

Transfer of cholesteryl esters and phospholipids as well as net deposition by microsomal triglyceride transfer protein

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Abstract Microsomal triglyceride transfer protein (MTP) activity is classically measured using radioactive lipids. We described a simple fluorescence assay to measure its triacylglycerol (TAG) transfer activity. Here, we describe fluorescence-based methods to measure the transfer of phospholipids (PLs) and cholesteryl esters (CEs) by MTP. Both transfer activities increased with time and MTP amounts and were inhibited to different extents by an MTP antagonist, BMS197636. We also describe a method to measure the net deposition of fluorescent lipids in acceptor vesicles. In this procedure, negatively charged donor vesicles are incubated with MTP and acceptor vesicles, and lipids transferred to acceptors are quantified after the removal of donor vesicles and MTP by the addition of DE52. Lipid deposition in acceptor vesicles was dependent on time and MTP. Using these methods, TAG transfer activity was the most robust activity present in purified MTP; CE and PL transfer activities were 60–71% and 5–13% of the TAG transfer activity, respectively. The method to determine lipid transfer is recommended for routine MTP activity measurements for its simplicity. ■ These methods may help identify specific inhibitors for individual lipid transfer activities, in characterizing different domains involved in transfer, and in the isolation of mutants that bind but cannot transfer lipids.—Rava, P., H. Athar, C. Johnson, and M. M. Hussain. **Transfer of cholesteryl esters and phospholipids as well as net deposition by microsomal triglyceride transfer protein.** *J. Lipid Res.* 2005. 46: 1779–1785.

Supplementary key words lipoprotein assembly • phosphatidylethanolamine • triacylglycerol • fluorescent lipids • apolipoprotein B • transfer assays • lipid transfer

Microsomal triglyceride transfer protein (MTP) is an essential chaperone for the assembly and secretion of apolipoprotein B (apoB) lipoproteins (for reviews, see 1–5). It is a heterodimeric protein consisting of 97 and 55 kDa polypeptides (5–8). The small subunit is the ubiquitous endoplasmic reticulum resident, protein disulfide isomerase, whereas the large subunit is unique and responsible for

the lipid transfer activity present in MTP. There is evidence to suggest that MTP functions in the biosynthesis of apoB lipoproteins through physical association (9–13) with nascent apoB and lipidation of apoB's hydrophobic, lipid binding β -sheets (14). In addition, MTP has been implicated in the import of neutral lipids into the endoplasmic reticulum lumen (15–17), the association with lipid droplets, and the fusion of lipid droplets with nascent apoB primordial lipoproteins (1, 2). In humans, the absence of MTP activity results in abetalipoproteinemia, which is characterized by very low plasma lipid levels and the absence of apoB lipoproteins (18).

The major defining function of MTP is its ability to transfer lipids between small unilamellar vesicles in vitro (7, 19–22). This activity is classically measured using radiolabeled lipids. In this procedure, donor vesicles containing radiolabeled lipids are incubated with acceptor vesicles in the presence of an MTP source (7, 19, 20). After incubation, donor vesicles and MTP are removed by the addition of cationic ion-exchange resins, and the amounts of radiolabeled lipids transferred to acceptor vesicles are quantified by liquid scintillation counting. Recently, we described a simple, rapid, and sensitive assay to measure triacylglycerol (TAG) transfer activity of MTP (23). In this assay, fluorescent TAG was incorporated into donor vesicles and incubated with acceptor vesicles in the presence of an MTP source. During transfer, MTP removes the quenched fluorescent lipids from donor vesicles, exposing the fluorescent moiety. This action is measured as an increase in fluorescence that is subsequently detected using a fluorimeter. Thus, a real-time increase in lipid transfer by MTP can be monitored using this procedure.

In addition to TAG, MTP is known to transfer several other lipids, such as phospholipids (PLs) and cholesteryl esters (CEs) (7, 19–22). Here, we describe simple and sensitive methods to measure the transfer of fluorescently la-

Abbreviations: apoB, apolipoprotein B; CE, cholesteryl ester; MTP, microsomal triglyceride transfer protein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipid; TAG, triacylglycerol.

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beled PL and CE by MTP. In addition, a method is presented to measure the net deposition of fluorescent lipids by MTP in acceptor vesicles.

