Modulation of the phospholipid transfer protein-mediated transfer of phospholipids by diacylglycerols

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Abstract **Previous studies have shown that diacylglycerols (DAG) are formed during triglyceride hydrolysis in very low density lipoproteins (VLDL), a process that is accompanied by an elevated phospholipid transfer protein (PLTP)-mediated transfer of phopholipids (PL) from VLDL to high density lipoprotein. Because PLTP has been also shown to transfer DAG, we hypothetized that DAG might modulate PL transfer through a mechanism of competition with respect to PLTP. To address this question we performed in vitro PL transfer assays using specifically designed PL donor particles. These were single bilayer vesicles (SBV) and large (EM-L) or small (EM-S) lipid emulsions, containing various proportions of DAG. The PLTP-mediated transfers of PL decreased as the volumes of the particle cores increased (SBV > EM-S > EM-L). In all cases, these transfers were inhibited by DAG in a concentration-dependent manner. We determined the core-to-surface distribution of DAG and we measured their relative affinity for PLTP by comparison with that of PL. From these parameters, we calculated the theoretical effects of DAG on PL transfers that would result from a competition mechanism. The experimental data showed that the inhibiting effects of DAG on PL transfers were much more important than those predicted from our calculations. Additional data showed that a large part of DAG effects was in fact due to their ability to increase the viscosity of the particle PL surfaces, as calculated from electron spin resonance experiments. These results show that DAG can modulate the PLTP-dependent PL transfers, both by competition with PL and by increasing the viscosity of the particle surfaces. These findings might be physiopathologically relevant in situations where elevated plasma concentrations of DAG might result from hypertriglyceridemia.**—Lalanne, F., C. Motta, Y. Pafumi, D. Lairon, and G. Ponsin. **Modulation of the phospholipid transfer protein-mediated transfer of phospholipids by diacylglycerols.** ***J. Lipid Res.*** **2001.** 42: **142–149.**

Supplementary key words PLTP • lipid particle viscosity • electron spin resonance • pyrene fluorescence • surface-to-core lipid distribution

The hydrolysis of circulating lipoprotein triglycerides (TG) results from the activities of both lipoprotein lipase and hepatic lipase. The initial products generated through the action of these two enzymes are diacylglycerols (DAG). In humans, the fasting plasma concentration of DAG is on the order of 10 μM (1). Their presence in human lipoproteins has been recognized only in more recent years (2–4), although they have long been known as major components of insect lipophorins (5). This is mainly due to the lack of specificity of routine enzymatic TG assays, which do not discriminate between mono-, di-, and triacylglycerols. Nonnegligible amounts of DAG were first described in high density lipoproteins (HDL) by Vieu et al. (2). Then, it was shown by Coffill et al. (3) that in HDL, DAG were the preferred substrate for hepatic lipase. These data prompted us to study the distribution of DAG in all lipoprotein classes from control subjects and from patients with type 2 diabetes, a situation with high atheromatous risk (6). Two important findings emerged from this work. First, DAG were found in all lipoproteins and their total concentration was highly correlated to that of plasma TG. Second, our data clearly showed that DAG mainly resulted from the TG hydrolysis occurring in very low density lipoprotein (VLDL). Thus, DAG present in other lipoprotein fractions had necessarily to arise from VLDL through a transfer process. Consistent with this concept, an interesting report has demonstrated that the phospholipid transfer protein (PLTP) was able to promote, in vitro, the transfer of DAG between reassembled HDL (7).

PLTP is a circulating plasma protein present in humans (8, 9) as well as in various animal species (10, 11), which exerts two different types of actions with respect to lipid metabolism. The first is a function of lipid carrier, while the second is that of an HDL conversion factor. The latter action has been unambiguously demonstrated by several

Abbreviations: CE, cholesteryl esters; CETP, cholesteryl ester transfer protein; DAG, diacylglycerols; E/M, excimer-to-monomer fluorescence ratio; EM-L, large lipid emulsions; EM-S, small lipid emulsions; HDL, high density lipoproteins; PL, phospholipids; PLTP, phospholipid transfer protein; Pyr-PC, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine; SA-DAG, 1-stearoyl-2-arachidonoyl-DAG; SBV, single bilayer vesicles; TG, triglycerides; VLDL, very low density lipoproteins.

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studies that indicated that PLTP was able to promote the conversion of small HDL_3 to larger HDL_2-like particles through a fusion process (12–16). As to its lipid transfer function, PLTP was first described to facilitate the transfer or exchange of phospholipids (PL) between lipoproteins (8, 9). Although it was later shown to be able to promote the transfers of other molecular species including unesterified cholesterol (17), lipopolysaccharides (18, 19) and α-tocopherol (20), the role of PLTP in PL metabolism appears to be essential.

