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Evidence that during very low density lipoprotein assembly in rat hepatocytes most of the triacylglycerol and phospholipid are packaged with apolipoprotein B in the Golgi complex

Joan A. Higgins

Dept of Biochemistry, Sheffield S10 2TN, England

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Rat liver lipids were labelled by an intraportal injection of [^{3}H]palmitic acid followed by isolation of rough and smooth endoplasmic reticulum or 'cis' or 'trans'-enriched Golgi fractions. The preparations were separated into membrane and contents and the apolipoprotein B of the content fractions was immunoprecipitated. More than 90% of the labelled triacylglycerol and phospholipid secreted into the blood immunoprecipitated with apolipoprotein B. Under the same experimental conditions 8, 12, 27 and 59% of the lipids of the rough, smooth, 'cis-Golgi' and 'trans-Golgi' contents, respectively, were immunoprecipitated. Thus, the 'trans-Golgi' region appears to be the major intracellular site of assembly of apolipoprotein B with triacylglycerol and phospholipid.

VLDL; Apolipoprotein B; Lipid assembly; Golgi; Endoplasmic reticulum; (Rat liver)

1. INTRODUCTION

The liver is the main site of secretion of very low density lipoproteins (VLDLs) the vehicle by which endogenous lipids are transported in the circulation [1–4]. Although the biosynthetic pathways involved in the synthesis of the components of VLDL are known, the organisation of hepatocytes in the packaging of these components remains to be elucidated. Morphological studies have demonstrated that VLDL-like particles initially appear in the cisternal space of the endoplasmic reticulum and are subsequently carried in membrane vesicles to the Golgi region where particles accumulate. The trans elements move to fuse with the plasma membrane at the sinusoidal surface releasing the VLDL into the blood space [5–7]. We have demonstrated that although VLDL-like particles isolated from the cisternae of the endoplasmic reticulum are precursors of those isolated from Golgi preparations these two groups of particles differ in size and lipid composition [8]. Most of the phospholipid and cholesterol components of the VLDL are packaged with the triacylglyceride in the Golgi region. We have also shown that isolated Golgi fractions have the enzymes which catalyse the final steps in the synthesis of phosphatidylcholine and that the methylation pathway is preferentially used for the synthesis of VLDL phosphatidylcholine [9]. We have now extended these studies to examine the intracellular site of assembly of lipid and apolipoprotein B. These investigations indicate that the major site of packaging of apolipoprotein B with lipid is the Golgi region.

Correspondence address: J.A. Higgins, Dept of Biochemistry, Sheffield S10 2TN, England

2. MATERIALS AND METHODS

2.1. *Materials*

Antiapolipoprotein B was purchased from Boehringer Mannheim. Using either radial immunodiffusion or immunoblotting on nitrocellulose we found that the antiserum, which is raised against human apolipoprotein B, crossreacts with rat serum and chylomicrons, VLDL and low density lipoprotein fractions prepared from rat serum by sequential centrifugation on potassium bromide gradients [10] but not with high density

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lipoprotein and bovine serum albumin. Non-immune sheep serum and anti-sheep IgG (donkey) were purchased from Scottish Antibodies Ltd. [^{3}H]Palmitic acid was purchased from Amersham International. Other chemicals were purchased from Sigma or British Drug Houses Ltd.

2.2. *Preparation of subcellular fractions*

Rough and smooth microsomes and Golgi 'trans-enriched' and 'cis-enriched' subfractions were prepared and the vesicular fractions were separated into membrane and content fractions by treatment with 100 mM sodium carbonate as described [8,9,11,12].

2.3. *Incorporation of [^{3}H]palmitic acid into lipids of rat liver*

[^{3}H]Palmitic acid bound to fatty acid free bovine serum albumin (0.1 ml of 1% albumin//2.5 μCi palmitic acid per 100 g body wt) was injected into the portal vein of rats anaesthetized with Nembutal for a range of times prior to removal of the liver and isolation of subcellular fractions.

2.4. *Immunoprecipitation of apolipoprotein B*

The association of labelled lipids with apolipoprotein B was determined by immunoprecipitation of the apoprotein followed by extraction of the lipid as described by Siuto-Mangano et al. [13] using antiapolipoprotein B as a primary antibody and anti-sheep IgG as a secondary antibody. Content fractions prepared from subcellular fractions were dialysed against 150 mM NaCl in 10 mM Tris buffer for 15 h and concentrated by ultrafiltration using an Amicon PM 10 filter. Antiapolipoprotein B (0.1 ml diluted 1:50) was added to 0.5 ml fractions (equivalent to 0.5 g of starting liver) and the samples allowed to stand at 4°C for 1–2 h. Sheep serum was used instead of antiapolipoprotein B in parallel control experiments. Anti-sheep IgG (0.2 ml diluted 1:5) was added and the immunoprecipitate allowed to form for 15 h. 0.5 ml of 0.5 M sucrose was layered beneath each sample and the tubes were centrifuged at 2000 rpm in a Mistral refrigerated centrifuge for 30 min. The immunoprecipitate which pelleted was washed twice by resuspension in 0.5 ml of buffer and centrifugation through 0.5 M sucrose.