MATERIALS AND METHODS

Materials

MTP was purified from bovine liver using the radioisotope assay (7, 19) and rat liver using a kit (Chylos, Inc., Woodbury, NY). Fluorescent (nitrobenzoaxadiazol)-labeled phosphatidylcholines (PCs) and unlabeled PC were purchased from Avanti Polar Lipids (Alabaster, AL). Nitrobenzoaxadiazol-labeled CE, phosphatidylethanolamine (PE), and TAG were from Molecular Probes (Eugene, OR). Thermo Labsystems (Franklin, MA) supplied the black 96-well microtiter plates. Isopropanol and other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Preparation of PL vesicles containing fluorescent PE and CE

Acceptor PC vesicles were prepared as described by Wetterau and associates (7, 19–21). Donor vesicles were also prepared by sonication as described before (7, 19–21, 23). Briefly, unlabeled PE and fluorescent PE were evaporated and sonicated under nitrogen for 45 min at 4°C. CE donor vesicles were prepared similarly using fluorescent CE and unlabeled PC. Vesicles, collected after centrifugation (50,000 rpm, 4°C, 10 min; SW55 Ti), were found to be stable for 1 month. Known amounts of fluorescent lipids were diluted in isopropanol to generate standard curves used to estimate the moles of fluorescent lipids incorporated in the donor vesicles.

Measuring lipid transfer activities

Assays were performed in triplicate on a black 96-well microtiter plate (23). A final reaction mixture (100 μ l) contained 3 μ l of donor vesicles (1.2 nmol of PC or PE and 100 pmol of fluorescent lipids), 3 μ l of acceptor vesicles (7.2 nmol of PC), and an MTP source in 10 mM Tris, pH 7.4, 0.1% BSA, and 150 mM NaCl buffer. The microtiter plate was incubated at 37°C, and at predetermined time points, samples were excited at 485 nm and fluorescence emission was measured at 550 nm using a Victor³ dual fluorimeter/luminometer (Perkin-Elmer). To determine the percentage of lipid transfer, fluorescence values obtained from control assays containing no MTP source (blanks) were subtracted from sample values and then divided by the total fluorescence present in the vesicles reduced by blanks. Blank values ranged from 10% to 25% of total fluorescence in various preparations. To obtain total fluorescence, 3 μ l of donor vesicles was incubated with 97 μ l of isopropanol for 5 min.

Measurement of net lipid deposition

To measure net lipid deposition, fluorescent TAG containing negatively charged donor vesicles was prepared (23). To introduce a negative charge, 67.5 nmol of cardiolipin (~7% of total lipids) was added before sonication (7, 19–21). Various amounts of MTP, as well as 3 μ l of donor vesicles and 3 μ l of acceptor vesicles, were incubated as described above. At predetermined times, fluorescence readings were recorded to quantify the TAG transfer. The reaction mixture was then transferred to microcentrifuge tubes containing 100 μ l of DE52 [equilibrated (1:1, v/v) with 15 mM Tris-Cl, pH 7.4, 1 mM EDTA, and 0.02% sodium azide buffer], rotated at 4°C for 5 min, and centrifuged (12,000 rpm, 5 min, 4°C). Supernatants (10 μ l) containing only acceptor vesicles were transferred to a microtiter plate, and fluorescence was measured at 5 min intervals after adding 90 μ l of isopropanol. Readings obtained at 20 min were used for calculations. The blank values obtained in the absence of MTP were subtracted

from the sample values, divided by the total fluorescence reduced by blanks, and multiplied by 100 to determine the percentage of lipids deposited to acceptor vesicles.

Determination of MTP activity in cells and tissues

HepG2 cells grown to confluence in T175 flasks were washed with PBS and then swelled by 2 min incubation at room temperature in hypotonic buffer (1 mM Tris-Cl, pH 7.4, 1 mM MgCl₂, and 1 mM EGTA) (23, 24). The buffer was aspirated, cells were scraped in 750 μ l of ice-cold hypotonic buffer containing protease inhibitors and homogenized (20 passages through a 21 gauge needle), the lysates were centrifuged (50,000 rpm, 4°C, 1 h; SW55 Ti rotor), and supernatants were used for lipid transfer assays and protein determination (25). For microsome preparation (7, 19, 23), mouse liver pieces were washed with PBS, homogenized in 50 mM Tris-Cl, pH 7.4, 5 mM EDTA, 250 mM sucrose, and 0.02% sodium azide using a Polytron homogenizer, and centrifuged (10,900 rpm, 30 min, 4°C; Beckman microcentrifuge). Supernatants were adjusted to pH 5.1 with concentrated HCl, mixed in the cold for 30 min, and centrifuged (13,000 rpm, 30 min, 4°C; Beckman microcentrifuge). Pellets were resuspended in 1 mM Tris-Cl, pH 7.6, 1 mM EGTA, and 1 mM MgCl₂, vortexed, incubated for 30 min at 4°C, and ultracentrifuged (50,000 rpm, 4°C, 1 h; SW55 Ti rotor), and supernatants were used for lipid transfer assays and protein determination.

