmic Reticulum	—	—
Crude Golgi Apparatus	—	±
PURIFIED GOLGI APPARATUS	±	+

Particles similar to those described by Hamilton et al. (3) were present in the isolated and purified Golgi apparatus (Fig. 1). Similar particles were not observed in the endoplasmic reticulum or mitochondrial fractions from the same sucrose gradients.

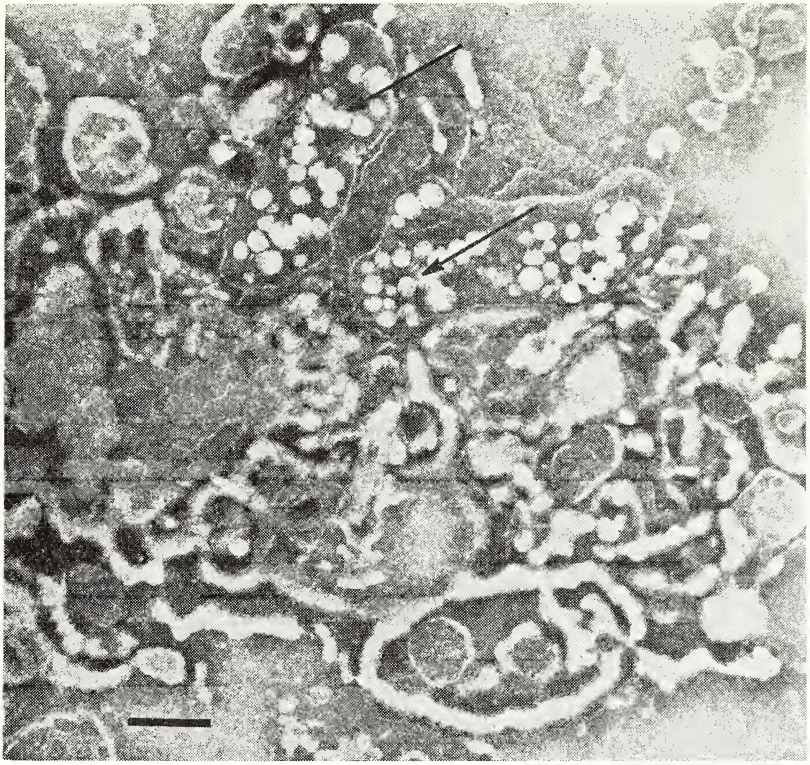

Figure 1. Electron micrograph of a portion of an isolated hepatocyte Golgi apparatus negatively stained with 1% phosphotungstic acid, pH 6.7. Clusters of small particles tentatively identified as very low density lipoprotein and surrounded by the limiting membrane of a secretory vesicle are shown by the arrows. Bar = 0.5 μ.

Discussion

The apoprotein of VLDL is probably synthesized, like other proteins for export, from messenger RNA associated with polyribosomes of the granular endoplasmic reticulum. Most of the 300 to 800 Å particles appear first in the smooth endoplasmic reticulum (6) which seems to correspond at least in part to the system of peripheral tubules and plate-like structures which are continuous with the Golgi apparatus cisternae (Fig. 1). These membranes are also considered as sites of triglyceride synthesis (6). During lipoprotein assembly, the conformation of the β-lipoprotein is altered (7) and it is possible that the tubular membrane system provides a mechanism facilitating the conformation changes attendant to lipoprotein synthesis. Antiginicity depends upon

conformation as well as chemical composition (7). Our failure to demonstrate conclusively VLDL in the total homogenate or crude Golgi apparatus fractions presumably is due to the dilution by extraneous protein of the small amounts of VLDL present. A similar explanation may account for the inability to associate VLDL with the supernatant and purified endoplasmic reticulum fractions. However, it is also possible to explain the unreactivity of the endoplasmic reticulum on the basis that the protein moiety of β-lipoprotein may exist in more than one antigenic state depending on conformation (7).

These observations are consistent with a role of the Golgi apparatus in VLDL assembly involving synthesis of the lipid moieties and conformation changes in the apoprotein. Since lipoproteins may actually be glycolipoproteins, it is conceivable, as suggested by Jones et al. (6), that the Golgi apparatus plays a further role in lipoprotein synthesis by addition of the carbohydrate moiety.

Summary

Particles tentatively identified as very low density lipoprotein (VLDL) were observed in electron micrographs of Golgi apparatus isolated from rat liver. Using immunochemical methods, only the Golgi apparatus obtained by centrifugation in a sucrose gradient gave a positive immunochemical precipitin reaction with antiserum prepared against purified plasma VLDL. Crude liver homogenates, purified mitochondrial and endoplasmic reticulum fractions and a supernatant fraction from rat liver did not react. These results provide additional evidence that the Golgi apparatus is a source of low density plasma lipoproteins in rat hepatocytes.

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