

MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN: A MULTIFUNCTIONAL PROTEIN

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1. ABSTRACT

Microsomal triglyceride transfer protein (MTP) is a heterodimeric protein that transfers neutral lipids between membranes *in vitro*. Absence of this lipid transfer activity in the microsomes of abetalipoproteinemia patients established its pivotal function in lipoprotein assembly. Recent studies indicate that the lipid transfer activity is involved in importing triglycerides into the lumen of the endoplasmic reticulum. In addition to its lipid transfer activity, MTP physically interacts with apoB. This led to speculation that MTP may act as a chaperone. It remains to be determined whether the binding of MTP to apoB plays a role in either proper folding or net lipidation of nascent apoB. Both functions, lipid transfer and apoB binding, may be involved in the initial step of lipidation of nascent apoB resulting in the synthesis of primordial lipoprotein particles. Furthermore, it has been shown that MTP stably associates with lipid vesicles. The lipid-associated MTP may be important in core expansion of primordial lipoproteins. In summary, three independent functions (lipid transfer, apoB binding and membrane association) of MTP have been identified. Here, we propose these functions are carried out by a combination of different structural motifs. Based on sequence homology with lipovitellin, the M subunit of MTP is predicted to contain three β -sheets (A, C, and N) and one α -helical domain. The A- and C-sheets may be involved in lipid transfer, the N-sheet and the helical domain in apoB binding, and the N- and A-sheets in membrane association. It is also speculated that MTP may function in physiologic processes beyond lipoprotein assembly.

2. INTRODUCTION

Microsomal triglyceride transfer protein (MTP) was identified based on its ability to transfer neutral lipids

between synthetic vesicles *in vitro* (42). The purified protein was shown to be a heterodimer of 97 (the M subunit) and 55 (the P subunit) kDa subunits (40). The P subunit is the well-characterized protein disulfide isomerase. Due to its microsomal location, it was hypothesized that MTP may play a role in lipoprotein assembly [for reviews, (4, 10-12, 17, 20, 23, 29, 33, 34, 41, 44)]. In this review, various functions of MTP will be emphasized along with speculations on how these functions contribute to lipoprotein assembly. In addition, we will discuss a structure based on its sequence homology with lipovitellin and assign functions to the different structural motifs in MTP.

3. FUNCTIONS OF MTP

3.1. Lipid transfer activity

Wetterau and associates showed that MTP enhances lipid transfer between vesicles by a shuttle mechanism (2, 3). In this mechanism, MTP extracts lipid molecules from one membrane then travels to another membrane to deliver them. Both during acquisition and delivery, MTP transiently interacts with donor and acceptor vesicles. They observed that MTP had optimum lipid transfer activity in the presence of neutrally charged membranes. Presence of negatively charged lipids in membranes decreased the lipid transfer activity of MTP. Kinetic studies suggest that MTP has two lipid-binding sites, one fast and one slow. The fast site is probably involved in lipid transfer and the slow site may function in membrane association (see below).

Three independent approaches provided significant evidence for the involvement of MTP's lipid transfer activity in lipoprotein assembly and secretion. Mutations leading to defective lipid transfer activity have

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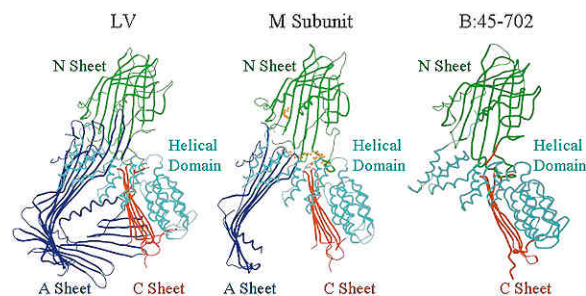


Figure 1. Comparison of the structures of lipovitellin, the M subunit of MTP and B:45-702: LV; The X-ray structure of lipovitellin as described by Anderson *et al.* (44). M subunit; Structure of the M subunit of MTP based on sequence homology with LV. B:45-702; Molecular model of the N-terminal 700 amino acids of apoB based on its sequence homology with LV. The sequence homology was performed using CLUSTAL W 1.8 as described before (3). During structural modeling emphasis was placed on the retention of various sheets and helices. Comparison shows the conservation of the N- and C-sheets as well as the helical domains in these proteins. The major difference is with regards to the A-sheet that is progressively truncated in MTP and absent in apoB.

been correlated with the absence of apoB in the plasma of abetalipoproteinemia patients (28, 35, 39). Co-expression of apoB and MTP in non-hepatic and non-intestinal cells that do not assemble lipoproteins results in the secretion of apoB-lipoproteins (13, 14, 25). Finally, MTP inhibitors that reduce *in vitro* lipid transfer activity decrease apoB secretion *in vivo* (8, 15, 22).