RESULTS

PL transfer activity

We previously described a simple, rapid, and sensitive assay to measure TAG transfer activity of MTP (23). Here, we determined whether the same procedure could be used to quantify the PL transfer activity of MTP (Fig. 1). Upon the incubation of different amounts of MTP with donor vesicles containing fluorescent PE and acceptor vesicles, fluorescence increased and saturated in a time-dependent manner (Fig. 1A). Each concentration gave a specific curve indicating MTP-dependent increases in fluorescence and was confirmed by plotting the 1 h data against varying amounts of MTP (Fig. 1B). PL transfer was linear between 0.1 and 0.3 μ g of MTP and saturated at higher amounts. Next, we studied the inhibition of PL transfer activity by BMS197636. The PL transfer activity was inhibited by ~60% (Fig. 1C). Increased inhibition was not achieved even when the inhibitor concentration was increased to 10 μ M (data not shown). Similar results have been described for another MTP antagonist, BMS200150, which is a potent inhibitor of TAG transfer activity but only partially inhibits PL transfer activity (26). The reproducibility of the assay was established by determining the intra-assay and inter-assay coefficients of variation. The transfer activity in six separate samples using 0.3 μ g of MTP was $11.9 \pm 1.4\%$, and variation was found to be 0.12. The average activity in three independent experiments using 0.25 μ g of MTP was $9.9 \pm 0.96\%$, and the coefficient of variation was 0.097. These studies indicate that the PL transfer activity of purified MTP could be measured using this method.

CE transfer activity

To study CE transfer, donor PC vesicles containing fluorescent CE were incubated with acceptor vesicles and pu-