A large part of the PLTP-dependent transfer of PL from VLDL to HDL takes place during postprandial TG hydrolysis (21). Because this process concomitantly results in the formation of DAG (6), the finding that PLTP can also transfer DAG (7) might be of particular interest. Indeed, during VLDL lipolysis, PL and DAG might well be available for PLTP at the same time, thereby creating the conditions of a competition for binding to PLTP. This concept might be important in certain physiopathological situations. For example, the increase in DAG in diabetic lipoproteins (6) might contribute to the disorders in PL exchanges between lipoproteins that have been previously observed in this pathology (22, 23). Thus, in principle, DAG might act as a regulator of PL transfer, at least from a qualitative viewpoint. The present work was undertaken to evaluate the influence of DAG on the PLTP-dependent PL transfers from model lipid particles to HDL, and to determine whether the effect observed could be quantitatively explained on the basis of a competition between DAG and PL for the binding to PLTP.

MATERIALS AND METHODS

All experiments were carried out with a buffer containing 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid, 30 mM NaN_3, and 150 mM NaCl, pH 7.4.

Materials

1-Hexanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine (Pyr-PC) was from Molecular Probes (Eugene, OR). Butylated hydroxytoluene, cholesteryl oleate, trioleine, egg L-α-phosphatidylcholine type III (egg PC), and 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SA-DAG) were obtained from Sigma (St. Louis, MO). 1-Stearoyl-2-[1-^{14}C]arachidonoyl-*sn*-glycerol ([^{14}C]SA-DAG; 55 mCi/mmol) was from Amersham Pharmacia Biotech (Uppsala, Sweden), as were high performance phenyl-Sepharose and heparin-Sepharose CL-6B. Q2 column and biologic fast protein liquid chromatography (FPLC) system were from Bio-Rad (Hercules, CA).

Preparation and compositional analysis of model lipid particles

Large (EM-L) and small (EM-S) lipid emulsions containing various proportions of DAG were prepared according to a procedure based on that previously described by Derksen and Small (24). [^{14}C]SA-DAG was added to the SA-DAG stock solution to a specific activity of 2×10^5 dpm/μmol. The initial mass proportions of lipids were 60% TG, 20% cholesteryl ester (CE), and 20% PL + SA-DAG for EM-L; and 15% TG, 5% CE, and 80% PL + SA-DAG for EM-S. Briefly, 80-mg samples of these lipid mixtures were evaporated and dried in the dark under a stream of nitrogen, and resuspended in 5 ml of buffer. They were then maintained in an ice bath and sonicated during six periods of 5 min each separated by 5-min periods of cooling, using a Vibra-Cell sonifier (Sonics, Newtown, CT) operated in pulse mode at 60% of active cycle. Then, 5 ml of water was added to the samples and the lipid particles formed were isolated after ultracentrifugation at 23,000 rpm for 40 min, at a temperature of 15°C, using an SW41 Ti swinging rotor (Beckman, Fullerton, CA).

Single bilayer vesicles (SBV) containing only egg PC and various proportions of SA-DAG were prepared by sonication in the presence of butylated hydroxytoluene as antioxidant and separated from multilamellar structures by centrifugation at 20,000 rpm (25).

SBV as well as EM-L and EM-S particles were kept in the dark at 4°C and used within 4–5 days. Their final compositions were determined with commercial kits for PL (Biomérieux, Charounieres-les-Baines, France) and for TG and CE (Boehringer Mannheim, Indianapolis, IN). Aliquots from all samples were counted for [^{14}C]SA-DAG radioactivity to permit the calculation of the SA-DAG content of each preparation of lipid particles.

The surface-to-core distribution of DAG was determined in a specific series of experiments in which the core and surface lipid components of lipid emulsions containing labeled DAG were separated as previously described (26), using an ultracentrifugation procedure based on that of Miller and Small (27, 28).

Physical characterization of small and large emulsions

The mean sizes of EM-S and EM-L were inferred from their composition. They were estimated from the calculation of the diameters of equivalent spheres containing neutral lipids in their core and phospholipids in their surface (29). The values of the molecular volumes of neutral lipids were assumed to be 1.09 and 1.60 nm^3 for CE and TG, respectively. For DAG, we used the same values as those of PL for molecular volumes (1.27 nm^3) as well as for the range of molecular surfaces (0.45–0.75 nm^2). Because the latter depends on the molecular packing of the phospholipid surface, a corresponding range of diameters was calculated for each particle from the same compositional data.