3.2. Formation and stabilization of lipid droplets in the lumen of endoplasmic reticulum

Recent studies led to the understanding that MTP may transport triglycerides into the lumen of endoplasmic reticulum (ER). First, Raabe *et al.* (31) created conditional knockout of MTP in mice and visualized their livers for lipid droplets using electron microscopy. They showed that normal hepatocytes had significant lipid droplets in the Golgi and some droplets in the smooth ER, but they did not contain appreciable amounts of lipid droplets in the cytosol. In contrast, MTP deficient hepatocytes were devoid of lipid droplets in the ER and Golgi area, but they contained significant amounts of lipid droplets in the cytosol. From these studies they concluded, "MTP is essential for bulk TG transfer into the ER lumen".

Second, Wang *et al.* (38) studied the effect of MTP inhibition on the transfer of lipids into the ER lumen. They observed that supplementation of McA-RH7777 cells with oleic acid increased triacylglycerol levels in the cytosol and microsomes. Within microsomes, increases were observed in both the lumen and membrane fractions. When MTP inhibitors were included with oleic acid supplementation, the accumulation of triglycerides in the cytosol was not affected and accumulation in the ER membrane fractions was slightly decreased. In contrast, the accretion of triglycerides in the microsomal lumen was completely inhibited in the presence of MTP inhibitors. In contrast to triglycerides, the accretion of

phosphatidylcholine in the cytosol and microsomes was not altered by the inhibition of the lipid transfer activity. This indicates entry of triglycerides into the ER lumen is dependent on MTP, but that of PC is not. From these studies, authors concluded that MTP plays a role in facilitating TG accumulation in microsomes, a step required for the post-translational assembly of TG-enriched lipoproteins. These studies have been extended to rat primary hepatocytes. Kulinski *et al.* showed that the inhibition of MTP's lipid transfer activity reduces the amount of luminal triglycerides associated and unassociated with apoB (24).

Third, we showed that MTP stably associates with lipid vesicles (5). In the absence of lipid vesicles, ^{125}I -MTP was recovered in the bottom fractions ($d > 1.21$ g/ml) corresponding to free proteins after ultracentrifugation. In contrast, MTP incubated with lipid vesicles was distributed in two peaks. One peak corresponded to the free protein. The other peak was recovered in fractions corresponding to a density of lipid vesicles (1.02-1.063 g/ml) indicating MTP was stably associated with lipid droplets. Furthermore, we showed that MTP exists associated with lipids in the lumen of ER.

In summary, there is evidence to suggest that the absence of MTP correlates with the absence of lipid droplets in the ER lumen and inhibition of MTP decreases triglyceride levels in the lumen. It has also been shown that MTP stably associates with vesicles. Thus, we propose, "MTP may play a role in the import of triglycerides, and the formation and stabilization of lipid droplets in the ER lumen."

3.3. ApoB binding, the chaperone activity

Three different experimental approaches have been used to document physical interactions between apoB and MTP [for review, see (20)]. Wu *et al.* (43), Patel and Grundy (30), and Mann *et al.* (27) used coimmunoprecipitation techniques to demonstrate interactions between apoB and MTP. We developed a solid-liquid inter-phase binding assay (19) to study protein-protein interactions between these proteins. In this assay, immobilized MTP was incubated with increasing concentrations of LDL, and bound LDL was then quantified using an enzyme-linked immunoassay for apoB (7, 21). These studies showed that human LDL binds with high affinity to immobilized MTP. To identify critical amino acid residues involved in protein-protein interactions, human LDL was subjected to chemical modifications (6). Modification of acidic residues by glycine methyl ester had no effect on apoB-MTP binding. However, acetoacetylation of lysine residues and cyclohexanedione modification of arginine residues completely abolished MTP binding. More importantly, regeneration of the modified arginine and lysine residues completely restored MTP binding. These studies indicated that lysine and arginine residues in apoB are important for interaction with the acidic residues in MTP.