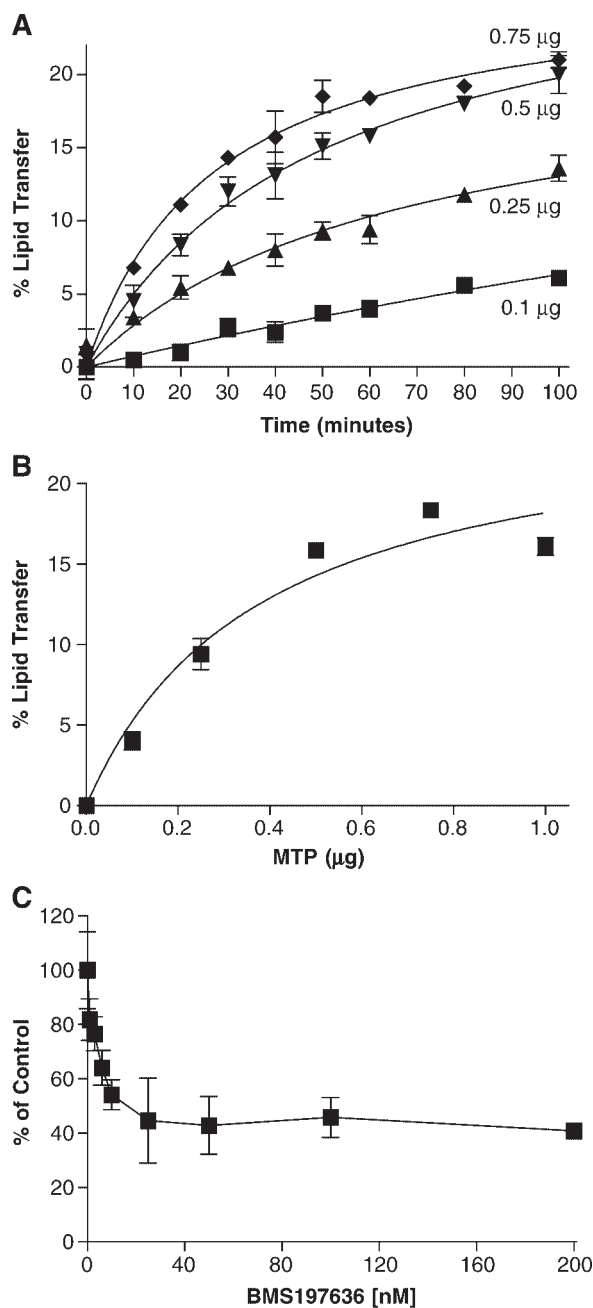


Fig. 1. Phospholipid transfer activity of microsomal triglyceride transfer protein (MTP). A: Different amounts of purified bovine MTP, in triplicate, were incubated with donor vesicles [1.2 nmol of phosphatidylethanolamine (PE) and 100 pmol of fluorescent PE] and with acceptor vesicles [7.2 nmol of phosphatidylcholine (PC)] in 100 μ l of 10 mM Tris-HCl buffer containing 0.1% BSA, 150 mM NaCl, and 2 mM EDTA at 37°C. Fluorescence at 550 nm was monitored over time. B: Data from the 60 min time point of A were replotted and subjected to nonlinear regression analysis ($r^2 = 0.9459$) using Prism. C: MTP assay was performed in the presence of the indicated amounts of the MTP inhibitor BMS197636, and fluorescence readings were recorded after 90 min. Line graphs and error bars represent means \pm SD. For some points, error bars are not visible because the deviations are smaller than the symbol sizes.

riated MTP (Fig. 2). The transfer of CE increased initially and then saturated with time for each of the MTP concentrations used (Fig. 2A). A concentration-dependent, linear increase in CE transfer followed by saturation was also observed using increasing amounts of MTP (Fig. 2B). The intra-assay coefficient of variation using 0.2 μ g of MTP was

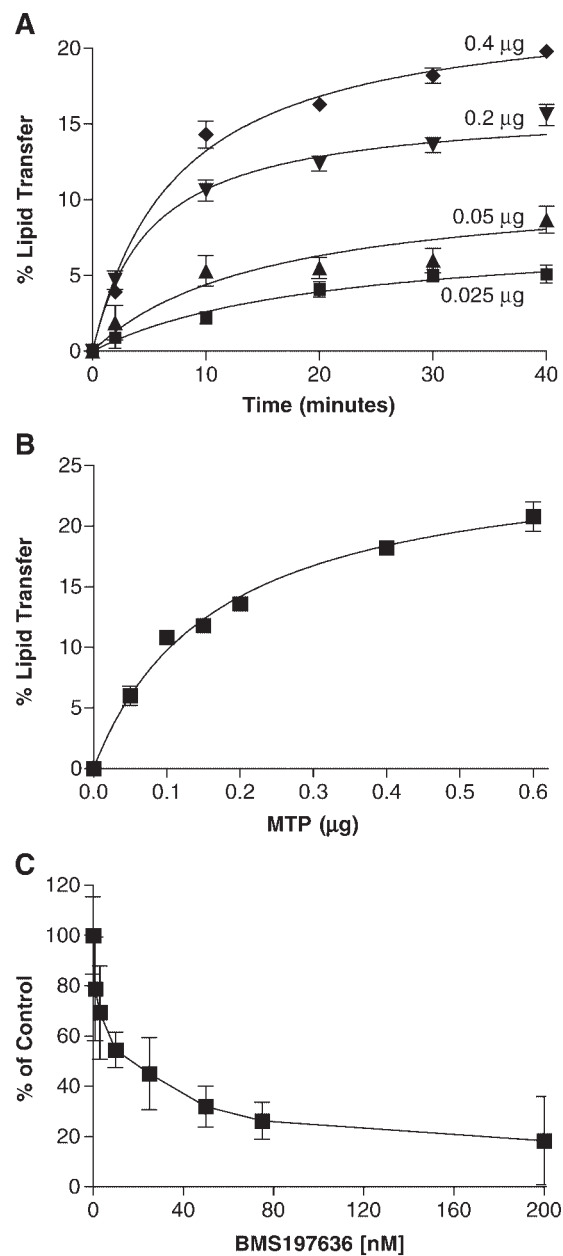


Fig. 2. Cholesteryl ester (CE) transfer activity of MTP. A: Different amounts of purified MTP were incubated with donor (1.2 nmol of PC and 100 pmol of fluorescent CE) and acceptor vesicles as described for Fig. 1. Increases in fluorescence emission at 550 nm were recorded at the indicated time intervals. B: Different amounts of MTP were incubated with donor and acceptor vesicles for 30 min, and the amounts of fluorescent CE being transferred were calculated. A nonlinear regression curve ($r^2 = 0.9842$) was generated using Prism. C: For inhibition, the indicated amounts of the MTP lipid transfer inhibitor BMS197636 were included in the reaction mixture, and fluorescence was measured after 1 h. Line graphs and error bars represent means \pm SD ($n = 3$).

