The Enzymatic and Non-enzymatic Roles of Protein-disulfide Isomerase in Apolipoprotein B Secretion*

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Secretion of apolipoprotein B (apoB) from mammalian cells requires the presence of functional microsomal triglyceride transfer protein (MTP). We previously reported that co-expressing the human intestinal form of apoB, B48, with both subunits of human MTP in oleate-treated SF21 cells led to a dramatic induction of B48 secretion. Deletion mutagenesis studies showed that the cysteine-enriched amino terminus of apoB was necessary for the MTP responsiveness (Gretch, D. G., Sturley, S. L., Wang, L., Dunning, A., Grunwald, K. A. A., Wetterau, J. R., Yao, Z., Talmud, P., and Attie, A. D. (1996) J. Biol. Chem. 271, 8682–8691). We therefore hypothesized that the small subunit of MTP, protein-disulfide isomerase (PDI), plays a role in apoB secretion by facilitating correct disulfide bond formation. To determine whether the enzymatic activities of PDI are important for MTP-stimulated apoB secretion, the wild type PDI subunit was replaced with an active site mutant, mPDI (Cys\(^{36}\) → Ser/Cys\(^{380}\) → Ser), lacking both disulfide shuffling and redox activities. MTP containing mPDI was fully functional in promoting apoB and triglyceride secretion. Therefore, the shufflase and redox activities of PDI are not necessary for the function of MTP. Since PDI exists in large molar excess over the other subunit of MTP, the role of free PDI (independent of the MTP complex) was investigated. PDI or mPDI was co-expressed with B48 and B17, a fragment encompassing the amino-terminal 17% of apoB. Mutant PDI significantly and specifically reduced the accumulation of the B17 and B48 both intracellularly and in the culture medium. The reduction was partially eliminated by the protease inhibitor N-acetyl-leucyl-leucyl-norleucinal, consistent with the clinical evidence obtained* This work was supported in part by Grant HL37251 from the NHLBI, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Many secretory and plasma membrane proteins have disulfide bonds. Correct disulfide arrangement is crucial not only for protein structure and function, but also for their progress through the secretory pathway beginning in the endoplasmic reticulum (ER).\(^1\) Although the redox conditions in the ER lumen permit spontaneous disulfide bonding (1), formation of the native disulfide pattern appears to be an enzyme-facilitated process (2).

Protein-disulfide isomerase (PDI, EC 5.3.4.1) is a multi-functional protein that plays a key role in the co- and post-translational modification of secretory and cell surface proteins in all eukaryote cells (2, 3). It is a major ER-resident protein. As an enzyme, PDI catalyzes the rearrangement of pre-existing disulfide bonds (referred to as the “shufflase” activity) as well as net formation or breakage of disulfide bonds, depending on the reduction potential of the environment (the “redox” activity). Although PDI can catalyze both types of enzymatic reactions, it is the shufflase activity that appears to be essential in eukaryotic cells (4).

PDI serves as a subunit in two known protein complexes. It is the \(\beta\) subunit of prolyl 4-hydroxylase, an enzyme involved in collagen biosynthesis (5). PDI also forms a heterodimer with a 97-kDa protein that has \textit{in vitro} lipid transfer activities, to form microsomal triglyceride transfer protein (MTP), located in the ER lumen of hepatocytes and intestinal cells (6–8). PDI is necessary to maintain the lipid transfer activity of MTP and to prevent aggregation of the 97-kDa subunit (7).

Mutations within the 97-kDa subunit of MTP are responsible for abetalipoproteinemia, a genetic disease distinguished by the inability to secrete triglyceride-carrying lipoproteins: chylomicrons and very low density lipoprotein (VLDL) (9–11). Hence, MTP is essential for the assembly and secretion of these lipoprotein particles in mammalian cells.