Particle surface viscosities were estimated from electron spin resonance measurements using a Bruker (Billerica, MA) ECS 106 spectrometer equipped with a TMH 269 resonance cavity. The labeling of lipid particles was performed with a solution of 1% spin label in dimethyl sulfoxide, to obtain a final ratio of spin label to PL of less than 0.5×10^{-3}. The spectra were obtained from five scans performed at 24°C. The viscosity of the most profound part of the PL monolayer of EM-S and EM-L was analyzed using 16-doxyl stearic acid (16-DSA) as the probe. The rotational correlation time, T_c, was calculated from the equation of Keith, Sharvoff, and Cohn (30):

$$T_c = (6.5 \times 10^{-10})\left\{W_0\left[\left(\frac{H_0}{H_{-1}}\right)^{1/2} + \left(\frac{H_0}{H_{+1}}\right)^{1/2} - 2\right]\right\} \quad \textit{Eq. 1)}$$

where H_0 and W_0 represent the amplitude and the line width of the central line, respectively. H_{+1} and H_{-1} represent the low field and the high field line amplitude, respectively.

According to the relation of Debye (31), T_c is directly proportional to viscosity:

$$T_c = \frac{4}{3}\pi a^3 \frac{n}{kT} \quad \textit{Eq. 2)}$$

where $4\pi a^3/3$ is a constant corresponding to the equivalent volume of the system, n is the viscosity, k is the Boltzmann constant, and T is the absolute temperature.

Preparation of PLTP

PLTP was isolated from fresh plasma, using a procedure adapted from Lagrost et al. (32). Briefly, plasma was adjusted to a density of 1.21 g/ml with solid KBr. After ultracentrifugation, the lipid supernatant was discarded. The resulting delipidated fraction was then successively subjected to hydrophobic, affinity, and ion-exchange chromatographies using high performance phenyl-Sepharose, heparin-Sepharose CL-6B, and a Bio-Rad Q2 column, respectively. All the chromatographic steps were performed at 4°C, using an FPLC system. The fractions of interest were assayed for PLTP and cholesteryl ester transfer protein (CETP) activities. PLTP was assayed by the radioisotopic PL transfer assay described by Damen, Regts, and Scherphof (25). CETP was determined as previously reported, using an isotopic assay in which the transfer of labeled CE from HDL to apolipoprotein B-containing lipoproteins was measured (33). The final PLTP preparation was free of CETP. For convenience, albeit only partially purified, it will be referred to as PLTP throughout the article. Accordingly, the PLTP masses used have been expressed in terms of protein mass of the final PLTP preparation.

Unidirectional fluorometric PL transfer assay

In this assay, SBV, EM-S, or EM-L, containing various proportions of DAG, were labeled with 4% (mol/mol) of Pyr-PC. These PL donor particles (200 nmol of PL) were incubated at 37°C with HDL (800 nmol of PL) in the absence or in the presence of PLTP (2.5 μg) in a total volume of 1.2 ml.

The fluorescence properties of Pyr-PC were used to monitor the kinetics of PL transfers. The fluorescence spectrum of pyrene moieties is a function of their microscopic concentration (34). The fluorescence emission below 420 nm corresponds to the monomer fluorescence (M). At high local concentrations, pyrene compounds form excited-state dimers that give an excimer fluorescence (E) at higher wavelengths. During the transfer process, the monomer and excimer fluorescence intensities increase and decrease, respectively, as Pyr-PC transfers from donor particles to HDL. Under given conditions, E/M may be considered to a first approximation as a linear function of the local pyrene concentration, thereby permitting the use of the time course of change in the E/M ratio to calculate the rate constant $K + 1$ for the transfer of Pyr-PC from donor particles to HDL (35). The fluorescence measurements were carried out at 37°C, using a PTI spectrofluorometer (Photon Technology International, Lawrenceville, NJ) equipped with an externally controlled temperature regulator. The excitation wavelength was set at 341 nm. The spectrofluorometer was operated in T mode to record simultaneously the E and M fluorescence intensities at 470 and 376 nm, respectively. $K + 1$ values were then determined from the plots of E/M against time. The PLTP-facilitated transfers of PL were calculated as the differences between the transfers measured in the presence and in the absence of PLTP, respectively.

Simultaneous determination of the PLTP-dependent transfers of both PL and DAG

To compare directly the abilities of PLTP to transfer PL and DAG, we performed duplicate transfer assays using donor particles of specific composition. These were SBV of egg PC containing both 4% (mol/mol) Pyr-PC and 4% (mol/mol) SA-DAG labeled with 1 μCi of [^{14}C]SA-DAG. SBV (1.25 μmol of PL) were incubated at 37°C with HDL (5 μmol of PL) in a total volume of 4 ml, both in the absence and in the presence of PLTP (15 μg). After 0, 20, 40, 60, 90, and 180 min of incubation, aliquots were withdrawn from the sample mixtures and cooled in iced water. SBV were separated from HDL after precipitation, using the heparin-$MnCl_2$ procedure (25). Finally, the samples were measured for both [^{14}C]SA-DAG radioactivity and Pyr-PC fluorescence. The tranfers were expressed as the percentages of each marker incorporated into HDL. The PLTP-dependent transfers of the two molecular species were calculated as the differences between the transfers measured in the presence and in the absence of PLTP, respectively.