To identify an MTP binding site in apoB, we subjected apoB to C-terminal truncations and studied the

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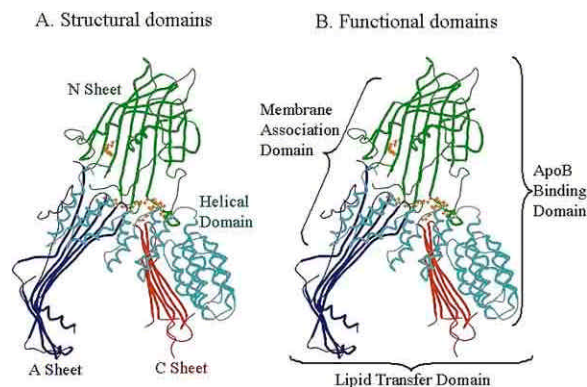


Figure 2. Structural and functional domains in the M subunit of MTP: A. Structural Domains; The M subunit consists of three β -sheets (A, C and N) and one helical domain. B. Functional domains; The M subunit is proposed to contain three functional domains: apoB binding, lipid transfer, and membrane association domains. A possible relationship between the structural and functional domain is illustrated. The functional domains are probably made up of different combinations of the structural domains present in the molecule.

binding of these chimeras to MTP at equi-molar concentrations (18). Truncation of apoB100 (B:1-4536) to apoB48 (B:1-1905) had no effect on MTP binding. However, truncation to apoB28 (B:1-1270) resulted in 3-fold increased binding to MTP. Further truncation to apoB17 (B:1-771) resulted in an additional 2-fold increase in binding. These studies showed that apoB17 contains the high affinity-binding site. To further characterize the binding site, we expressed apoB sequences (B:1-300 and B:270-570) as FLAG chimeras in COS cells which do not assemble lipoproteins (19). The amounts of secreted chimeras were quantified by studying their binding to immobilized anti-FLAG monoclonal antibody, M2. Both chimeras were secreted to a similar extent. Next, we studied their binding to immobilized MTP. B:270-570 bound to MTP, but B:1-300 did not. From this we concluded that MTP binds to B:270-570. In independent studies, Mann *et al.* (27) used a yeast two-hybrid system to show that B:1-152, B:349-583, and B:512-721 bind to MTP. Thus we can conclude that “the N-terminal B:1-771 (B17) contains an MTP binding site.”

Several studies have indicated that the apoB binding or chaperone activity of MTP plays an important role in lipoprotein assembly [reviewed in (20)]. First, we have identified a specific inhibitor of apoB-MTP binding which decreases apoB secretion (7). Second, Shoulders and associates showed that abolishing apoB-MTP binding by site-directed mutagenesis results in decreased apoB secretion (9, 27). In addition, Liang and Ginsburg observed that deletion of B:1-210 decreased apoB secretion and suggested that apoB-MTP binding might be important for apoB secretion (26). Thus, specific inhibitor studies, site-directed mutagenesis, and deletion analysis provide evidence for the physiologic importance of apoB-MTP binding in lipoprotein assembly.

4. STRUCTURES OF MTP AND APOB17

So far, three functions of MTP have been discussed. Now, let's turn to MTP structure. Shoulders and associates showed that MTP and N-terminal apoB sequences are homologous to lipovitellin (36). Lipovitellin is the major lipid-protein complex present in egg yolk. Banaszak and associates have described a structure of lipovitellin at 2.8 Å resolution (1, 32, 37). According to this structure lipovitellin consists of three β -sheets (A, C, and N) and one helical domain (Figure 1).