Apolipoprotein B (apoB) is an essential protein component of chylomicrons and VLDL (12, 13). The full-length apoB molecule (apoB100 or B100) has 4536 amino acid residues and multiple proposed lipid binding regions. In humans, VLDL is secreted from the liver carrying B100, while the intestine produces chylomicrons with a truncated form of apoB, B48 (the amino-terminal 48% of B100), due to an RNA editing event that converts the codon for Gln\(^{2153}\) to a stop codon (14, 15). Studies using non-hepatic/non-intestinal cell lines suggest that heterologous expression of MTP is required for the secretion of B100 as well as apoB carboxyl-terminal truncations such as B53 and B48 (16–19), consistent with the clinical evidence obtained

\(^1\) The abbreviations used are: ER, endoplasmic reticulum; PDI, protein-disulfide isomerase; MTP, microsomal triglyceride transfer protein; VLDL, very low density lipoprotein; apoB, apolipoprotein B; DTT, dithiothreitol; LDL-R, low density lipoprotein receptor; apoE3, apolipoprotein E3; m.o.i., multiplicity of infection; hpi, hours post-infection; ALLN, N-acetyl-leucyl-leucyl-norleucinal; PAGE, polyacrylamide gel electrophoresis; WT, wild type; RBP, retinol-binding protein; DHFR, dihydrofolate reductase; mPDI, mutant PDI.
from studying abetalipoproteinemia patients. However, secretion of shorter apoB truncations such as B17 does not require MTP (16–19).

We have previously reconstituted the mammalian lipoprotein assembly pathway in Sf21 insect cells by using baculovirus-mediated expression of human genes encoding apoB and the MTP subunits (18). Sf21 cells can secrete some B48 even in the absence of human MTP, suggesting that they possess an endogenous MTP-like function. However, expression of human MTP greatly enhanced B48 secretion in these cells. Deletion analyses of apoB demonstrated that the MTP responsiveness requires both the amino terminus and a sufficient length of apoB (18). Although a physical interaction between MTP and the amino terminus of apoB has been detected (20, 21), the mechanisms by which MTP mediates lipoprotein assembly and secretion are not well understood.

The amino terminus of apoB is enriched in cysteine residues: out of the 25 cysteines in B100, 12 are clustered within the first 11% of the protein, forming six disulfide bonds (22). There are only two more disulfide bonds in the rest of the molecule. In Sf21 cells, most apoB fragments without the amino terminus were secretion-incompetent (18). In hepatoma cells, treatment with dithiothreitol (DTT) interrupts secretion of newly synthesized apoB. These observations are consistent with a role for the amino terminus of apoB in the proper folding of the protein and its assembly with lipids (23).

The importance of the disulfide-rich amino terminus of apoB led to our hypothesis that PDI may be involved in apoB secretion by assisting in correct disulfide bond formation. In the present study, this hypothesis was tested by expressing an enzymatically inactive form of human PDI. We first asked if the catalytic activities of PDI are required for the MTP heterodimer to facilitate apoB secretion. As we have shown previously, the effect of MTP cannot be detected unless human PDI is expressed along with the 97-kDa subunit in Sf21 cells (18). This result implied that the endogenous insect PDI cannot form a functional complex with the 97-kDa subunit of MTP. Hence, this system provided us with the unique opportunity to explore the role of human PDI in the MTP complex without interference from the endogenous enzyme.

In addition to its role in the MTP complex, PDI also exists in a free form in high abundance in the ER lumen. Since Sf21 cells can secrete some apoB in the absence of human MTP and secretion of short apoB truncations can be MTP-independent, the second question we asked is whether the enzymatic activities of PDI are necessary at steps in the apoB secretion pathway not involving MTP.

PDI has two active sites that are homologous with the active site of another thiol-reactive enzyme, thioredoxin (2). The active sites contain the sequence -WCGHCK-. Since the amino-terminal cysteine within each active site has a relatively low pKₐ and is more solvent-accessible, it acts as the nucleophile that initiates the PDI-catalyzed reactions (24, 25). This cysteine residue is sufficient for the shufflase activity (4, 24, 26, 27). To catalyze disulfide bond formation and breakage, both cysteine residues within at least one of the active sites are required; the second cysteine residue functions to displace the mixed disulfide between the first cysteine and the substrate of PDI (24, 25).