RESULTS

Comparison of DAG and PL transfers

SBV containing 4 mol% of both [^{14}C]SA-DAG and Pyr-PC were used as donor particles to determine simultaneously the respective effects of PLTP on the kinetics of transfer of these two molecular species to HDL (**Fig. 1**). These data were used to calculate the initial velocities of PLTP-dependent transfers. The resulting value of the rate constant $K + 1$ for the transfer of SA-DAG was lower than that obtained for the transfer of Pyr-PC (0.35×10^{-4} vs. 0.60×10^{-4} pool/s). The ratio of these two values indicated that the relative transferability of SA-DAG for PLTP was 0.58 with respect to that of Pyr-PC.

Effect of DAG on the PLTP-dependent PL transfer

Because PLTP was able to transfer both DAG and PL, we performed a series of experiments designed to study the quantitative effect of DAG on the PLTP-dependent PL transfer. Two emulsions (EM-S and EM-L) differing by their surface-to-core lipid ratio and containing various proportions of SA-DAG were prepared by sonication in the presence of Pyr-PC and used as the donor particles in the PL transfer assay. Because DAG may partition between

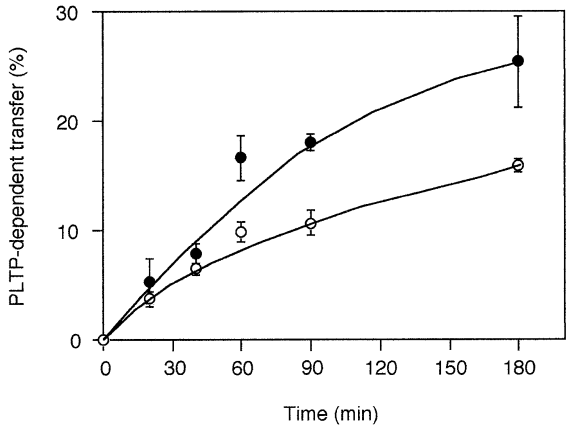

Fig. 1. Comparison of PLTP-dependent transfers of [^{14}C]SA-DAG (open circles) and Pyr-PC (solid circles). The transfers were measured from SBV of egg PC containing both 4% (mol/mol) Pyr-PC and 4% (mol/mol) SA-DAG labeled with 1 μCi of [^{14}C]SA-DAG. SBV (1.25 μmol of PL) were incubated at 37°C with HDL (5 μmol of PL) in a total volume of 4 ml, both in the absence and in the presence of PLTP (15 μg). At given times, SBV were separated from HDL and the samples were measured for both [^{14}C]SA-DAG radioactivity and Pyr-PC fluorescence. The PLTP-dependent transfers of the two molecular species were calculated as the differences between the transfers measured in the presence and in the absence of PLTP, respectively. They were expressed as the percentage of the total amounts of the corresponding markers.

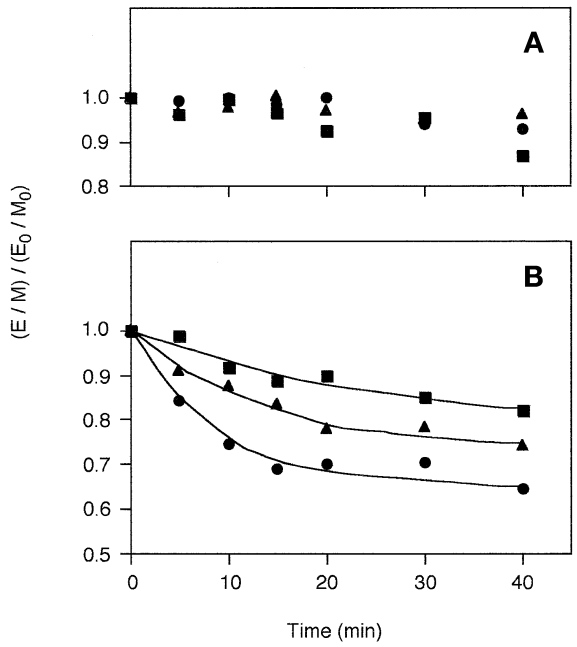

Fig. 2. Kinetics of Pyr-PC transfers from emulsions to HDL in the absence (A) or in the presence (B) of PLTP. Representative kinetics obtained with EM-S containing 0% (solid circles), 5.6% (solid triangles), or 9.8% (solid squares) of SA-DAG are shown as examples. The transfer of Pyr-PC was monitored by following the change in the E/M fluorescence ratio, relative to that determined at t_0 (E_0/M_0).

