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Review

Microsomal triglyceride transfer protein

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Abbreviations: MTP, microsomal triglyceride transfer protein; PDI, protein disulfid isomerase; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; apoB, apolipoprotein B; TG, triglyceride; CE, cholesteryl ester; PC, phosphatidylcholine; DG, diacylglycerol; ER, endoplasmic reticulum; SUV, small unilamellar vesicle; STZ, streptozotocin; C, cysteine; D, aspartic acid; E, glutamic acid; G, glycine; H, histidine; K, lysine; L, leucine; S, serine; W, tryptophan.

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1. Introduction

The microsomal triglyceride transfer protein (MTP) which was originally isolated from the microsomal fraction of bovine liver, accelerates the transport of triglyceride (TG), cholesteryl ester (CE), and phosphatidylcholine (PC) between synthetic membranes [1]. The protein was subsequently found within the lumen of microsomes isolated from both the liver and intestine [2]. The tissue distribution and subcellular location of MTP, in conjunction with its ability to transport TG, led to the hypothesis that MTP is involved in the assembly of TG-rich plasma lipoproteins, very low density lipoproteins (VLDL) and chylomicrons. This hypothesis was subsequently shown to be correct when it was discovered that individuals with abetalipoproteinemia (genetic defect in the production of VLDL and chylomicrons) have mutations in the gene encoding MTP which result in a loss of MTP protein.

Here we have reviewed MTP, its lipid transport properties, mutations in MTP which cause abetalipoproteinemia and what has been learned from them, and the regulation of MTP and its activity. We have tried to identify areas of controversy and areas which require further elucidation. In addition, what is known about the role of MTP in lipoprotein assembly is discussed, as well as our speculation regarding what available information may be telling us about the role of MTP in the assembly process.

2. Isolation and characterization of MTP

MTP from bovine liver has been isolated and extensively characterized [3–5]. The initial step in the purification of MTP is the isolation of the microsomal fraction. The isolated microsomes are suspended in 1 mM Tris and the pH is adjusted to 8.6 to disrupt the microsomal membrane and allow the lumenal contents, including MTP, to be released. Following removal of the membrane fraction, highly pure MTP is obtained by conventional column chromatography (ion exchange, gel permeation, and hydroxyapatite). Starting with the soluble proteins released from the microsomal fraction, the calculated purification factor for obtaining pure MTP is typically in the range of 100–200. This indicates that MTP is present in high concentrations within the lumen of the endoplasmic reticulum, representing up to 0.5 to 1% of the total protein content of the lumen. Using a modification of early purification protocols, Ohringer et al. [6] isolated bovine MTP which readily crystallizes. The crystals diffract to better than 3.2 ångström resolution.

Purified bovine MTP has two protein components with mobilities on SDS polyacrylamide gel electrophoresis that are consistent with proteins of molecular weight 58 000 and 88 000 [3]. By sedimentation equilibrium, the purified protein has a molecular weight of 150 000 [5], indicating that the transfer protein is a heterodimer of the two proteins. Sequence analysis and immunological characterization of the low molecular weight component of MTP demonstrated that it is identical to a previously described protein, protein disulfide isomerase (PDI) [4]. PDI is an ubiquitous protein which has multiple functions within the lumen of the endoplasmic reticulum (ER) [7]. Its disulfide isomerase activity is used to properly pair cysteine residues of newly synthesized proteins into disulfide bonds. PDI also has chaperone activity independent of its disulfide isomerase activity [8]. In addition to being a subunit of MTP, PDI is also a component of the tetrameric enzyme, prolyl 4-hydroxylase [9]. Prolyl 4-hydroxylase contains two alpha and two beta subunits. The beta subunit is identical to PDI.

