Assembly of Very Low Density Lipoprotein: A Two-Step Process of Apolipoprotein B Core Lipidation1,2

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ABSTRACT The liver plays a primary role in lipid metabolism. Important functions include the synthesis and incorporation of hydrophobic lipids, triacylglycerols and choleseryl esters into the core of water-miscible particles called lipoproteins and the secretion of these particles into the circulation for transport to distant tissues. In this article, we present a brief overview of one aspect of the assembly process of very low density lipoproteins, namely, possible mechanisms for combining core lipids with apolipoprotein B. This is a complex process in which apolipoprotein B interacts with core lipids to form very low density lipoproteins by a two-step process that can be dissociated biochemically. J. Nutr. 129: 463S–466S, 1999.

KEY WORDS: • apolipoproteinB-HDL • Brefeldin A • hepatoma cells • microsomal triglyceride transfer protein • secretion

The liver plays a primary role in lipid metabolism. Important functions include the synthesis and incorporation of hydrophobic lipids such as triacylglycerols and choleseryl esters into lipoproteins and the secretion of these particles into the circulation. Plasma lipoproteins consist of a hydrophobic core of neutral lipid surrounded by a monolayer of amphipathic phospholipids and free cholesterol in which a single molecule of apolipoprotein (apo)B resides. Apolipoprotein B is unique among the plasma apolipoproteins because of its hydrophobicity, high molecular weight and inability to transfer between lipoproteins (Kane 1983). Apolipoprotein B occurs in two forms, apoB100 and apoB48 (Kane et al. 1980). The larger apoB100 consists of 4536 amino acids, whereas apoB48 corresponds to the N-terminal 48% of apoB100. Both proteins are coded for by the same gene (Innerarity et al. 1996, Scott 1989, Young 1990), and the apoB100 mRNA is cotranscriptionally edited by a deaminase that converts codon 2153 into a translational stop codon (Chen et al. 1987, Powell et al. 1987). In humans, apoB100 is virtually the only form of apoB that is expressed in liver, whereas apoB48 is the dominating form of apoB that is synthesized in the intestine (Glickman et al. 1986). However, both apoB100 and apoB48 can assemble VLDL when expressed in the liver of certain animals such as rats (Innerarity et al. 1996). In this article, we present a brief overview of the assembly process of VLDL based on our studies of cultured human (HepG2) and rat (McA-RH7777) hepatoma cells. This is a complex process in which apoB interacts with lipids to form lipoprotein particles.

The secretory pathway of the cell has important roles in the intracellular sorting of proteins, and the molecular mechanisms for the segregation of secretory and membrane proteins have been elucidated in great detail (Pfeffer and Rothman 1992). However, the assembly and secretion of plasma lipoproteins is a complex process that is still not completely understood. This process involves the interaction between apoB and the membrane of the endoplasmic reticulum and, subsequently, with an amphipathic monolayer of phospholipid and cholesterol-containing surface that covers a core of neutral lipids that constitutes a lipoprotein particle. Apolipoprotein B exists in three forms in the secretory pathway as follows (Fig. 1): I) a form that is associated with the microsomal membrane (Borén et al. and 1993b), II) a more dense form (characterized as a lipoprotein particle with

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4 Abbreviations used: ALLN, N-acetyl-Leu-Leu-norleucinal; apo, apolipoprotein; BFA, Brefeldin A; L-FABP, liver fatty acid-binding protein; MTP, microsomal triglyceride transfer protein.
high density) and III) VLDL-sized particles that correspond to the particles that are secreted from the cells.

To further investigate the membrane-associated form of apoB, we developed a new extraction procedure based on a combination of carbonate and a small amount (0.025%) of deoxycholate (Rustaeus et al. 1998). The deoxylcholic acid/carbonate procedure had two major advantages compared with the standard carbonate method. First, it improved recovery of VLDL from the secretory pathway. Second, virtually all apoB was extracted from the microsomal membrane. The standard carbonate method typically yields a major amount of membrane-associated apoB, making this latter finding strikingly different and unexpected. The concentration of deoxycholate used in the extraction method is well below the critical micellar concentration. Thus, the detergent does not interfere with the membrane structure, and integral membrane proteins such as calnexin and ribophorin are left membrane bound (Rustaeus et al. 1998). Furthermore, control experiments demonstrated that deoxylcholic acid/carbonate did not interfere with the structure of VLDL. Characterization of the apoB that was released from the membranes with the deoxylcholic acid/carbonate method showed that it could be isolated on lipoprotein particles with high density. These results showed that apoB is not integrated into the microsomal membrane as a classical membrane protein but instead is associated with the membrane in a different way (Rustaeus et al. 1998).

While the dense apoB100-containing particle is retained in the secretory pathway of the cell, the dense apoB48-containing lipoprotein particle is secreted (we will refer to these dense particles as “apoB48 HDL”) (Borén et al. 1994). This difference allowed a comparison between the membrane-associated apoB48 HDL that were extracted by the deoxylcholate/carbonate method and apoB48 HDL that were secreted from the cell. The results indicated that the apoB48 HDL extracted from the membrane with the deoxylcholate/carbonate technique had the same density as apoB48 HDL secreted from the cells (Rustaeus et al. 1998). Moreover, our results indicated that both of these forms of apoB48 were associated with lipids. These data indicate that the membrane-associated apoB48 is partially lipidated. Kinetic experiments using pulse-chase methodology indicated that the partially lipidated membrane-associated form of apoB48 was a precursor to both the dense apoB48 HDL and apoB48 VLDL (Borén et al. 1994). These results indicate that the first event in the formation of VLDL is formation of a dense, partially lipidated precursor particle that is loosely associated with the microsomal membrane (Fig. 2). The molecular mechanism through which this precursor particle is associated with the membrane is still unclear, but we speculate that its retention is mediated by chaperones.