particle core and surface lipids, we compared the PL donor properties of EM-S and EM-L with those of SBV containing 0–10% SA-DAG with respect to PL. The decrease in E/M fluorescence ratio that occurs during the transfer of Pyr-PC was used to monitor the kinetics of PL transfers. **Figure 2** shows as examples the transfers obtained both in the absence (Fig. 2A) and in the presence (Fig. 2B) of PLTP, using as donors EM-S particles containing three different proportions of SA-DAG. While DAG had no apparent effect on the spontaneous transfer of PL, they clearly induced a concentration-dependent decrease in the PLTP-mediated PL transfer. From these kinetics, we determined the apparent rate constants of transfers, $K + 1_{app}$. The rate constants for the PLTP-facilitated PL transfer were calculated as the differences between those obtained, respectively, in the presence and in the absence of PLTP (**Table 1**). In our three types of particles, the facilitating effect of PLTP on PL transfer was progressively inhibited as the proportion of SA-DAG increased. When SBV were used as donors, the spontaneous PL transfer was also decreased by SA-DAG. Those observed with both EM-S and EM-L were too low to permit any accurate detection of SA-DAG effect.

To appreciate the extent to which the effect of SA-DAG might be explained on the basis of a competition mechanism, we assumed that only the fraction of SA-DAG present in the PL surface of the emulsions had to be taken into account. Core SA-DAG, if any, were considered to be unable to affect the initial velocity of PL transfer through a competitive binding to PLTP. Thus, we analyzed the core/surface partition of SA-DAG, using lipid emulsions comparable to those used in the transfer assays. Five independent experiments were carried out in which core and surface lipids were separated according to Borel et al. (26). The concentrations of SA-DAG in surface and core lipids were practically identical (1.37 ± 0.10 and 1.32 ± 0.04 mol%; mean ± SEM). We therefore considered for subsequent calculations that, in our emulsions, the core-to-surface DAG ratios were the same as the core-to-surface total lipid ratios. From these data, we calculated the respective amounts of SA-DAG present in the surface ($SA\text{-}DAG_{surf}$) and in the core ($SA\text{-}DAG_{core}$) of each particle (Table 1).

TABLE 1. Compositional analysis of SBV, EM-S, and EM-L particles, and the rate constants for the transfer of Pyr-PC

Particle Type	SA-DAG	Particle Composition PL:DAG_{surf}:DAG_{core}:TG:CE	Size Range	$K + 1_{spt}$ ($\times 10^4$)	$K + 1_{PLTP}$ ($\times 10^4$)
	nmol%	*nmol%*	*nm*	*pools/s*	
SBV	0	100:0:0:0:0		2.49	4.40
	2.27	97.8:2.27:0:0:0		2.08	3.75
	5.03	95.0:5.03:0:0:0		1.06	2.36
	7.23	92.8:7.23:0:0:0		1.56	1.31
	8.97	91.0:8.97:0:0:0		1.08	1.17
EM-S	0	40.1:0:0:48.5:11.4	22–34	0.13	2.67
	3.13	40.9:1.32:1.81:44.7:11.3	20–31	0.34	2.13
	5.64	36.1:2.16:3.48:47.9:10.3	23–36	0.12	1.80
	7.18	40.2:3.11:4.07:44.1:8.5	20–31	0.15	1.54
	9.79	34.5:3.74:6.05:47.2:8.5	23–36	0.23	0.86
EM-L	0	21.2:0:0:53.5:25.3	47–75	UM	0.83
	0.82	18.5:0.15:0.67:55.8:24.9	54–88	0.16	0.87
	1.63	17.0:0.28:1.35:56.6:24.7	59–96	0.06	0.78
	2.55	15.3:0.40:2.15:56.4:25.8	65–106	UM	0.71
	5.28	19.9:1.11:4.17:54.4:20.4	48–77	UM	0.45

The proportions of SA-DAG, respectively, contained in the surface (DAG_{surf}) and in the core (DAG_{core}) of the particle were calculated after determination of their partition coefficient. The size ranges of EM-S and EM-L were inferred from their compositional analysis. The rate constants for Pyr-PC transfers were determined both in the absence and in the presence of PLTP. $K + 1_{spt}$ corresponded to the spontaneous Pyr-PC transfers, while $K + 1_{PLTP}$ were calculated from the difference between the values obtained in the presence of PLTP and those of $K + 1_{spt}$. In three cases, $K + 1_{spt}$ were unmeasurable (UM).

The competitive effect of SA-DAG on the velocities of PL transfer was then theoretically predicted as follows:

$$V_{prd} = \frac{[PL]}{[PL] + [SA\text{-}DAG_{eff}]}(K + 1_0)[PL] \quad \textit{Eq. 3)}$$

where V_{prd} is the predicted initial velocity of the PLTP-facilitated PL transfer, [PL] is the concentration of donor PL in the transfer assay, and $K + 1_0$ is the apparent rate constant observed in the absence of SA-DAG. $[SA\text{-}DAG_{eff}]$, defined as the efficient $[SA\text{-}DAG_{surf}]$ concentration in the transfer assay, was calculated as

$$[SA\text{-}DAG_{eff}] = 0.58[SA\text{-}DAG_{surf}] \quad \textit{Eq. 4)}$$

where 0.58 is the relative affinity of SA-DAG for PLTP with respect to that of Pyr-PC (see above).