The interaction between PDI and the large subunit of MTP appears to be irreversible [10]. To date, attempts to reconstitute MTP from its individual components or exchange exogenous PDI into the MTP complex have not been successful. If the two components of MTP are dissociated by treatment with low concentrations of denaturants, a nondenaturing detergent, or chaotropic agents, the large subunit of MTP is insoluble. Based upon the purification factors for MTP and PDI, it is estimated that the free PDI concentration in the lumen of the endoplasmic reticulum of bovine liver exceeds that of MTP by at least five-fold [4]. In vitro, PDI has been found to self-associate into dimers and tetramers [11]. The insoluble nature of the large subunit of MTP suggests that it either co-translationally associates with PDI or that the addition of the large subunit of MTP to PDI is mediated by a chaperone protein-large subunit complex intermediate. Expression studies showing

that the carboxy-terminus of the MTP large subunit is required for the formation of the MTP complex suggests that the entire protein must be translated before it forms a stable complex with PDI [12].

The importance of PDI to the MTP complex was illustrated in expression studies in insect Sf9 cells using the baculovirus expression system [12]. When Sf9 cells were infected with viruses expressing the large subunit of MTP alone, MTP activity was not detectable. Western blot analysis of subcellar fractions demonstrated that the expressed protein was an insoluble aggregate. In contrast, when Sf9 cells were co-infected with viruses expressing PDI and the large subunit of MTP, MTP activity was detectable and both PDI and the large subunit of MTP were found in the soluble fraction. These findings confirmed that PDI is essential to maintain the structure and activity of MTP.

In an assay which measures the folding of reduced and denatured ribonuclease, the PDI component of MTP expresses disulfide isomerase activity only about one-tenth that of free PDI [4]. When PDI is dissociated from the MTP complex, its disulfide isomerase activity increases to a level comparable to that of authentic PDI. PDI contains two thioredoxin-like active sites (amino-acid sequence WCGHCK) located at amino acids 35–40 and 379–384 of the 491 amino-acid protein. These sites confer the disulfide isomerase activity to the protein. Apparently the association of PDI with the large subunit of MTP blocks accessibility to the two active sites of PDI, or the large subunit of MTP alters the conformation of PDI in a fashion which results in a loss of PDI activity. Whether the PDI in MTP still has its chaperone or peptide binding activities is not known. The peptide binding site on PDI is located in the carboxy terminal portion of the protein [13]. It is not known if the peptide binding site on PDI plays a role in its association with the MTP large subunit.

The contribution of PDI to the transfer activity of MTP is not known. Clearly, PDI plays a structural role in MTP by maintaining the large subunit in a soluble form. It may also play a role in targeting MTP to the endoplasmic reticulum. The MTP complex probably utilizes the carboxy terminal KDEL ER retention sequence found on PDI [9] to help retain it in the endoplasmic reticulum. This sequence of amino acids which is found on the carboxy terminus of several resident ER proteins, mediates binding to a receptor found in a post ER, Golgi-like compartment [14]. Once bound, a protein is transported back to the endoplasmic reticulum where it is released. A similar ER retention sequence is not found on the large subunit of MTP [15].

Baculovirus expression studies were used to demonstrate that the disulfide isomerase activity of PDI does not play a role in the lipid transfer activity of MTP. Lamberg et al. [16] prepared baculoviruses expressing either wild-type PDI or an inactive PDI in which the two thioredoxin-like sites of PDI were mutated from CGHC to SGHC. Viruses expressing the large subunit of MTP were then co-infected in insect cells with viruses expressing either the inactive or wild-type PDI. Comparable levels of MTP and TG transfer activity were observed using viruses expressing active or inactive PDI.

The amino-acid sequence of the large subunit of human MTP has been deduced from cDNA sequence [15]. The full length protein has 894 amino acids and a predicted molecular weight of 99 kDa. The amino terminal portion of the protein is hydrophobic in nature, consistent with it being a signal peptide. Using the algorithm of Von Heijne [17], cleavage of the signal peptide is predicted to occur following amino acid 19. This cleavage site is supported by amino terminal sequence analysis of the large subunit of bovine MTP (unpublished observation). The predicted molecular weight of the mature large subunit is 97 kDa. The large subunit is highly conserved between species. There is 86% identity between the deduced amino-acid sequence of the human protein and that of bovine [15], hamster [18], or mouse [19]. High levels of homology are found throughout the protein.