In this study, we expressed a PDI mutant devoid of both enzymatic activities. The amino-terminal cysteines of both active sites were mutated to serines: Cys⁵⁶ → Ser/Cys⁸⁰ → Ser; mPDI (28). We discovered that within the MTP complex, the enzymatic activities of PDI were not necessary to obtain the induction of apoB and triglyceride secretion. However, in the absence of adequate free fatty acid supplementation, expres-
continuous incubation with radioactive tracers—cells were infected as described above with a total m.o.i. of 10. At 27 hpi, fresh media containing 0.5% bovine serum albumin complexed with 1 mM oleate was added. Seventeen hours later, lipids were extracted and cell samples were loaded on the gel as not the same percentage of the whole culture. Lipids were extracted, separated by thin layer chromatography, and quantitated by scintillation counting. The ratios of secreted to total lipids were calculated and the results were normalized to the value from B48 + WT. Values represent the means of three or four determinations ± standard deviation. B48 is shown in representative immunoblots probed by a polyclonal antibody against apoB. In the immunoblot, the medium and cell samples loaded on the gel are not the same percentage of the whole culture (10% of total medium and 0.5% of total cell extract); thus, the percent secretion cannot be estimated directly from the band intensity.

Continuous Incubation with Radioactive Tracers—Cells were infected as described above with a total m.o.i. of 10. At 35 hpi, the media were removed and cells were washed with phosphate-buffered saline (pH 6.2). This was followed by incubation with methionine/cysteine-free medium for 1 h at 27 °C. Then the cells were pulse-labeled with the methionine/cysteine-free medium containing 0.1 mCi/ml [35S]methionine/cysteine for 5, 10 and 15 min. The protease inhibitor N-acetyl-leucyl-leucyl-norleucinal (ALLN) was dissolved in ethanol and added to the methionine/cysteine-free medium as indicated at a final concentration of 10 μg/ml. The same volume of ethanol was added to control plates. At each time point, cells were washed, collected, and lysed by sonication. Aliquots of cell lysate were used for protein assay and immunoprecipitation as described (18). Immunoprecipitates were subjected to SDS-PAGE fractionation. Bands corresponding to B17 and B48 were excised and radioactivity was quantitated in a liquid scintillation counter.

RESULTS

Assay for the Shufflase Activity of Human PDI Produced in Sf21 Cells—To study the importance of the enzymatic activity of PDI in apoB secretion, recombinant baculoviruses encoding the wild type and active site mutated human PDI were generated and used to infect Sf21 insect cells. Wild type and mutant PDI (mPDI) were each purified from infected cells and assayed for the shufflase activity by testing their ability to refold scrambled ribonuclease A in vitro (34). In a control experiment, a sample isolated from mock-infected cells with the same purification strategy was used. Only the wild type PDI was able to catalyze the reactivation of ribonuclease A (Fig. 1). Neither the control nor the mutant PDI displayed any shufflase activity. The lack of activity in the control sample indicated that the endogenous insect PDI was not purified with the procedure for human PDI. Since the lack of shufflase activity will ensure the absence of the redox activity (34), the latter was not assayed.

Effect of Enzymatically Inactive PDI Subunit on MTP-facilitated ApoB Secretion—We previously observed a dramatic induction of B48 secretion from Sf21 cells upon co-expression with MTP in the presence of oleate (18). In contrast, B17 (the amino-terminal 17% of apoB100) had little response to either oleate or MTP. To determine whether the stimulation of B48 secretion by MTP requires an enzymatically active PDI subunit, B48 was co-expressed with either the wild type or mutant form of human PDI, together with the 97-kDa subunit. B48 was co-expressed with individual subunits of MTP or wild type baculovirus (WT) were used as controls. All cells were treated with 1 mM oleate.

Cells expressing both MTP subunits secreted at least 4-fold more B48 than control cells infected with either an irrelevant virus (WT) or recombinant baculovirus encoding only one subunit of MTP (Fig. 2). MTP with the mutant PDI induced B48 secretion to the same level as compared with the wild type MTP. This result suggests that the mutant PDI is fully capable

FIG. 2. Effect of mutant PDI on MTP-facilitated apoB secretion. B48 was co-expressed with the indicated proteins in 1 mM oleate-treated Sf21 cells and analyzed by quantitative immunoblotting, as described under “Experimental Procedures.” Wild type baculovirus (WT) was used as a control. The ratios of m.o.i. for individual viruses are: B48:WT or PDI or mPDI or 97-kDa = 6.4 and B48:PDI or mPDI: 97-kDa = 6:2.2. Percent secretion = (secreted B48/total B48 accumulation in both medium and cells) × 100. Values represent the mean of four determinations ± standard deviation. B48 is shown in representative immunoblots probed by a polyclonal antibody against apoB. In the immunoblot, the medium and cell samples loaded on the gel are not the same percentage of the whole culture (10% of total medium and 0.5% of total cell extract); thus, the percent secretion cannot be estimated directly from the band intensity.