2.6. *Extraction and analysis of lipids*

Lipids were extracted from membrane suspensions, supernatants and immunoprecipitates and analysed as described previously [8,9,14].

3. RESULTS AND DISCUSSION

3.1. *Composition of membrane and content fractions of rough and smooth microsomes*

Smooth microsomes have a triacylglyceride content relative to phospholipid approx. 4 times that of rough microsomes (table 1). Approx. 70% of the triacylglyceride appeared in the content fraction together with a small amount of phospholipid. The mole ratio of triacylglyceride to phospholipid of the smooth microsomal content fraction was 8.7 and that of the rough microsomal content fraction was 1.2. These results are consistent with morphological studies of whole liver and isolated microsomes which have shown that there is a concentration of lipoprotein-like particles in the smooth membranes compared with the rough membranes [5–7,12].

Table 1

Composition of membranes and contents of rough and smooth microsomes

	Rough microsomes	Smooth microsomes
mol of triacylglycerol per mol of phospholipid		
Microsomes	0.052 ± 0.01 (4)	0.21 ± 0.042 (4)
Membrane fraction	0.036 ± 0.01 (7)	0.071 ± 0.016 (7)
Content fraction	0.036 ± 0.01 (7)	8.67 ± 0.291 (7)
% loss of phospholipid into content fraction	2.89 ± 0.41 (12)	1.89 ± 0.49 (12)
% loss of triacylglycerol into content fraction	72.50 ± 4.44 (12)	66.00 ± 6.06 (12)

Microsomes were isolated and separated into membrane and content fractions as described in section 2. Results are expressed as mean ± SD (number of observations)

3.2. *Incorporation of [^{3}H]palmitic acid into lipids of rough and smooth microsomal membrane and content fractions*

[^{3}H]Palmitic acid injected intraportally was rapidly incorporated into the triacylglyceride of rough and smooth microsomes (fig.1). Between 75% and 85% of the radioactive label was in the triacylglyceride fraction which had a specific activity 10-fold greater than that of the phospholipid. The specific activities of the triacylglyceride of both the rough and smooth membranes peaked at 2 min after injection of [^{3}H]palmitic acid (fig.1). The specific activity of the triacylglycerides of the rough microsomes was greater than that of the smooth microsomes by a factor of 1.7. However, taking into account the pool sizes of triacylglycerol (table 1) incorporation of palmitic acid into triacylglyceride was approximately equal in both rough and smooth microsomal membranes. The specific activity of the triacylglyceride of the rough microsomal con-

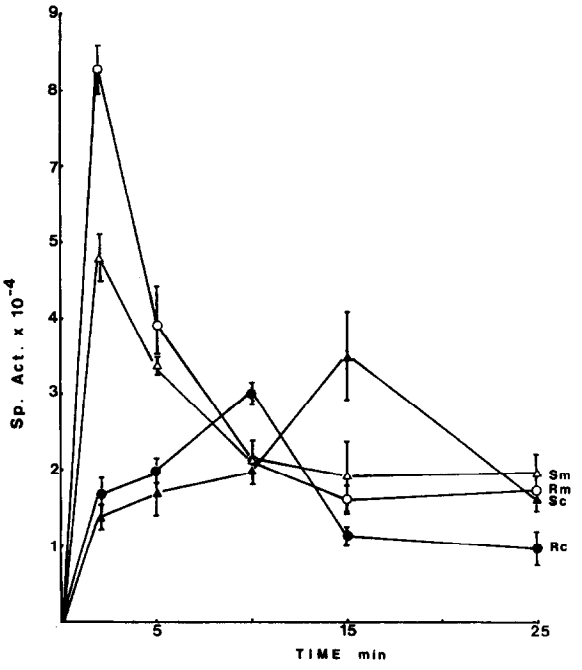

Fig.1. [^{3}H]Palmitic acid bound to albumin was injected intraportally into rats. Smooth and rough membrane and content fractions were prepared, the lipids extracted and the specific activities of the triacylglycerol (dpm/μmol) determined. Each time point is the mean of four experiments ± standard deviation. Sm, smooth membranes; Sc, smooth contents; Rm, rough membranes; Rc, rough contents.

tent rose after that of the membrane peaking 10 min after injection of palmitic acid, while the specific activity of the triacylglycerides of the smooth microsome content peaked after 15 min. As reported previously and not illustrated in fig.1 the specific activity of the Golgi content triacylglyceride rose between 10 and 15 min and levelled by 30 min after injection of [^{3}H]palmitic acid [8]. [^{3}H]Palmitic acid was also incorporated into the membrane and content phospholipids. The specific activities of the phospholipids rose to level at 5 min after injection and were similar in rough and smooth membranes and content fractions.

