Two Types of Lipoprotein Particles in Golgi Apparatus of Rat Liver[1]

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Abstract

Lipoprotein particles were examined in thin sections of rat liver. Particles within cisternae of endoplasmic reticulum and in vesicles at one face of the Golgi apparatus were larger than those in free secretory vesicles and in vesicles at the opposite face of the Golgi apparatus. On this basis, the Golgi apparatus face containing large particles was identified as the forming face, whereas that face associated with vesicles containing the smaller particles was identified as the maturing face.

Very low density lipoproteins (VLDL) of serum transport triglycerides (8, 16) and serve as precurse molecules to lipoprotein of other classes (1). Of the two sites of VLDL synthesis, liver and intestine, the liver is quantitatively the most important (18, 23).

In electron micrographs of liver cells, lipoprotein appears as osmiophilic particles within smooth endoplasmic reticulum and in vesicles of Golgi apparatus (3, 5, 7). The particles isolated from fractions rich in Golgi apparatus have the electron microscopic appearance and immunological and chemical properties of serum VLDL (2, 9, 10). The lipoprotein enters Golgi apparatus vesicles from smooth endoplasmic reticulum via tubular connections (2, 12, 13, 22). Yet, vesicles and tubules which contain lipoprotein particles are observed at both faces of individual dictyosomes (3, 7). Released secretory vesicles migrate through the cytoplasm to the cell border at the sinusoidal space (5, 7). Vesicle membranes and plasma membrane then fuse to release lipoproteins to the circulatory system (5, 7). We report observations on relationships between vesicles at each of the two dictyosome faces, especially as they regard distinguishing between vesicles of the forming face and those of the secreting face.

Materials and Methods

Male rats (Holtzman Co., Madison, Wisc.), 200 to 300 g were given a standard diet (Purina Laboratory Chow) and drinking water *ad libitum*. The rats were killed by cervical dislocation, and the livers were drained of blood and excised. Pieces of liver (1 mm^3) were fixed for 16 hours in osmium tetroxide (1% in 0.1 M sodium phosphate buffer, pH 7.2) at 4° C, rinsed in buffer, dehydrated through an acetone series and embedded in Spurr's (19) epoxy resin mixture. Thin sections were mounted on formvar-covered, carbon-coated grids, stained with lead

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citrate (17) and viewed with a Philips EM-200 at 60 KV. A 54,864 line-per-inch diffraction grating replica (Ladd Research Industries, U.S.A.) was used as the magnification standard. Diameters of lipoprotein particles were measured from the projected images of electron image plates at a magnification of 76,200.

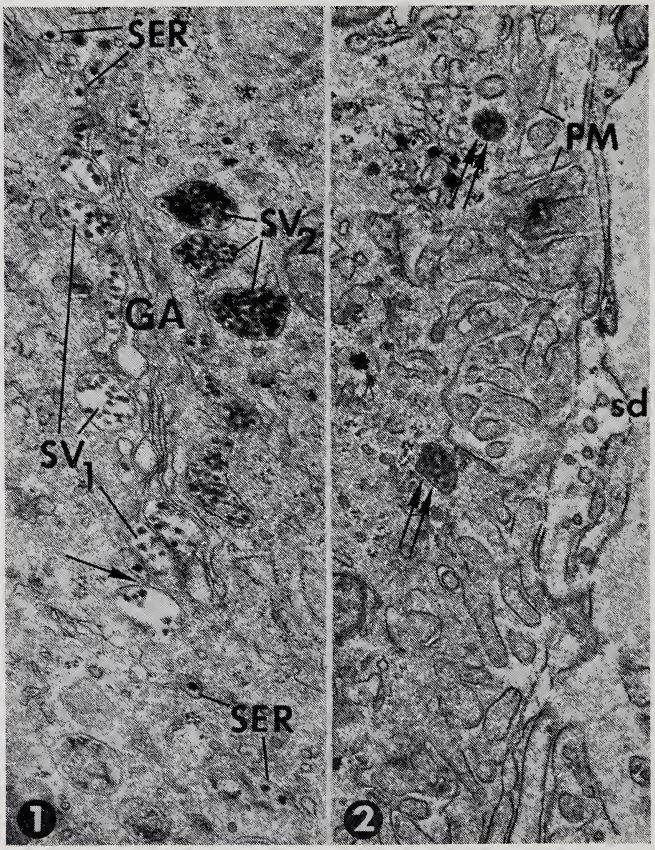

FIGURE 1. *Electron micrograph of a rat liver Golgi apparatus (GA) and neighboring smooth endoplasmic reticulum (SER). Vesicles at one face (SV_1) have a light matrix and the larger, disperse lipoprotein particles. Vesicles at the opposite face (SV_2) have a dark matrix and are densely packed with smaller lipoprotein particles. At the arrow, a smooth endoplasmic reticulum tubule is continuous with a light matrix vesicle. X 27,000.*