FIG. 3. Effect of mutant PDI on MTP-mediated triglyceride secretion. [3H]Oleate-labeled triglyceride was secreted from cells expressing the indicated proteins. Cells were infected as described in the legend of Fig. 2 and labeled as described under “Experimental Procedures.” Lipids were extracted, separated by thin layer chromatography, and quantitated by scintillation counting. The ratios of secreted to total triglyceride were calculated and the results were normalized to the value from B48 + WT. Values represent the means of three or four determinations ± standard deviation.
of forming a functional complex with the 97-kDa subunit of MTP to enhance apoB secretion. The role of PDI in the MTP complex therefore involves a function that is intrinsic to its association with the 97-kDa subunit, but does not require the known enzymatic activities of PDI.

**Effect of Enzymatically Inactive PDI Subunit on MTP-mediated Triglyceride Secretion**—When supplemented with oleate, Sf21 cells can synthesize triglyceride and store it as intracellular droplets. However, they are unable to secrete large quantities of triglyceride unless the cells express both B48 and MTP (18). To assess whether the enzymatic activities of PDI are necessary for MTP-mediated triglyceride mobilization, we measured triglyceride secretion from cells labeled with [3H]oleate where indicated, as described under “Experimental Procedures.” Secretion and intracellular accumulation of the apoB constructs, apoE3, and LDL-R354, were analyzed by quantitative immunoblotting and are shown in the representative blots under each bar graph. Levels of each protein were normalized to the values obtained in the presence of wild type PDI without oleate treatment. Values represent the mean of three determinations ± standard deviation.

**Effect of Mutant PDI on ApoB Secretion Independent of the MTP Complex**—The enrichment of cysteine residues near the amino terminus of apoB might indicate a role for disulfide bonds in stabilizing apoB structure and facilitating lipoprotein assembly and secretion. Since the role of PDI in the MTP complex does not reside in its enzymatic activities, we explored the possibility that free PDI might play a role in apoB secretion at steps not involving MTP. This was done by expressing the enzymatically inactive form of PDI in the absence of the 97-kDa subunit of MTP. Since our expression system did not permit us to express the 97-kDa subunit at low levels, it was necessary to express mPDI by itself to prevent it from being sequestered into an MTP complex. Two carboxyl-terminal truncated apoB constructs, B17 and B48, were used.

Co-expression of mPDI with B17 or B48 resulted in a dra-

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**FIG. 4.** Effect of free mutant PDI and exogenous oleate on the secretion and intracellular accumulation of apoB and control proteins. B17, B48, and B88-100 were expressed along with wild type or mutant PDI respectively, at an m.o.i. ratio of 5:5 (A–C). ApoE3 and the truncated LDL receptor (LDL-R354) were used at the same m.o.i. ratio to test the specificity of the PDI effect (D). Cells were treated with 1.0 mM oleate where indicated, as described under “Experimental Procedures.” Secretion and intracellular accumulation of the apoB constructs, apoE3, and LDL-R354, were analyzed by quantitative immunoblotting and are shown in the representative blots under each bar graph. Levels of each protein were normalized to the values obtained in the presence of wild type PDI without oleate treatment. Values represent the mean of three determinations ± standard deviation.
matic decrease in the secreted and intracellular levels of both apoB fragments when compared with values obtained in the presence of the wild type PDI (Fig. 4, A and B). The level of apoB was similar in cells expressing wild type PDI and cells expressing no exogenous PDI (data not shown). Additionally, total protein synthesis and secretion were not affected by the mutant PDI.