The large subunit of MTP is not highly homologous to any previously described protein. Shoulders et al. [20,21] identified several regions with low levels of sequence homology to lipovitellin, the protein component of a lipoprotein complex found in egg-laying animals. Lipovitellin is formed from the cleavage of vitellogenin. Based upon sequence analysis, molecular modeling, and a comparison of gene structures, they proposed that the MTP large subunit is a member of the vitellogenin gene family. The lipovitellin complex is comprised of three peptides, LV1, LV2, and PV with molecular weights of 66 800,

40750, and 35200, and about 15% lipid. The polypeptides have beta-sheet domains that form a cavity which can accommodate 32 phospholipid molecules [22].

3. Lipid transport properties of MTP

3.1. Lipid transfer assay

MTP accelerates the transport of lipid molecules between synthetic small unilamellar vesicles (SUV) and between low density lipoproteins (LDL) and high density lipoproteins (HDL) [3]. MTP activity is routinely determined by measuring the rate of transfer of radiolabeled TG from donor SUV to acceptor SUV [23]. A typical transfer reaction mixture contains donor vesicles (40 nmol egg PC, 0.25 mol% radiolabeled TG, and 7.5 mol% cardiolipin), acceptor vesicles (240 nmol egg PC and 0.25 mol% unlabeled TG) and 5 mg bovine serum albumin in a total of 0.9 ml buffer. The negative charge in the donor vesicles due to the presence of cardiolipin facilitates the separation of donor and acceptor membranes. Following a transfer reaction, a DEAE-cellulose suspension is added to selectively bind the negatively charged donor vesicles. The DEAE-cellulose and bound donor membranes are then pelleted by low speed centrifugation. The acceptor SUV concentration in the assay mixture is kept in excess over that of the donor SUV to minimize back transfer of radiolabeled lipid from acceptor to donor vesicles. First-order kinetics are used to quantitate total lipid transfer. This corrects for the dilution of labeled lipid in the donor vesicles as the transfer reaction proceeds. The transfer activity is generally expressed as a percent of the donor lipid transferred per unit of time.

3.2. Exchange versus mass transport

Lipid transfer proteins may either exchange lipid molecules between membranes or promote the net movement of lipid molecules from one population of membranes to another. Although there are exceptions, in the in vitro assays used to evaluate lipid transfer, lipid transfer proteins generally promote the exchange of lipid molecules between membranes. When there are equal concentrations of TG in the donor and acceptor membranes, MTP promotes the exchange of TG between membranes [24]. However, when there is an imbalance between the neutral lipid content in the donor and acceptor membranes, for example, when only the donor membranes contain TG or CE, neutral lipid is still transported to acceptor particles [3,24], suggesting MTP is capable of net TG and CE transport. Whether there is an equal molar transfer of PC from the acceptor to donor membranes is not known. Thus it is not clear if in the in vitro assays used to characterize MTP, MTP is capable of promoting net lipid transport, or if it is exchanging one lipid molecule for another.

3.3. Lipid transport mechanism

Enzyme kinetics were used to investigate the mechanism by which MTP transports lipid molecules between membranes [24]. The analysis was complicated by acceptor membranes acting as both a substrate and an inhibitor in the transfer reaction. To simplify the analysis, initial velocities were measured as a function of substrate concentration when both donor and acceptor membrane concentrations were varied simultaneously. This allowed the equations used to determine the kinetic mechanism to be simplified. MTP-mediated lipid transport consistent with ping pong bi bi kinetics suggests that MTP binds and shuttles lipid molecules between membranes. For ping pong bi bi kinetics, the kinetic equation is:

$$V_{max}/v = 1/[\mathrm{DON}](K_D + K_A) + (1 + K_D/K_i)$$

where K_A and K_D are steady state constants and K_i is the dissociation constant for an acceptor vesicle-MTP complex. This predicts a 1/initial velocity vs. 1/substrate concentration double reciprocal plot is a straight line. Alternative kinetic models considered predicted that a similar plot would be a concave line [24]. The double reciprocal plot was a straight line, indicating that MTP transports lipid molecules between membranes by a shuttle mechanism.