Based on the sequence homology, it has been proposed that lipovitellin, MTP, and apoB may be structurally similar (9, 27). We also observed sequence homology between these molecules using CLUSTAL W program as described before (20). Based on this homology, we deduced a structure for MTP and apoB17 (Figure 1). MTP contains three β -sheets (A, C and N) and one helical domain. ApoB17 contains N- and C-sheets as well as one helical domain. Comparison of these structures shows that the helical domain is fairly well conserved between the three molecules. Similarly, the N- and C-sheets are also well conserved. The major difference is with regards to the A-sheet, it is truncated in MTP and absent in apoB. In the M subunit of MTP, truncation of the A-sheet results in a larger cavity compared to that present in lipovitellin. This might explain the ability of MTP to bind a larger number of lipid molecules compared to lipovitellin. Absence of the A-sheet may explain the inability of apoB to transfer lipids. However, apoB contains the other hydrophobic C-sheet and it has been implicated in membrane association (16).

5. STRUCTURE FUNCTION RELATIONSHIP

As discussed above, MTP has three functions and four structural motifs, N-sheet, A-sheet, C-sheet, and one helical domain. How do the four structural domains of the M subunit carry out these functions? Different structural motifs may coordinate to form functional domains in MTP. Based on the studies of Shoulders and associates, it is likely that the lipid transfer domain is surrounded by the A- and C-sheets. The helical domain and N-sheet may comprise the apoB-binding domain. We propose that the N-sheet and the A-sheet may form the membrane association domain (Figure 2).

Inherent in this assignment is the assumption that different domains function independent of each other. Significant evidence exists to suggest and support these assumptions. Studies involving specific inhibitors provide the evidence for independent lipid transfer and apoB binding domains. We have shown that inhibitors of lipid transfer activity do not affect apoB-MTP binding (7). Similarly, an inhibitor of apoB-MTP binding has no effect on the lipid transfer activity of MTP indicating that these might be independent functional domains (7). Modulation of apoB-MTP binding by lipids provides evidence for independent apoB binding and membrane association domains. We have shown that MTP associates with lipid vesicles and this association increases the binding of MTP

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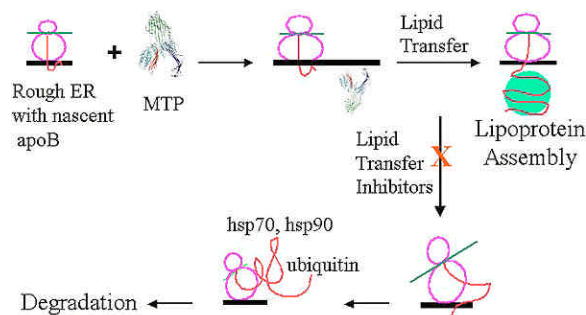


Figure 3. Functional importance of different domains of MTP: A. Lipid transfer activity; Nascent apoB is shown associated with the ER membrane. MTP is shown binding to the ER membrane. MTP can pick up lipid molecules from the membrane, transfer them to apoB and promote lipoprotein assembly. Inhibition of the lipid transfer activity results in the binding of hsp70, hsp90, and ubiquitin enhancing the degradation of apoB.

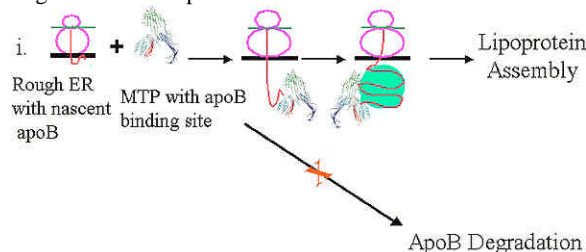


Figure 4. ApoB binding activity; MTP is shown to interact with nascent apoB resulting in the partial dislocation of nascent apoB from the ER membrane. This may provide a hydrophobic region in apoB for MTP to deliver lipids. Extensive lipidation may result in successful assembly of lipoproteins. Inhibition of apoB-MTP binding may increase apoB degradation.