Our results are consistent with the cysteine-rich amino terminus of apoB having a requirement for the enzymatic activities of PDI for optimal secretion. This interpretation would predict that expression of a segment of apoB not including the amino terminus would not be responsive to mPDI. We tested this prediction by co-expressing with mPDI a carboxyl-terminal fragment of apoB, B88-100 (18). Neither the secretion nor the intracellular accumulation of B88-100 was affected by mPDI (Fig. 4C). Since B88-100 contains two free cysteines that are not disulfide-bonded, it is likely that only the regions of apoB that have disulfide bonds are sensitive to mPDI.

To assess the specificity of the mPDI effect, we co-expressed two other proteins with mPDI. Human apoE3 has no disulfide bonds and therefore was used to reveal general toxicity or an indirect effect of mPDI on protein secretion. The other test protein, the soluble extracellular domain of the LDL receptor, LDL-R354 (18), has 24 disulfide bonds. The secretion and intracellular accumulation of both test proteins were unaffected by co-expression with mPDI (Fig. 4D). Thus, the effect of mPDI on apoB was highly selective.

Reversal of the mPDI Effect on ApoB by the Protease Inhibitor ALLN—The reduction in B17 and B48 levels resulting from mPDI co-expression could be due to decreased synthesis and/or increased degradation. In short-term metabolic labeling experiments, the rate of $^{35}$S-apoB accumulation in the cells was decreased in the presence of mPDI (Fig. 5A). However, this result is compatible with either a lower translation rate or rapid co- or post-translational degradation. To distinguish between these two possibilities, we measured the rate of net apoB synthesis in the presence of ALLN, a calpain inhibitor that has been shown to protect apoB from degradation in some mammalian cell systems (21, 36–40). At 10 $\mu$g/ml ALLN, the difference in intracellular accumulation of apoB between PDI- and mPDI-expressing cells was partially abolished, suggesting that rapid co- or post-translational degradation of newly synthesized apoB contributes to the decrease in apoB accumulation in cells expressing mPDI (Fig. 5B). The data do not allow us to rule out an effect of mPDI on apoB synthesis.

Effect of Oleate on ApoB Secretion in the Presence of Mutant PDI—In hepatoma cells, free fatty acids induce apoB secretion by a process that diminishes the proportion of apoB subjected to co- and/or post-translational degradation (41, 42). However, in primary rat hepatocytes and perfused liver, oleate does not increase apoB secretion even though total triglyceride secretion is stimulated (43–45). Our previous studies in Sf21 cells showed oleate induction of apoB secretion in the absence of rescue from degradation (18). In this system, expression of mutant PDI affects apoB secretion as well as intracellular accumulation (Fig. 4). The latter could be due to intracellular degradation. The fact that mPDI-expressing cells can still secrete some apoB may be attributed to the action of endogenous insect PDI that shares the well conserved enzymatic activities with human PDI.

Although mammalian lipoprotein-producing cells express MTP, they experience wide variations in free fatty acid flux. We tested if physiological concentrations of oleate could alter the inhibitory effect of mPDI on apoB secretion. B48 was co-expressed with wild type or mutant PDI in cells treated with oleate at concentrations between 0 and 1.0 mM. Oleate was able...
The shufflase activity of PDI is only detected in a guanidine HCl-treated MTP sample but not in the intact complex (46). Therefore, the active sites of PDI may not be accessible to substrates when it is associated with the 97-kDa MTP subunit. Interestingly, insect PDI cannot support MTP function as do its mammalian counterparts, although PDI is a well-conserved protein among all eukaryotes (48). This could be due to a failure to form a complex or to the inability to adopt the conformation necessary to maintain the lipid transfer activity of MTP.

Our data provide the first in vivo evidence that MTP does not require the enzymatic activities of PDI to stimulate triglyceride or apoB secretion. This is consistent with the observation that the enzymatic activities of PDI are unnecessary for the in vitro lipid transfer activity of MTP (46). It has also been shown in vitro that the shufflase activity of PDI is only detected in a guanidine HCl-treated MTP sample but not in the intact complex (47). Therefore, the active sites of PDI may not be accessible to substrates when it is associated with the 97-kDa MTP subunit. Interestingly, insect PDI cannot support MTP function as do its mammalian counterparts, although PDI is a well-conserved protein among all eukaryotes (48). This could be due to a failure to form a complex or to the inability to adopt the conformation necessary to maintain the lipid transfer activity of MTP.