0.09 (n = 6), and the percentage transfer per hour observed in those conditions was $17.4 \pm 1.6\%$. Comparing data from three independent experiments using $0.15 \mu\text{g}$ of MTP revealed a transfer of $15.0 \pm 1.9\%$ (n = 9) and interassay coefficient of variation of 0.127. CE transfer was inhibited by $>80\%$ upon increasing concentrations of BMS197636 (Fig. 2C). The IC_{50} values for CE transfer ranged between 15 and 25 nM and were similar to those observed for the inhibition of TAG transfer activity using this inhibitor (23, 26). These studies attest to the suitability of the method for determining CE transfer activity of purified MTP.

Fluorescent lipid transfer measured in cell homogenates and liver microsomes

We then used these assays to study lipid transfer activities in cellular and tissue homogenates. All lipid transfer activities (TAG, CE, and PL) could be measured in HepG2 cell homogenates (Fig. 3A). Lipid transfer activities showed time-dependent increases and reached maxima between 20 and 30 min of incubation. The initial rates and maximum amounts of CE transfer were lower than those ob-

served for TAG. PL transfer profiles were similar to those of CE and TAG transfer. The major difference was that PL transfer activity reached a significantly lower maximum transfer.

Next, we measured lipid transfer activity present in mouse liver microsomes (Fig. 3B). All three lipid transfer activities could be measured in microsomal samples using these assays. Again, the major activity observed was TAG transfer followed by CE and PL transfer activities. The initial rates and the maximum amounts of TAG transfer were significantly higher compared with those of CE and PL. These studies indicate that the efficiency of lipid transfer is greatest for TAG followed by those of CE and PL transfer in mouse liver microsomes.

Relative lipid transfer activities in MTP

Subsequently, we sought to compare the relationship between TAG, CE, and PL transfer activities measured in purified MTP preparations as well as in cellular and tissue homogenates (Table 1). In purified bovine and rat MTP preparations, CE and PL transfer activities were 59–60% and 6–5%, respectively, the TAG transfer activity. These are similar to the relative activities noted by Wetterau et al. (7, 19) in purified bovine MTP using a radioactive assay. In HepG2 cell lysates and liver microsomes, the CE and PL activities were 42–55% and 13–27%, respectively, compared with the TAG transfer activity. Thus, although the relative CE transfer activity in mouse liver microsomes was similar to that of the purified protein, HepG2 cell lysates demonstrated less CE transfer activity compared with purified MTP preparations. This suggests that proteins or other soluble factors in cells or tissues may interfere with this transfer. In contrast, relative PL transfer activities observed in HepG2 cells and liver microsomes were 2- to 4-fold higher than those observed in purified MTP preparations. This is most likely attributable to the presence of other PL transfer proteins, such as PC and phosphatidylinositol