The theoretical effects of increasing SA-DAG concentrations on V_{prd} are shown in **Fig. 3** for SBV, EM-S, and EM-L particles, together with the experimental values of PL transfer velocities determined as

$$V_{exp} = K + 1_{app}[PL] \quad \textit{Eq. 5)}$$

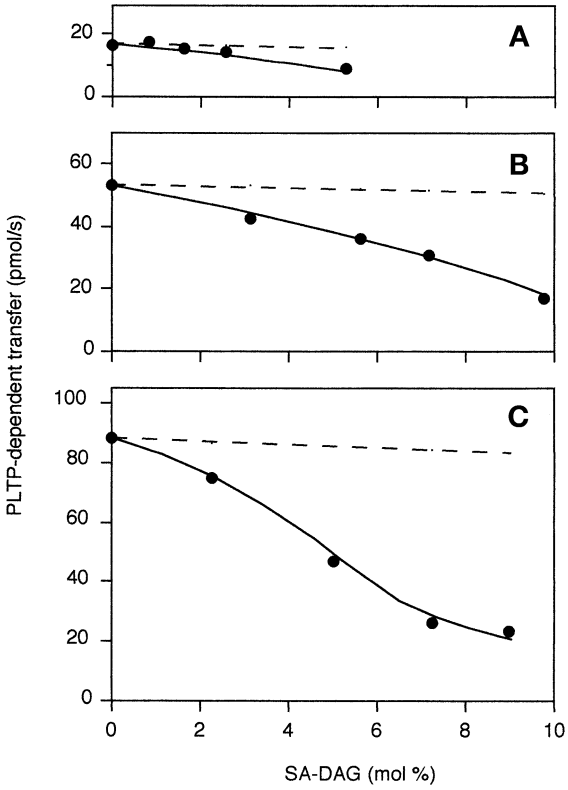

Fig. 3. Effects of SA-DAG on the velocities of PLTP-dependent transfers of Pyr-PC. EM-L (A), EM-S (B), and SBV (C) containing various proportions of SA-DAG were used as Pyr-PC donors. They were incubated (200 nmol of PL) with HDL (800 nmol of PL) in the presence or in the absence of PLTP (2.5 μg) in a total volume of 1.2 ml, at 37°C. The kinetics of Pyr-PC transfers were monitored as shown in Fig. 2. The experimental velocities of the PLTP-dependent transfers (solid circles) were then determined and compared with the theoretical velocities (– – –) that were calculated on the basis of a simple binding competition between SA-DAG and Pyr-PC with respect to PLTP.

where $K + 1_{app}$ values are the apparent rate constants for the PLTP-facilitated PL transfers described in Table 1.

The results clearly indicated that the inhibiting effects of SA-DAG on V_{exp} had a magnitude much more important than that predicted, irrespective of the type of the donor particles, that is, EM-L (Fig. 3A), EM-S (Fig. 3B), or SBV (Fig. 3C). In addition, we observed that in the absence of SA-DAG, the PL transfer velocities decreased as the proportion of core lipids increased (V_{exp} in SBV > V_{exp} in EM-S > V_{exp} in EM-L). In an attempt to normalize the data, we plotted the V_{exp}/V_{prd} ratios as a function of SA-DAG concentrations (**Fig. 4**). Under these conditions, the latter appeared to affect PL transfers in a noticeably similar way for the three types of particles.

Effect of DAG on the physical properties of emulsion particles

In addition to their competitive binding to PLTP, we considered the possibility that DAG might affect the physical properties of the model emulsion particles. The particle sizes of EM-S and EM-L, inferred from their compositions, are shown in Table 1. Because the molecular surfaces of PL are known to vary according to their environment, we determined the range of possible values for each particle. As expected, the size of EM-L was about twice as large as that of EM-S. In contrast, the concentration of SA-DAG in the particles had no apparent effect on their size.

The viscosities of the particle surfaces were determined by electron spin resonance, using 16-DSA as the probe. In each particle type, the results clearly indicated that the increase in SA-DAG concentration determined the elevation of the particle surface viscosity (**Fig. 5A**). We therefore considered the possibility that the SA-DAG-induced decrease in PL transfer velocity might be related to their effect on the particle viscosity (Fig. 5B). For both EM-S and EM-L, we found an approximately linear relation between the V_{exp}/V_{prd} ratios and the particle surface viscosities. However, the decrease in PL transfer velocity began at a higher viscosity in EM-S than in EM-L particles.