Hepatoma cells expressed prolyl 4-hydroxylase, the latter enzyme was secreted (28). Thus, one role of PDI in the complex might be to ensure proper localization within the ER where lipid transfer occurs. In the absence of PDI, the 97-kDa subunit also forms aggregates (7). Thus, PDI might be necessary to maintain the stability of the 97-kDa subunit. The 97-kDa subunit of MTP has been shown to have a stable interaction with apoB (20, 21). PDI might be necessary for this interaction by virtue of its ability to influence protein folding even in the absence of its enzymatic activities (7).

Since B17 contains most of the disulfide bonds in apoB (22), the requirement for B17 in apoB secretion might be related to the necessity for the formation of a domain with the correct disulfide bonds. This inference is consistent with the observation that treating hepatoma cells with DTT leads to ER retention and rapid degradation of newly synthesized apoB (23). However, if DTT was not applied until the chase period, apoB became much more resistant to the effects of DTT, suggesting that formation of disulfide bonds in the amino-terminal segment is critical for apoB stability in the ER (23). Even in cells treated with DTT prior to pulse labeling, a portion of apoB was secreted with at least some of its disulfide bonds intact (23). These results are all consistent with the hypothesis that disulfide bond formation is essential for the stability and secretion of apoB. The experiments also imply that there is heterogeneity in the stability of the disulfide bonds within the amino terminus of apoB.

Expression of a mutant form of human PDI devoid of both enzymatic activities had a strong inhibitory effect on B17 and apoB secretion. Both B17 and B48 contain cysteine-rich amino terminus of apoB, implying that this region confers on apoB its dependence upon the enzymatic activities of PDI. This interpretation is supported by an additional experiment in which we showed that B88-100, a carboxyl-terminal fragment of apoB devoid of disulfide bonds, was not sensitive to the effect of mPDI. To further test the specificity of the mPDI effect, we co-expressed two other human proteins with mPDI, apoE3 and the LDL receptor ligand binding domain (LDL-R354). ApoE3 has only one cysteine residue so that it should not be directly affected by the enzymatic activities of PDI. The absence of an effect on apoE3 suggests that mPDI is not acting as a general inhibitor of protein secretion. LDL-R354 is highly enriched in disulfides; 48 out of the 354 amino acid residues are cysteines, and all are disulfide-bonded. One would expect this protein to be particularly sensitive to the presence of mPDI. Nevertheless, under conditions where apoB secretion was drastically affected, there was no significant change in the secretion of LDL-R354. This result shows that the detrimental effect of mPDI on apoB secretion is selective and does not occur with every protein bearing numerous disulfide bonds. ApoB appeared to be uniquely sensitive to the presence of enzymatically inactive PDI.

The intracellular accumulation of B17 and B48 was also specifically reduced by co-expression with the mutant PDI. This reduction was partially reversed by the calpain/proteasome inhibitor ALLN and the specific proteasome inhibitor MG132 (data not shown). We propose that the mutant human PDI competed with the endogenous enzyme for substrates, leaving a population of improperly processed apoB to be degraded. The ER possesses multiple mechanisms for targeting misfolded proteins for degradation (50). Recent studies have identified a novel pathway in which proteins are retro-translocated from the ER lumen to the cytosol where they are degraded by the proteasome in a ubiquitin-dependent manner (51, 52). The demonstration that apoB is ubiquitinated in
HepG2 cells (53) raises the possibility that a similar pathway may exist for apoB degradation in certain experimental systems. Alternatively, some separate proteolytic system(s) might also be responsible for degrading misfolded apoB (54, 55).

The assembly and secretion of apoB-containing triglyceriderich lipoproteins by the liver and intestine is tightly linked to the availability of triglyceride (56). In hepatoma cells, oleate, a substrate for triglyceride synthesis, enhances apoB secretion as a consequence of rescue from early intracellular degradation (41, 42). In contrast, such an effect was not detected in hepatocytes or perfused liver (43–45).