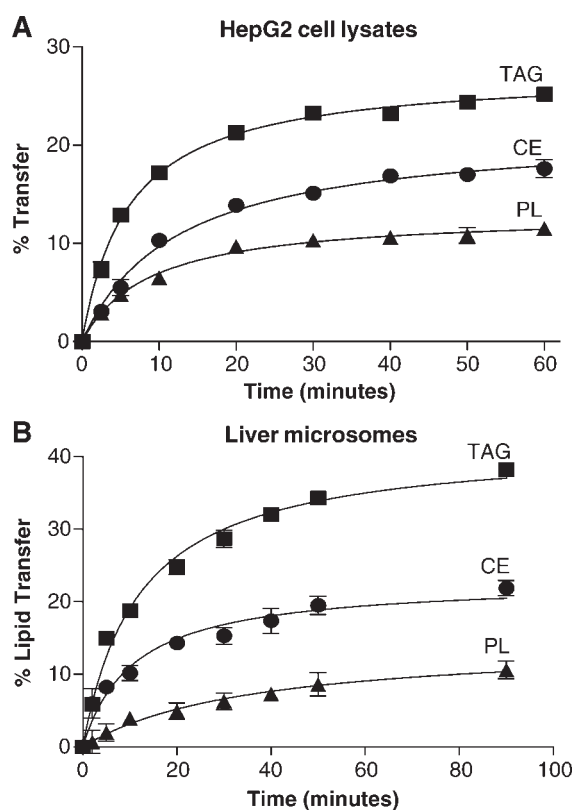


Fig. 3. Lipid transfer activities in HepG2 cells and mouse liver microsomes. A: HepG2 cell lysates were prepared as described in Materials and Methods and used to perform lipid transfer assays in triplicate. Each assay contained $42 \mu\text{g}$ of protein. Data are expressed as line graphs and error bars representing means \pm SD. TAG, triacylglycerol. B: Mouse microsomal contents were prepared as described in Materials and Methods. TAG, CE, or PE lipid transfer activities were measured in triplicate using $21 \mu\text{g}$ of protein. Mean values are drawn as line graphs and SDs as error bars. Nonlinear regression curve fits were determined using Prism.

TABLE 1. Specific and relative lipid transfer activities of MTP

MTP Source	Specific Activities (Relative Activities)		
	TAG	CE	PL
Purified bovine	901 ± 36 (100)	533 ± 53 (59)	56 ± 4 (6)
Purified rat	735 ± 45 (100)	438 ± 175 (60)	34 ± 5 (5)
Mouse liver			
microsomes	9 ± 0.2 (100)	5 ± 1 (55)	1 ± 0.1 (13)
HepG2 cell lysate	4 ± 0.5 (100)	1.5 ± 0.1 (42)	1 ± 0.1 (27)

CE, cholesteryl ester; MTP, microsomal triglyceride transfer protein; PL, phospholipid; TAG, triacylglycerol. Lipid transfer assays were performed using fluorescent lipids as described for Figs. 1–3. The initial rates of lipid transfer (nmol lipid/mg/h) were obtained using low amounts of purified MTP (0.025 , 0.04 , and $0.1 \mu\text{g}$ of protein for determining TAG, CE, and PL transfer, respectively) that produced a linear curve for at least 30 min of the assay. Specific activities (% transfer/mg protein/h) were then calculated using time points falling in the linear range for each assay. The absolute rate of lipid transfer (nmol lipid transferred/mg protein/h) was determined by comparing the fluorescence with standard curves. Dividing the specific activity of the lipid transfer in question by the specific activity of TAG transfer and multiplying by 100 provided the relative activities (in parentheses).

transfer proteins (27), in cells and tissues that might transfer fluorescent PE.

Measuring net lipid deposition

To measure the net deposition of lipids in acceptor vesicles, we required a method to separate acceptor vesicles from the donor vesicles and MTP present in the assay. Wetterau et al. (7, 19–21) used cardiolipin and DE52 to achieve this in their radiolabeled assay. First, we determined that the addition of cardiolipin had no effect on the incorporation of TAG in the donor vesicles. The total fluorescence incorporated was $17,821 \pm 112$ and $17,202 \pm 1,162$ for donor vesicles with and without cardiolipin, respectively. Second, we confirmed that >99% of the donor vesicles and MTP could be removed from the reaction mixture after incubation with DE52. Third, we determined the effect of the presence of cardiolipin in donor vesicles on the TAG transfer activity of MTP. For this purpose, we performed parallel measurements of TAG transfer with donor vesicles containing, and free of, cardiolipin (Fig. 4A). Even though both assays contained the same amounts of acceptor and donor vesicles, as well as MTP, the TAG transfer by MTP from donor vesicles containing cardiolipin was 50% less compared with that obtained with donor vesicles with no cardiolipin and is consistent with published studies (5, 19, 28).

Next, we determined the net lipid deposition to acceptor vesicles. For this, donor as well as acceptor vesicles and MTP were incubated for different times, and fluorescence readings were taken to determine the transfer of TAG. The reaction was then stopped, donor vesicles and MTP were precipitated by the addition of DE52, and the TAG deposited in acceptor vesicles was quantified. The TAG transfer slowly increased with time (Fig. 4B), similar to that observed in the presence of cardiolipin (Fig. 4A). The net deposition of fluorescent TAG to acceptor vesicles increased for 120 min and remained unchanged until 180 min. At saturation, ~50–60% of the TAG was deposited in acceptor vesicles.