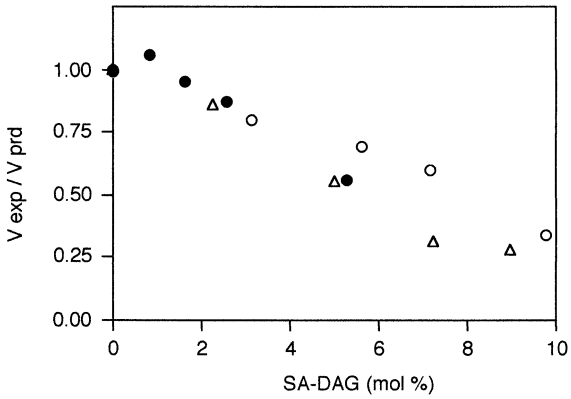

Fig. 4. Relationship between the SA-DAG contents of EM-S (open circles), EM-L (solid circles), and SBV (open triangles) and the ratios of experimental (V_{exp}) to predicted (V_{prd}) velocities of Pyr-PC transfers. For each type of particles, the V_{exp}/V_{prd} ratios were calculated from the data shown in Fig. 3.

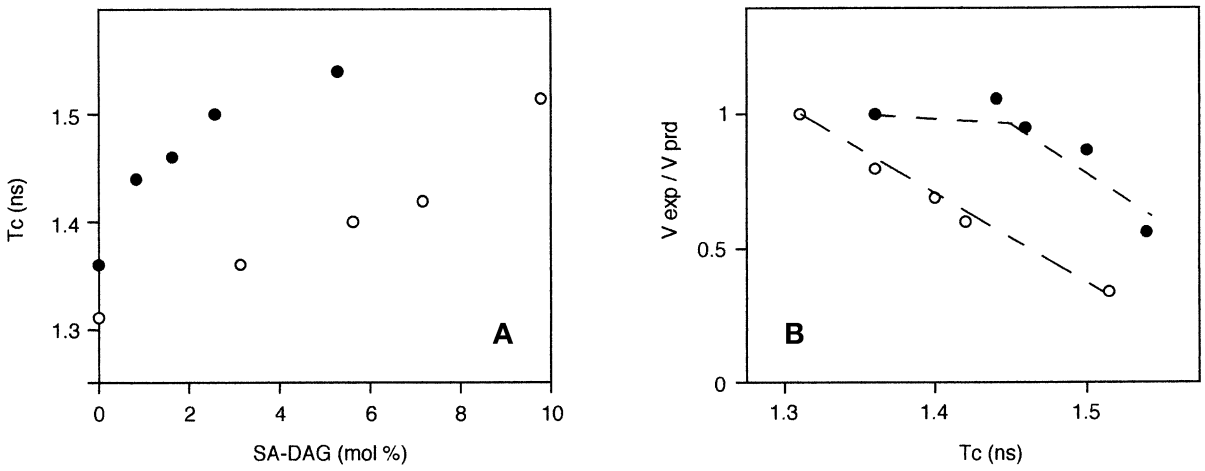

Fig. 5. Relationship between the SA-DAG contents of EM-S and EM-L, the particle surface viscosities, and the velocities of Pyr-PC transfers. Viscosities of the particle surfaces were expressed as the rotational correlation times (T_c) of 16-doxyl stearic acid calculated from electron spin resonance experiments. In both EM-S (open circles) and EM-L (solid circles), T_c progressively increased as a function of SA-DAG concentrations in the particle surfaces (A). The ratios of experimental (V_{exp}) to predicted (V_{prd}) velocities of Pyr-PC transfers were found to decrease as T_c increased (B).

DISCUSSION

The present work was designed to study the possibility that the presence of DAG in lipoproteins might induce a predictable decrease in PLTP-mediated PL transfer through a mechanism involving a competition between DAG and PL for the binding to PLTP. This hypothesis was based on three considerations deriving from several independent works. First, the TG hydrolysis in VLDL results in part from the formation of DAG that distribute in all lipoprotein subfractions (2, 6). Second, a large part of the PLTP-facilitated transfer of PL from VLDL to HDL occurs during the process of VLDL lipolysis (19). Third, in vitro studies have shown that PLTP was able to promote the transfer of DAG from reassembled lipoprotein particles (7). Collective consideration of these data led us to undertake the first quantitative study of the effects of DAG on PLTP-mediated PL transfer. To this end, we used model lipid particles containing various concentrations of DAG in the same range as those previously described in native VLDL (6). The levels of PL transfer were noticeably different according to the types of particles used as donors, irrespective of DAG concentrations. PL transfer velocities decreased as the core-to-surface lipid ratio increased. Although this work was not designed to study this question, we can speculate that elevated PL transfers from particles containing large proportions of core lipids would necessarily result in major reorganizations of the particles to protect their neutral lipids from the aqueous environment. From this viewpoint, one could consider that PL transfer from particles with large cores might be a thermodynamically unfavorable process in the absence of concomitant lipolysis.

