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FACILITATED TRANSFER OF CHOLESTERYL ESTER BETWEEN ROUGH AND SMOOTH MICROSOMAL MEMBRANES BY PLASMA LIPID TRANSFER PROTEIN

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The accessibility of intracellular membrane cholesteryl esters to removal was tested with plasma lipid transfer protein as a tool. Incubation of a mixture of non-radioactive smooth microsomes + rough microsomes prelabeled with cholesteryl ester resulted in slight movement (2-4%) of radioactive cholesteryl ester into smooth microsomes. With the addition of increasing amounts of plasma lipid transfer protein to the mixture, the % transfer of cholesteryl ester into smooth microsomes progressively increased until a plateau was reached at 14%. Movement of cholesteryl ester in the reverse direction was examined with non-radioactive rough microsomes as an acceptor and smooth microsomes prelabeled with cholesteryl ester as a donor. The pattern of the % cholesteryl ester transferred in the reverse and forward direction was almost identical in the presence of plasma lipid transfer protein, showing bidirectional movement of cholesteryl ester between membranes.

Cholesteryl esters are formed intracellularly in several types of tissues by acyl-CoA:cholesterol acyltransferase (EC 2.3.1.26) (1-5). This enzyme is found predominantly in the rough endoplasmic reticulum (6,7) and is located topologically on the cytoplasmic surface (6,8). In vitro studies indicate that cholesteryl esters are bound to the microsomal membrane and are not released in the aqueous medium (9) nor are they easily extractable by smooth microsomal membrane vesicles (6). These observations suggested that cholesteryl esters are trapped or otherwise inaccessible. Despite these observations cholesteryl esters in rough endoplasmic reticulum appear to be

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mobilized in vivo since the concentration of cholesteryl esters in other subcellular components (smooth endoplasmic reticulum and Golgi membranes) are higher than in the rough endoplasmic reticulum (6). The nature of this process is unknown. One of several possibilities were considered, namely, that a cytosolic protein(s) might be involved in the intracellular movement of cholesteryl ester. Before undertaking the isolation of this putative material it seemed prudent to establish whether cholesteryl esters in the rough endoplasmic reticulum can be removed and transferred to other membrane vesicles by using plasma lipid transfer protein as a tool. We present evidence that this protein can mediate the transfer of cholesteryl ester between rough and smooth microsomal membranes.

MATERIAL AND METHODS

Chemicals were obtained from the sources indicated: sucrose grade 1, Sigma (St. Louis, MO), cesium chloride, J.T. Baker (Phillipsburg, NJ), Handifluor, Mallinckrodt (Paris, KY), [1-^{14}C]palmitoyl-CoA, RoseChem Prod., (Hollywood, CA).

Male Wistar rats (300-400 g) were decapitated. The liver was perfused with 0.25 M sucrose through the portal vein and the perfusate was allowed to escape through a cut in the ventricles. The liver was minced with a razor blade and homogenized in eight volumes of 0.25 M sucrose with a Teflon-glass homogenizer by three downward strokes of a motorized pestle. Rough and smooth microsomes were isolated by centrifugation in a discontinuous sucrose/CsCl gradient according to the procedure of Andersson et al (10). The identity of the microsomal components were confirmed by the marker enzyme composition and by the lipid and RNA content (6).

Transfer of cholesteryl ester between rough and smooth microsomes by plasma lipid transfer protein. The transfer of cholesteryl ester from rough microsomes to smooth microsomes was measured from the transfer of radioactive cholesteryl ester from rough microsomes (donor) into smooth microsomes (acceptor) after incubation of a mixture of these organelles. The transfer of this ester in the reverse direction was measured in a similar manner

using non-radioactive rough microsomes as the acceptor and smooth microsomes labeled with cholesteryl ester as a donor particle. For the sake of simplicity the procedure will be given only for the assay in the direction of rough to smooth microsomes since the assay for movement in the reverse direction is virtually the same except for the donor and acceptor.

Rough microsomes (1.5 mg protein) were labeled with cholesteryl ester by incubation in 3 ml 0.1 M Tris buffer (pH 7.4) containing 0.1% albumin and 32 μM [1-^{14}C]palmitoyl-CoA (spec. act., 13 μCi/umole) at 37°C for 30 min. After incubation the mixture was diluted with 0.25 M sucrose and centrifuged at 100,000 x g for 90 min in a 40-rotor (Beckman-Spinco) to recover the microsomes. The microsomal pellet was washed free of residual albumin and palmitoyl-CoA by suspension in 0.25 M sucrose-50 mM Tris buffer, pH 7.4, and centrifugation at 100,000 x g for 90 min. The rough microsomes were suspended to a concentration of 5 mg protein/ml. The protein was determined by the method of Lowry et al (11).