We then determined the relative net lipid deposition of various lipids to acceptor vesicles. Donor vesicles containing fluorescent TAG, CE, or PE, as well as cardiolipin, were made (Fig. 4C). Net deposition to acceptor vesicles was measured after the removal of donor vesicles and MTP. The relative activities were $100 \pm 4.8\%$, $71.0 \pm 8.5\%$, and $13.5 \pm 5.2\%$ for TAG, CE, and PL, respectively. These relative values are similar to those observed based on lipid transfer measurements (Table 1). Thus, both assays gave similar results concerning relative lipid transfer activities.

DISCUSSION

Wetterau et al. (7, 19–21) had published an *in vitro* method for measuring lipid transfer in microsomal fractions using radiolabeled lipids. Recently, a fluorescent assay was described for MTP that evaluates the transfer of TAG between membrane vesicles (23). This procedure, although rapid and sensitive, also precludes the use, as well

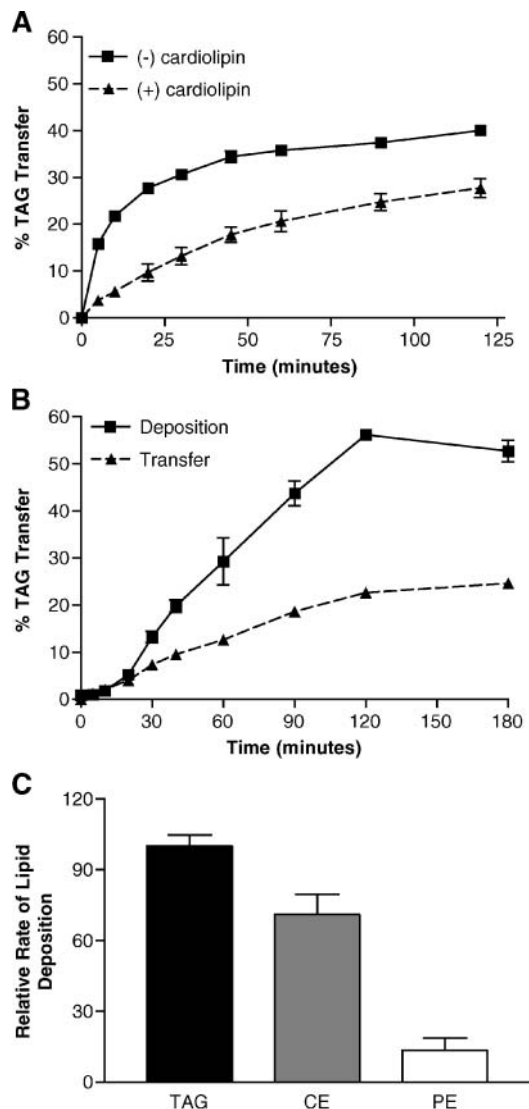


Fig. 4. Measurement of net lipid deposition: A: Effect of cardiolipin on the TAG transfer activity of MTP. Donor vesicles made with and without cardiolipin were used. Each assay in triplicate contained 0.25 μg of purified bovine MTP, 3 μl of donor vesicles (100 pmol of fluorescent TAG, 1.2 nmol of PC with or without 0.081 nmol of cardiolipin), and 3 μl of PC acceptor vesicles, as described for Fig. 1. The microtiter plate was incubated at 37°C, fluorescence was monitored over time, and percentage transfer was determined as described previously. B: Measurement of net deposition of lipids by MTP. Transfer assays were set up in triplicate as described for A containing 0.25 μg of MTP, 3 μl of donor vesicles, and 3 μl of acceptor vesicles in 100 μl assay volume. Percentage lipid transfer was measured as described for Fig. 1. To measure lipid deposition, 100 μl of DE52 anion-exchange resin was added to the reactions at the predetermined times. After centrifugation, 10 μl of supernatant was transferred to a 96-well black microtiter plate. Fluorescence was measured after the addition of 90 μl of isopropanol. C: Relative net lipid deposition by MTP. Net lipid transfer assays were set up in triplicate as described for B. Assays contained 0.25 μg of purified bovine MTP, donor vesicles (100 pmol of different fluorescent lipids, 1.2 nmol of PC, and 0.081 nmol of cardiolipin), and acceptor vesicles. Percentage net lipid deposition was determined at 1 h for TAG as well as CE and at 1.5 h for PE. The specific activity (% transfer/mg protein/h) was then calculated. Dividing the individual specific activities with the specific activity of TAG lipid transfer and multiplying by 100 provided relative net lipid transfer activities. Bar graphs and error bars represent means \pm SD.

as the disposal, of radioactivity. Here, we describe methods to measure CE and PL transfer by MTP using fluorescent lipids. In addition, we describe a procedure to determine the net deposition of fluorescent lipids to acceptor vesicles.