3.3. *Immunoprecipitation of [^{3}H]palmitate-labelled lipids of microsomal and Golgi content fractions by antiapolipoprotein B*

30 min after injection 90% of the [^{3}H]palmitic acid in the blood serum was in triacylglycerol and 10% in phospholipid. Approx. 90% of the labelled triacylglycerol and phospholipid was immunoprecipitated by antiapolipoprotein B (table 2). [^{3}H]Palmitic acid is therefore rapidly cleared from the blood and recirculated mainly as triacylglycerol in VLDL.

Under the same experimental conditions 15 min after injection of [^{3}H]palmitic acid, 8.5% and 12.2% of the labelled lipids of rough and smooth microsomal contents, respectively, were precipitated with apolipoprotein B. 30 min after injection of [^{3}H]palmitic acid, 27.4% and 59.3% of the 'cis-enriched' and the 'trans-enriched' Golgi contents, respectively, were precipitated under the same conditions (table 2). These results suggest that packaging occurs as the lipids and apoprotein move through the membrane compartments involved in secretion. The contents of the 'trans-enriched' Golgi, the equivalent of secretory vesicles in hepatocytes, still contain a significant amount of lipid which is not associated with apolipoprotein B consistent with other observations which suggest that the 'trans'-Golgi elements contain precursors in addition to completed VLDL [8,15].

Table 2

Immunoprecipitation of ^{3}H-labelled lipids with antiapolipoprotein B

Fraction	% labelled lipid immunoprecipitated		
	Total lipid	Phospho-lipid	Triacyl-glycerol
Serum	90.1 ± 1.58	88.4 ± 4.1	88.4 ± 2.39
'trans-enriched' Golgi	59.3 ± 6.1	58.1 ± 6.6	55.5 ± 2.51
'cis-enriched' Golgi	27.4 ± 4.0	26.0 ± 6.6	26.5 ± 5.5
Smooth micro-somes	12.2 ± 2.7	10.2 ± 1.7	12.9 ± 0.8
Rough micro-somes	8.5 ± 1.1	8.9 ± 0.2	7.2 ± 0.9
Total micro-somes	6.9 ± 0.4	5.6 ± 4.7	7.6 ± 0.8

Apolipoprotein B was immunoprecipitated from content fractions and from blood serum removed 30 min after injection of [^{3}H]palmitic acid and the lipids extracted as described in section 2. The amount of label associated with the immunoprecipitate in control experiments in which sheep serum was substituted for antiserum was always less than 7% and has been subtracted from each figure. Results are expressed as % of each labelled lipid immunoprecipitated ± mean standard deviation for four separate experiments

Alexander et al. [16] using immunocytochemical methods to detect apolipoprotein B in situ observed that although the apolipoprotein was associated with the rough endoplasmic reticulum membranes and with bound ribosomes it was not present in lipid particles in the smooth endoplasmic reticulum cisternae. VLDL-like particles in the Golgi cisternae did contain apolipoprotein B. This has also been demonstrated by isolation of Golgi preparations and separation of their contents for analysis [17,18]. Investigations of the sequence of appearance of labelled precursors in VLDL secreted by oestrogen-induced chick liver cells have also suggested that triacylglyceride is first packaged with a small amount of phospholipid followed by apolipoprotein B and that the remaining phospholipid is added to the VLDL just prior to secretion [13,19]. This sequence of events fits our observations closely. However, by isolation and analysis of the endoplasmic reticulum and Golgi subcellular fractions we have been able to identify the morphological sites of the assembly steps.

Using [^{3}H]leucine to label newly synthesized proteins we could detect labelled apolipoprotein B by immunoprecipitation in the cisternal contents of Golgi membranes but could not detect the protein in the cisternal contents of the endoplasmic reticulum. This may be because small amounts of protein are involved and we are currently developing more sensitive assay methods. However, it has been reported that in HEP-G2 cells newly synthesized apolipoprotein B binds to the endoplasmic reticulum to a greater extent than other secreted proteins [20,21]. It may be therefore that the apoprotein is transported as a membrane component to the Golgi region for packaging with lipid.

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