Several years ago, we found that there was an inverse relationship between the secretion of apoB48 VLDL and apoB48 HDL (Borén et al. 1994). Incubating the cells with oleic acid could influence this relationship. Thus, the secretion of apoB48 VLDL was highly dependent upon the availability of fatty acids. We believe that the results could be explained in the following way (Fig. 3): the apoB48 VLDL assembly consists of two discrete steps, the first being the formation of partially lipidated apoB48 HDL. In the second step, bona fide apoB48 VLDL are formed from these pre-VLDL particles by the addition of the bulk of the lipid. Thus, apoB48 HDL can be converted into apoB48 VLDL in the presence of oleic acid, but these pre-apoB48 VLDL (apoB48 HDL) particles can also be released into the secretory pathway and secreted when the second step is not active, i.e., in the absence of oleic acid. The
The microsomal triglyceride transfer protein (MTP) is involved in the assembly of the VLDL precursor but is not involved in the second step to a major extent. The first step is MTP dependent during the translation-translocation of apolipoprotein B and for a short period after the translation is completed.

The results of these pulse-chase studies indicated that there was a post-translational step during which the assembly process was MTP dependent. This step occurred before the major amount of lipid was added to the particle. We propose that the role of MTP is to cotranslationally assemble an “apoB100 precursor” and to post-translationally adjust the structure of this particle so that it can interact with the second step. Thus, a functional definition of the first step would be the step that is dependent on MTP and during which a precursor particle that can interact with the second step assembly process(es) is assembled (Fig. 3).

The second step has the potential to be important for regulating the assembly process because membrane-associated apoB100 that fails to assemble VLDL is degraded. Inhibition of the second step with a low concentration of BFA resulted in the accumulation of membrane-bound apoB100 precursor particles, and kinetic studies showed that membrane-associated apoB100 was sorted to post-translational degradation when the VLDL assembly was blocked with BFA (Rustaeus 1998). We have previously demonstrated that the calpain inhibitor N-acetyl-Leu-Leu-norleucinal (ALLN) (Adeli et al. 1997) did not inhibit the degradation of the standard carbonate-unextractable form of apoB100 (i.e., the apoB100 precursor form). This means that the precursor is degraded via an ALLN-insensitive protease system. This is interesting because it suggests the presence of two sites for intracellular degradation of apoB100 in the secretory pathway, i.e., an early ALLN-sensitive pathway, most likely via proteasomes, and a late ALLN-insensitive degradation of apoB100 precursor. There may be a difference in the mechanisms regulating apoB100 and apoB48 degradation because the apoB48 HDL particles can be secreted, in contrast to the dense apoB100 precursor (see above).

The importance of the second step in the regulation of lipoprotein assembly is also indicated by recent studies of the liver fatty acid binding protein (L-FABP) on the assembly and secretion of apoB100 VLDL (K. Lindberg, this group, unpublished observations). Liver fatty acid binding protein inhibited the VLDL secretion without interfering with the biosynthesis of apoB100. The effect of L-FABP appeared to be an inhibition of the second step, thereby promoting a “late” intracellular degradation of apoB100. This effect of L-FABP could be explained by a diversion of fatty acids from the second step. It should be pointed out that this effect of L-FABP was not due to changes in the biosynthesis of triglycerides in the cell but appeared to be a direct effect on the second step.

There appears to be a structural requirement for apoB to undergo the second step in the assembly process. Experiments with cycloheximide to produce truncated, nascent apoB polypeptides released by puromycin demonstrated that it was necessary for apoB to reach a length of at least apoB40 (i.e., 200 kDa) in order to go through the second step, whereas a size of 80–120 kDa was sufficient to form a dense “apoB HDL” particle (Boreén et al. 1992, P. Stillemark, this group, unpublished observations).

Recently, apoB was demonstrated to associate with calnexin, a chaperone present in the secretory pathway (Chen et al. 1998). Most intracellular apoB100 occurs in the membrane-associated precursor form in cultured hepatocytes and hepatoma cells. The observation that this is the first synthesized form of full-length apoB100 and that it requires MTP to fold correctly indicates that this form undergoes an active folding that requires chaperones. However, chaperones may also be required for the “second step.” To investigate this.
hypothesis, we isolated lipoprotein particles with a density of \( \rho < 1.010 \text{ g/mL} \) from rat liver microsomes and characterized the proteins that were associated with these lipoproteins. The proteins were purified by polyacrylamide gel electrophoresis under denaturing conditions and identified by mass spectrometry after trypsin treatment (Larsson et al. 1997). The results indicated that lipoprotein particles with \( \rho < 1.010 \text{ g/mL} \) were also associated with chaperones, a finding that suggests that the second step is a highly active process supported by chaperones.

In summary, the second step in the VLDL assembly process has a key role in the regulation of apoB secretion and is a very active process that can be inhibited by BFA.

**LITERATURE CITED**