Although the mechanism associated with MTP lipid transfer has yet to be fully elucidated, it is suggested that MTP binds transiently to lipid membranes, extracts lipid, and ultimately transfers this lipid to another membrane or possibly apoB (21, 22). The assays described here are capable of evaluating the two key events in this process, lipid transfer by MTP and the subsequent deposition of such lipids to acceptor vesicles. The availability of these two methods may be useful for determining whether the two steps occur independent of each other and/or through different domains of MTP.


The first method monitors MTP's capacity to bind and extract lipids from a membrane in the presence of acceptor vesicles. Fluorescence is quenched when lipids are in unilamellar (one PL bilayer) membrane vesicles. Upon association with MTP, the lipid fluorophore is unquenched and detected by the fluorimeter. In this process, the real-time lipid transfer is measured and found to be time- as well as concentration-dependent. Furthermore, the process is saturable. The saturation of lipid transfer might indicate that all of the MTP molecules are actively involved in the process.

The second method measures the deposition of fluorescent lipids to acceptor vesicles and is based on the procedure of Wetterau et al. (7, 19–21). In this method, fluorescent lipids deposited in acceptor vesicles are quantified after the removal of MTP and donor vesicles by anion-exchange resin. The amounts of lipids deposited were far greater than those measured during lipid transfer, mostly reflecting the large excess of acceptor vesicles present in the assay. There are two major drawbacks associated with this method. First, it involves an additional step of separating acceptor vesicles from donor vesicles and MTP. Second, the incorporation of negatively charged lipids in the donor vesicles decreases the sensitivity of the assay. Thus, this assay is recommended only when there is a need to measure the process of lipid deposition. Under normal conditions, the measurement of lipid transfer is recommended to measure individual MTP activities. For the routine determination of MTP activity in cell lysates, we recommend measuring TAG transfer.

We observed that PL transfer could not be completely inhibited by MTP antagonists (Fig. 1). Jamil et al. (26) also reported that PL transfer cannot be completely inhibited by MTP antagonists. Three possible reasons were considered. First, we do not think that the incomplete inhibition is attributable to nonfacilitated transfer of PL. All assays have blanks consisting of donor and acceptor vesicles in the absence of MTP. Blank values were not affected by the MTP antagonists, and increases in fluorescence were not observed in blanks. Furthermore, blank values were subtracted during activity calculations, eliminating the contribution, if any, of nonfacilitated transfer. Second, contamination of MTP preparations with PL transfer proteins was considered. Plasma contains PL and CE transfer

proteins, whereas cells are known to contain PC and phosphatidylinositol transfer proteins. The purified MTP preparations consisted mainly of two polypeptides corresponding to the known 97 and 55 kDa subunits. None of the known cellular and plasma PL transfer proteins was present in the preparations. Third, BMS197636 might be a specific inhibitor for neutral lipids. Kinetic studies have demonstrated that MTP might contain two PL binding sites and one neutral lipid transfer site (22). The neutral lipid transfer site might also transfer PL. It is possible that the inhibitor might inhibit only one site and not the other, resulting in partial inhibition.

There are some caveats concerning the measurement of PL and CE transfer activities using cell and tissue homogenates. Cells contain other PL transfer activities that contribute to PL transfer activity. The determination of PL transfer activity in cell homogenates is further complicated by the <60% inhibition of this activity by the available MTP inhibitors. The extent of CE transfer is comparatively lower than that of TAG and is only linear under a small concentration range of MTP. Thus, time course and concentration curves should be measured in the presence and absence of MTP inhibitors to determine the CE transfer activity of MTP in homogenates.

In short, we describe two methods for evaluating lipid transfer activities of MTP using fluorescent lipids. These assays are fast and sensitive and can be used to detect inherent differences in the transfer of various lipids by MTP. The methods measuring the deposition of lipids to acceptor vesicles may be useful in determining the unidirectional transfer of lipids to various acceptors. These methods may be valuable in identifying MTP mutants that are able to bind lipids but cannot transfer them to acceptor vesicles, or those deficient in a specific lipid transfer activity. The availability of methods for measuring different lipid transfer activities of MTP may facilitate the discovery of inhibitors specific for individual lipid transfer activities. The identification of such inhibitors may be valuable in delineating the importance of various lipid transfer activities of MTP in apoB lipoprotein assembly and secretion. 

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