In spite of these differences in PL transfer levels between SBV, EM-S, and EM-L, a clear inhibiting effect of DAG was observed in the three types of particles. We therefore attempted to determine to what extent this effect might be due to the putative competition between DAG and PL for their binding to PLTP. To this end, we had to estimate two parameters specifically concerning the particular molecular species of DAG used in the present work. The first parameter was the relative affinity of SA-DAG for PLTP in comparison with that of PL. We found that SA-DAG had a relative affinity for PLTP of 0.58 with respect to that of Pyr-PC. This value was slightly lower than expected on the basis of the previous work of Rao et al. (7), who reported that the affinity of DAG for PLTP was better than that of Pyr-PC. This minor difference is likely due to the fact that the molecular species of DAG used by these authors as the marker was 1-palmitoyl-2-[10-(1-pyrenyl)decanoyl]-glycerol, confirming that various DAG species may have different transferabilities. This emphasizes the necessity to specifically trace the DAG species used with its homologous labeled counterpart to obtain accurate data. The second parameter that we had to estimate was the coefficient for the partition of DAG between the core and surface lipids of our model particles. The data available on this question are rather controversial and likely vary according to the molecular species of DAG. Vieu et al. (2) suggested that most of the DAG molecules are associated with the lipoprotein surface, which contrasted with insect lipophorins, in which DAG are essentially contained in the core of the particles (36). In this work, we found a partition coefficient not statistically different from 1, thereby indicating that the core/surface partition of SA-DAG was approximately the same as that of total lipids. On the basis of our estimates, we calculated the theoretical influence of SA-DAG on the PLTP-mediated transfers of PL. For all types of particles, the experimental inhibiting effects of DAG on PL transfers were much more pronounced than those predicted from our calculations. The difference was too large to be simply explained by the uncertainties of our estimates of the competition parameters mentioned above.

Because, in addition to the competition mechanism, other SA-DAG effects had to be considered to account for their inhibition of PL transfer, we examined their influence on the physicochemical characteristics of our model particles. As expected, the calculated size of EM-L was larger than that of EM-S. These sizes were in no way influenced by the DAG content of the particles. In contrast, a quantitative relationship was established between the latter and the viscosity of the particle surface. The direct plots of the inhibiting effects of DAG on the PLTP-mediated PL transfers as a function of their effects on the particle viscosity revealed a clear negative relationship. Although this was true for both EM-L and EM-S, the decrease in PL transfer began at a more elevated viscosity in the latter than in the former. No particular data from our work can explain this difference. One can only observe that the different sizes of these two types of particles determine different curvatures of the particle surfaces, which might influence their properties with respect to PL transfer.

A putative effect of the viscosity/fluidity properties of particle surface on lipid transfer has been previously considered (37). Nonesterified fatty acids known to modulate CETP-dependant lipid transfers were shown to decrease the viscosity of lipoprotein surface, suggesting that the latter might directly affect CETP activity. Thus, particle viscosity might emerge as a parameter governing the transfers of lipids between lipoproteins, whether mediated by CETP or by PLTP. In our experiments, two nonexclusive explanations could account for the negative effect of particle surface viscosity on PL transfer. In the first, the energy required for the desorption of a given PL molecule might increase according to the degree of packing of the particle surface, thereby limiting the transfer rate. In the second explanation, the particle surface packing might govern the binding affinity of PLTP and thus its efficiency as a PL carrier. Although not ruling out the latter possibility, we observed that the spontaneous PL transfer from SBV was lowered as the SA-DAG content increased. This finding suggests that the activation energy for the desorption of PL molecules increased in the presence of DAG, independent of the PLTP action.

In addition to those mentioned above, another mechanism might participate to the overall effect of DAG on PL transfer. In our model particles, the progressive increase in DAG content was necessarily balanced by a decrease in PL. Albeit moderate, this decrease might have induced some changes in the electrostatic charge properties of the lipid surface, which is an important parameter governing the interaction of PLTP with lipoproteins (38). However, the question as to whether DAG might have a significant effect on the electrostatic properties of natives lipoproteins is presently unanswered. Thus, the present work does not totally clarify the mechanism of action of DAG on PL transfer. However, it clearly establishes that the proportion of DAG present in lipoproteins in physiological conditions may result in a strong decrease in PL transfer, both by competition with respect to PLTP and by increasing the particle surface viscosity. These observations might be of particular interest in physiopathological situations with elevated plasma TG concentrations. For example, we have shown in previous works that the hypertriglyceridemia accompanying type 2 diabetes resulted in an increase in plasma DAG concentrations (6), while the same pathology was characterized by a lowered PL transfer (22). Both these studies were performed with fasting plasma samples. To establish a relationship of physiopathological relevance between plasma DAG concentrations and PLTP-facilitated PL transfers, it would now be interesting to measure these two parameters in the same diabetic plasma samples withdrawn during the postprandial state.

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