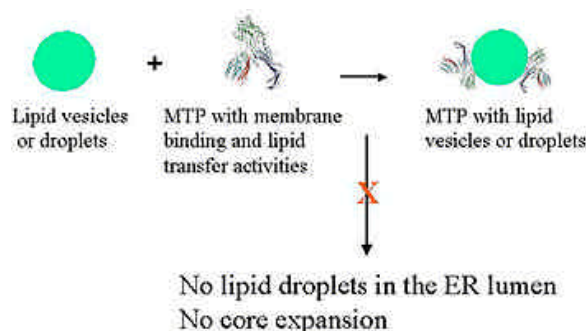


Figure 5. Membrane binding activity; Several molecules of MTP can associate with lipid droplets and stabilize them. The formation and stabilization of lipid droplets may involve lipid transfer and membrane association domains. It is speculated that inhibition of the binding of MTP to lipid vesicles might decrease the number of lipid droplets in the ER lumen and impair core expansion of primordial particles.

to apoB (5). Currently, it is not known whether lipid association affects lipid transfer activity of MTP.

6. ROLE OF DIFFERENT FUNCTIONAL DOMAINS OF MTP IN LIPOPROTEIN ASSEMBLY

6.1. The lipid transfer domain

The lipid transfer domain of MTP can play an important role in lipoprotein assembly by two independent mechanisms. First, it can pick up lipids from the ER membrane and transfer them to nascent apoB (Figure 3). Several rounds of transfer would result in the formation of a primordial particle. Inhibitors that bind the lipid-transfer domain would attenuate the lipidation process. Insufficiently lipidated apoB is recognized by hsp90, hsp70, and ubiquitin leading to enhanced degradation. Second, as discussed before, the lipid transfer domain may play a role in the formation and stabilization of lipid droplets. Thus, inhibition of the lipid transfer activity could also affect the import of triglycerides and formation of lipid droplets jeopardizing the formation of lipoprotein particles.

6.2. The apoB binding domain

MTP binds the N-terminus of apoB exposed to the luminal side of the ER (Figure 4). This apoB binding, or the so-called chaperone, activity of MTP may promote lipoprotein assembly in two ways. First, it may be involved in the release of nascent apoB from the ER membrane. As the apoB is pulled away from the membrane, the hydrophobic lipid binding domains in apoB can become available for lipidation. Second, apoB-binding may also provide a mechanism for the specific net transfer of lipids to nascent apoB instead of a futile transfer between membranes. Extensive lipidation may result in the formation of an intermediate lipoprotein particle that still has MTP associated with it. At some point MTP will be released resulting in the formation of a primordial lipoprotein particle. It is known that the inhibition of apoB-MTP binding results in decreased apoB secretion (7). It remains to be determined whether decreased secretion is secondary to increased proteosomal degradation.

6.3. The membrane association domain

The membrane-association domain may be involved in the formation and stabilization of lipid droplets (Figure 5). MTP associated with lipid droplets could be involved in the maturation of lipoprotein assembly by delivering lipids as a bolus. In addition, lipid-associated MTP can be important in the biogenesis of primordial lipoprotein particles. We have shown that lipid-associated MTP has higher affinity for apoB (5). Binding of lipid-associated MTP to nascent apoB may result in the availability of lipid surface for nascent apoB to encircle and wrap around. This would result in the formation of a primordial particle in a single step avoiding sequential addition of lipid molecules. This process is anticipated to predominate during increased lipid availability. Inhibition of the membrane association domain of MTP may decrease the formation of lipid droplets in the ER lumen and impair core expansion of the primordial particles.

7. CONCLUDING REMARKS

As discussed above and in several other reviews, the requirement of MTP in lipoprotein assembly is well

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established. However, various steps in lipoprotein maturation that are assisted and coerced by MTP have not yet been fully appreciated. The aim of this review was to emphasize that MTP is a multi-functional protein and to invigorate research toward the identification of novel functions of MTP. Presently, all the identified functions are believed to play important roles in lipoprotein assembly and secretion. It is quite possible that MTP may also be required in physiologic processes beyond lipoprotein assembly and secretion. For example, MTP may act as a chaperone inducing proper folding of proteins other than apoB. In addition, MTP may play a role in lipid secretion/mobilization pathways not involving apoB.

8. ACKNOWLEDGMENT

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Abbreviations: Apolipoprotein B, apoB, Endoplasmic reticulum, ER, Lipovitellin, LV, Microsomal triglyceride transfer protein, MTP.

Key Words: Apolipoprotein B, apoB, Apolipoprotein, Lipoprotein, Microsomal triglyceride transfer protein, Mtp, Biosynthesis, Review

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