FIGURE 2. *Two free secretory vesicles (double arrows) with a dark matrix and containing small lipoprotein particles. The vesicles will eventually fuse with the plasma membrane (PM) to release lipoprotein secretory product into the space of Disse (sd) X 27,000.*

Results

In whole tissue, vesicles containing osmiophilic lipoprotein particles occurred at both faces of the dictyosome (Fig. 1). At one face the

vesicles were dilated and the particles were dispersed within a light matrix (SV_1). At the opposite face, the particles were densely packed in undilated vesicles containing an osmiophilic matrix (SV_2). Vesicles located between the two dictyosome faces were intermediate between these two extremes. Vesicles free in the cytoplasm (Fig. 2) were morphologically similar to the dictyosome vesicles containing the densely packed particles (SV_2).

Size of lipoprotein particles also differed across the dictyosome. Particles in the dilated vesicles at one face averaged only slightly smaller than particles within the smooth endoplasmic reticulum (Table 1). Particles in vesicles at the opposite face were considerably smaller and corresponded most closely to the particles in vesicles free in the cytoplasm. Not only does lipoprotein particle diameter decrease across the stack of dictyosome cisternae, but the particles in vesicles released from the dictyosomes appear smaller than those in vesicles still attached to the mature face (Table 1).

TABLE 1. *Lipoprotein particle diameters in rat liver cell components.*

Cell Component	Mean Particle Diameter (Å)	±	Standard Deviation
Smooth endoplasmic reticulum	590	±	70
Golgi app. light matrix vesicles	550	±	70
Golgi app. dark matrix vesicles	465	±	70
Secretory vesicles (free)	420	±	60

Discussion

In the process of membrane transformation and product compartmentalization at the Golgi apparatus, input of membrane and product occurs at or near the forming (proximal) face; maturation of membrane and compartmentalization of product occurs across the stack to the secreting (distal) face (12, 14, 15). Thus the proximal and distal faces of dictyosomes can be identified by recognizing either product compartmentalization (functional polarity) or changes in cisternal morphology (morphological polarity) from the proximal to the distal face. In cells in which an electron-dense secretory product appears in large secretory vesicles at only one face of the dictyosome, the faces are identified easily. In systems which secrete mucopolysaccharides, like the Brunner's gland of the duodenum (20), the dictyosome is obviously polar. In other cell types (4, 6, 12, 14, 15), changes in membrane thickness and/or staining intensity have been used to distinguish one face from the other.

In the liver, secretory product is visible in vesicles associated with both faces of the dictyosome (Fig. 1). A complex and hitherto not reported functional polarity is described which provides both a qualitative and quantitative basis for the indentification of the forming and secreting faces of the liver dictyosome.

Comparisons of lipoprotein particle concentration and dimensions and vesicle matrix density reveal a clear polarity across the stacked

cisternae (Fig. 1, Table 1). The dictyosome vesicles with small particles and a dark matrix are similar to the free secretory vesicles with respect to particle size and morphology. Thus the secreting face of the dictysome is that face associated with densely packed vesicles with small lipoprotein particles and a dark matrix. The forming face has dilated vesicles with a light matrix and the larger, more disperse particles, similar to those in smooth endoplasmic reticulum.

To ascribe different functions to the forming face *vs.* mature face vesicle, it will be important to isolate each vesicle type. Morphological differences between vesicles not only distinguish the forming face from the mature face in whole tissue, but may help to identify the origin of secretory vesicles observed after cell fractionation (11).

We reported previously a potential role of secretory vesicles in product glycosylation in rat liver (11) and in pollen tubes (21). Secretory vesicles associated with the dictyosome not only compartmentalize secretory product, but also may take part in the modification of secretory product. Even though all lipoprotein particles in the liver are within the size range of the very low density lipoprotein of serum (280-800Å, 8) there is an overall reduction of 24% in particle diameter, comparing particles in vesicles from the forming face of the Golgi apparatus and in free secretory vesicles. Lipoprotein particle sizes differ across the stack of dictyosome cisternae, and a small additional decrease appears to occur after the vesicles have been released from the dictysome. This suggests that lipoprotein particles are trimmed and/or condensed within vesicles at the dictyosome as well as within vesicles free in the cytoplasm.

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