An intriguing observation in the present study was the attenuation of the mPDI effect on B48 secretion by oleate. The fact that lipid availability could overcome the effect of mPDI might indicate a convergence between the roles of PDI and triglyceride. Quite possibly, both may assist in the formation of disulfide bonds in apoB to make it secretion-competent.

Triglyceride may affect the kinetics and/or the thermodynamics of disulfide bonding. The polarity of the environment surrounding unfolded polypeptides has been shown to affect the rate of disulfide bond formation; disulfide bonds form much more quickly in non-polar environments (57). In addition, disulfide bonds are thermodynamically more stable in less polar environments if the surrounding polypeptide has non-polar side chains (58). The proposed amphiphatic secondary structure of apoB (59) might be more stable in a disulfide cross-linked form upon acquisition of triglyceride. As shown in Fig. 6, the inhibitory effect of mPDI on B48 secretion diminished with increasing oleate concentrations. This could be explained by a decrease in the polarity of the environment in which apoB is synthesized and processed for secretion.

Oleate failed to modify the mPDI effect in the case of B17. This is likely due to the inability of B17 to combine with substantial amounts of triglyceride to form buoyant lipoprotein particles (60–63). Our results are also supported by the observation that oleate was unable to stimulate the secretion of short amino-terminal fragments of apoB (42). In a previous report, we showed that B17 secretion was relatively unresponsive to MTP. However, when B17 is fused to a long internal segment of apoB, the resulting protein is MTP-responsive. If the triglyceride effect on B48 involves promotion of proper disulfide bond formation, then a critical length of apoB might be necessary to incorporate triglyceride into a nascent particle and to provide the environment required for disulfide bonds to form.

There is precedent for the stabilization of disulfide bonds by lipids. Retinol-binding protein (RBP) is partially retained in the ER of retinol-deficient cells (64). In the presence of retinol, RBP is much more resistant to DTT-mediated disulfide bond reduction than in the absence of its lipid ligand (65). In fact, DTT inhibits RBP secretion only in retinol-deficient cells, suggesting that the retinol-bound form of RBP is properly folded in such a manner that its disulfides are not accessible to DTT (65).

In the case of apoB, there are two possibilities; either, like RBP, apoB must be lipid-associated to form stable disulfide bonds, or conversely, apoB must be properly folded with correct disulfide bonds to bind to lipid. Our results are compatible with the first possibility.

Protein translocation across the ER membrane requires translocated proteins to be in an unfolded state. If protein translocation is bi-directional, as has been suggested recently (51, 52, 66, 67), interaction with triglyceride might prevent retrograde translocation of apoB by stabilizing and/or accelerating the formation of its amino-terminal disulfide bonds. A similar phenomenon has been reported for the enzyme dihydrofolate reductase (DHFR). Binding of DHFR to methotrexate stabilizes its folded conformation (68). Since this interaction occurs in the cytosol and proteins must unfold to be translocated into the mitochondrial matrix, the interaction of DHFR with methotrexate blocks it from entering the mitochondria (68).

Our results contribute new information to an emerging model of apoB secretion, in which the amino terminus of apoB is translocated and undergoes two possible fates. In HeLa cells and Chinese hamster ovary (CHO) cells, which do not express MTP or apoB, transfection of B53 resulted in the secretion of B17 (17, 38). In the presence of protease inhibitors, a microsome-bound form of B53 can be detected, suggesting that in the absence of triglyceride transfer, apoB translocation is arrested after B17 has been translocated (38). Consistent with this interpretation is the observation that patients with abetalipoproteinemia also secrete a B17-like protein (69). In fact, even cells expressing MTP secrete some B17, which is detected in the bloodstream of normal human subjects (39). Based on our data, secretion (and possibly translocation) of B17 requires the enzymatic activities of PDI. Alternatively, in cells undergoing triglyceride transfer (MTP-expressing cells or cells with MTP-like activity receiving a threshold level of triglyceride), the translocation arrest allows apoB to combine with lipid to form a stable lipoprotein particle and get secreted. Our studies suggest that the enzymatic activities of PDI are necessary for the stability and secretion of apoB when intracellular triglyceride is limiting.

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