A shuttle mechanism for lipid transport predicts that a stable MTP-lipid complex is an intermediate in the transfer reaction. Evidence for formation of a stable reaction intermediate is provided by lipid binding studies. If MTP is incubated with donor vesicles containing radiolabeled lipid molecules and then re-isolated, the MTP contains up to three molecules of

bound lipid [25]. MTP forms a stable complex with a variety of neutral lipids (TG, CE, diacylglycerol, and squalene) and phospholipids (PC, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidic acid) [25,26]. Fluorescent assays capable of measuring lipid transport from donor membranes to MTP demonstrated that MTP rapidly binds (within minutes) fluorescent analogs of TG, CE, and PC [24,25].

The binding and transfer of TG by MTP is dependent upon the concentration of TG in the donor membrane. An increase in TG concentration in membranes increases both binding of TG to MTP and the number of TG molecules transported from donor to acceptor membranes [25]. This suggests that the ability of MTP to transport lipid molecules is related to its ability to bind them. When the binding of a variety of different lipid molecules (TG, CE, diacylglycerol, squalene, and PC) to MTP was investigated, a linear relationship was observed between the different lipid molecules binding to MTP and their relative rates of transport by MTP [26]. Thus, the ability of MTP to bind lipid molecules at the membrane surface appears to govern their rate of transport by MTP. The close relationship between the ability of MTP to bind and transport lipid molecules is consistent with the proposed shuttle mechanism of lipid transport.

3.4. Substrate specificity

The ability of MTP to transfer different types of lipid molecules has been investigated in detail to determine how changes in the structure of a lipid molecule affects its transport by MTP. To compare the transfer rates for different lipid molecules, the rates are expressed as percent lipid transported per time. This allows one to identify the selective transport of a particular class of lipid molecules. However, the substrate membranes used to characterize MTP-mediated lipid transfer contain a vast excess of PC so that when the actual mass of lipid transported is calculated, the mass of PC transferred exceeds that of the lipid molecules found in low concentrations in the membranes.

Jamil et al. [26] incorporated different neutral lipids and phospholipids into donor membranes at a 0.25 mol% concentration and then measured their initial rates of transfer. The concentration of the lipid molecules studied was kept low so that there would be minimal changes in the physiochemical properties of the donor membranes with the different lipids studied. Under these conditions, relative transfer rates should reflect MTP-lipid molecule interactions at a membrane surface. Lipid transport rates were decreased in order of TG > CE > diacylglycerol (DG) > PC. MTP showed a strong preference for transporting neutral lipid between membranes. Initial rates of transfer of TG, CE, and DG were 24-, 16-, and 2.5-fold higher than that of phosphatidylcholine, respectively. A striking finding was the sensitivity of the transfer rate to changes in fatty acid content and hydrophobic nature of a lipid molecule. The removal of a fatty acid chain from TG, CE, or PC to form DG, cholesterol, or lyso-PC, respectively, resulted in a 90% decrease in the rate at which the lipid molecule was transported, indicating that the size of the hydrophobic moiety, regardless of the lipid class, plays an important role in MTP-mediated transfer.

MTP has been shown to transport a wide variety of phospholipid molecules (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, and a positively charged synthetic phospholipid analog, ethyl PC) [26]. Phospholipid molecules of varying head group structure or charge (negative, neutral, or positive) are all transported at similar rates, suggesting that there are no specific, structural or charge–charge interactions between MTP and the polar head group of a phospholipid molecule [26]. The lipid binding pocket on MTP which binds and transports lipid molecules appears to be flexible and hydrophobic in nature in that it can bind a variety of both neutral and polar lipid molecules.

3.5. Two classes of lipid molecule binding sites

Lipid binding and transport studies have shown that there are two different classes of lipid molecule binding sites on MTP [25]. MTP has been reported to bind up to three phospholipid molecules, whereas the highest reported value for TG binding to MTP is 0.2–0.25 mol per mol MTP. Differences in the neutral lipid and phospholipid binding properties of MTP are observed by measuring the transport of radiolabeled lipid from MTP to acceptor vesicles. TG and CE are transported rapidly (within minutes) from

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