Rabbit plasma lipid transfer protein was partially purified in D.B. Zilversmit's laboratory according to the referenced procedure through the Phenyl-Sepharose step (12). The cholesteryl ester transfer activity of this preparation from human low-density lipoprotein to high-density lipoprotein was 9.0 μg cholesteryl ester transferred /124 μg protein/3 hr at 37°C under conditions described previously (12).

Assay for the transfer of cholesteryl ester from rough to smooth microsomes. The incubation medium consisted of 0.3 ml 50 mM Tris buffer (pH 7.4) containing a mixture of rough microsomes prelabeled with cholesteryl ester (100 μg protein) and non-radioactive smooth microsomes (100 μg protein). The mass of plasma lipid transfer protein added to the medium and the time of incubation at 37°C varied with the experiment. For a control, rough microsomes prelabeled with cholesteryl ester alone were carried through the procedure. After incubation the mixture was cooled in ice-water and the mixture was overlayed onto a discontinuous sucrose gradient composed of 0.6 M sucrose and 1.3 M sucrose, both containing 15 mM CsCl and centrifuged at 100,000 x g

for 4 h at 4°C. The smooth microsomes at the 0.6 M/1.3 M sucrose interface and the rough microsomal pellet in 1.3 M sucrose were removed washed individually by suspension in 0.15 M NaCl 0.1 M Tris buffer (pH 7.4) and centrifugation at 100,000 x g for 90 min. Lipids of each of the microsomal components were extracted by the procedure of Bligh and Dyer (13) and the lipid was fractionated on silica gel G by thin-layer chromatography with 5% diethyl ether-95% petroleum ether (b.p. 60-110°C) as the developing solvent. The area corresponding to the location of cholesteryl palmitate standard was scraped into a counting vial and the powder was suspended in Handifluor: water (10:4) and assayed for radioactivity in a liquid scintillation spectrometer.

RESULTS AND DISCUSSION

The influence of lipid transfer protein on the movement of cholesteryl ester between microsomal components with time is presented in Fig. 1. Rough microsomes, prelabeled with cholesteryl ester, were used for a donor and nonradioactive smooth microsomes for an acceptor of cholesteryl ester. The release of cholesteryl ester from rough microsomes was measured by the incorporation of radioactive cholesteryl ester into nonradioactive smooth microsomes after incubation of a mixture of these microsomal subfractions. In the absence of lipid transfer protein, smooth microsomes incorporated at most only 1.3% of the radioactive cholesteryl ester originally present in rough microsomes (15,332 cpm/100 μg protein). Addition of lipid transfer protein (25 μg protein/ml) to the mixture of microsomal components promoted the transfer of cholesteryl ester into smooth microsomes linearly with time, attaining a maximum of 12%.

The effect of varying the concentration of lipid transfer protein was examined (Fig. 2). The percent transfer of cholesteryl ester from rough microsomes to smooth microsomes (•) during a 90 min incubation progressively increased to 14% as the concentration of the lipid transfer protein in the incubation medium was increased to 100 μg/ml, showing within this range a qualitative relationship between the % transfer and concentration of the

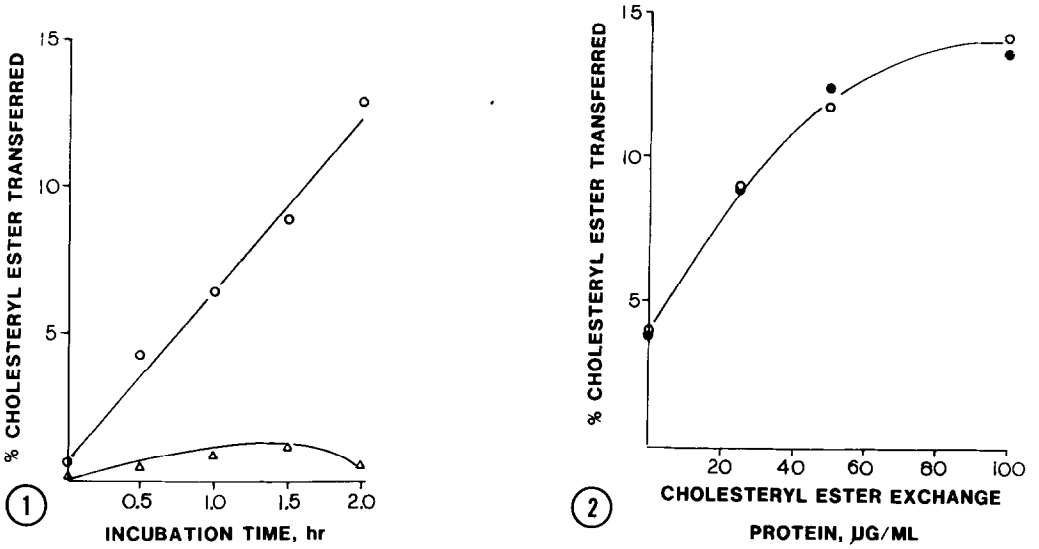

Fig. 1. Influence of time on the transfer of cholesteryl ester from rough microsomes to smooth microsomes in the presence of plasma lipid transfer protein. Incorporation of radioactive cholesteryl ester into smooth microsomes was used as a measure of transfer after incubation of a mixture of non-radioactive smooth microsomes + rough microsomes prelabeled with cholesteryl ester at 37°C for varying time intervals. % Cholesteryl ester transfer refers to the % of radioactive cholesteryl ester in rough microsomes transferred to smooth microsomes in the presence (o) or absence (Δ) of plasma lipid transfer protein. Each symbol is a mean of two experiments.

Fig. 2. Influence of plasma lipid transfer protein on the movement of cholesteryl ester between rough and smooth microsomes. The transfer of cholesteryl ester from rough to smooth microsomes (•) was measured by the incorporation of radioactive cholesteryl ester into non-radioactive smooth microsomes from rough microsomes prelabeled with cholesteryl ester after incubation of these microsomal components in the presence of varying amounts of plasma lipid transfer protein. Transfer in the reverse direction (o),i.e., smooth to rough, was measured in a separate experiment using non-radioactive rough microsomes as the acceptor and smooth microsomes prelabeled with cholesteryl ester as a donor. Transfer of cholesteryl ester is expressed as % of radioactive cholesteryl ester in the donor transferred to the acceptor during a 90 min incubation at 37°C. Each value is a mean of two separate experiments.

transfer protein. Since the assay procedure involved the reisolation of the microsomal components on a discontinuous sucrose gradient for the analysis of radioactive cholesteryl ester in smooth microsomes it is alternatively possible that there was no facilitated movement of cholesteryl ester to smooth microsomes by the transfer protein but instead a conversion of rough microsomes prelabeled with cholesteryl ester to a less dense particle having the density of smooth microsomes. This possibility was considered in a similar experiment in which movement in the reverse direction was measured by the incubation of nonradioactive rough microsomes with smooth microsomes

prelabeled with cholesteryl ester (Fig. 2). The incorporation of radioactive cholesteryl ester into rough microsomes from smooth microsomes (o) was the same as in the forward direction when the data were expressed as % of the radioactive cholesteryl ester originally present in smooth microsomes (2330 cpm/100 μg protein). The results showed that the lipid transfer protein facilitated the transfer of cholesteryl ester in both directions. Since the concentration of cholesteryl ester in the rough and smooth microsomes are not the same, there could be a difference in transfer of mass between microsomal particles provided that the label is equilibrated with endogenous cholesteryl ester in the membrane. There is no compelling reason to think that there is equilibration since there may be lipid domains in the membrane. Bidirectional movement of cholesteryl ester between the microsomal components rules out the possibility that donor vesicles labeled with cholesteryl ester are converted to vesicles having the density characteristics of the acceptor vesicles since such an event would require formation of a less dense particle in one direction and formation of a more dense particle than the donor in the reverse direction.

Cholesteryl ester within the cell is probably due almost entirely to synthesis, since exogenous cholesteryl ester via the uptake of lipoproteins is hydrolyzed by the lysosomes. The location of cholesteryl ester is not confined to the rough endoplasmic reticulum where it is synthesized but is distributed among other organelles. These observations suggest that there is intracellular movement of cholesteryl ester. It is conceivable that cholesteryl ester may: (i) diffuse within the bilayer of contiguous membranes from rough endoplasmic reticulum to smooth endoplasmic reticulum and the latter vesiculate and subsequently fuse to Golgi membranes; (ii) be carried along with the transport of membrane material during the biogenesis of endomembranes (14); and/or (iii) transferred to organelles by a cytosolic transfer protein having the characteristics similar to plasma lipid transfer protein. The present experiments suggest that the latter mechanism is plausible. The presence of such a protein was found by one of the authors

(D.B.Z); other lipid bearing cytosolic proteins have been reported (15-18).

Plasma lipid transfer protein has the property to exchange cholesteryl ester between lipoproteins (19) and between lipoproteins and liposomes (20). The present study shows transfer between subcellular membranes as well.

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