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Transfer of Pyrene-Dietherphosphatidylcholine to Serum Lipoproteins

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The nonhydrolyzable fluorescent diether analog of phosphatidylcholine, 1-*O*-hexadecyl-2-0-pyrenedecyl-*sn*-glycero-3-phosphocholine, has been synthesized as a stable probe for the determination of phospholipid transfer to different lipoprotein classes with potential phospholipase activities. After incubation of total human serum with the new probe at 37°C for 3 hours a characteristic partition equilibrium between the main lipoprotein fractions was observed. The fluorescent lipid was not degraded under these conditions and, therefore, served as a marker for choline glycerophospholipid distribution between and transport to serum lipoproteins.

KEY WORDS: atherosclerosis; lipoprotein (a); low density lipoprotein; high density lipoprotein; phospholipid vesicles.

Phospholipids are surface components of plasma lipoproteins. They are subject to exchange between the different lipoprotein classes. In particular, phosphatidylcholine transport is facilitated by the action of transfer proteins that can be isolated from the lipoprotein-free serum fraction [1]. When partitioning and transfer of phospholipids to lipoproteins have to be determined in the presence of hydrolytic enzymes, a nonhydrolyzable phospholipid analogue is required. A radiolabeled diether analogue has already been used to determine the exchange of choline glycerophospholipid among lipoproteins, vesicles, and cells in the presence and the absence of a phospholipid transfer protein from serum [2,]. We used a fluorescent diether analogue of phosphatidylcholine, 1-*O*-hexadecyl-2-*O*-pyrenedecyl-*sn*-glycero-3-phosphocholine (pyrene-diether-PC) (Fig. 1) [3], carrying a pyrene reporter group at the omega end of the hydrophobic chain in position 2 of glycerol. This

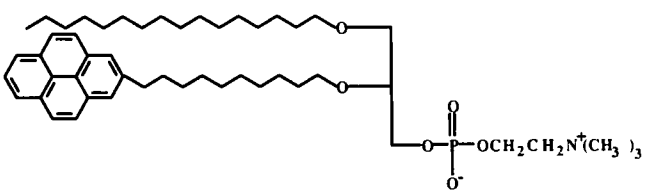

Fig. 1. Chemical structure of 1-*O*-hexadecyl-2-*O*-pyrenedecyl-*sn*-glycero-3-phosphocholine.

new compound not only is a suitable lipid analogue for the rapid and continuous analysis of phosphatidylcholine transfer, but also provides information on the concentration and lateral distribution of the label in lipid aggregates.

Upon incubation of pyrene-diether-PC vesicles with a 100-fold excess of human serum lipoproteins, incorporation of the labeled phospholipid into the particle surface was followed by continuous monitoring of the increase in pyrene monomer fluorescence intensity at 400 nm. Vesicles consisting of the labeled lipid show predominantly excimer fluorescence, which decreases in favor of monomer fluorescence if pyrene lipid is trans-

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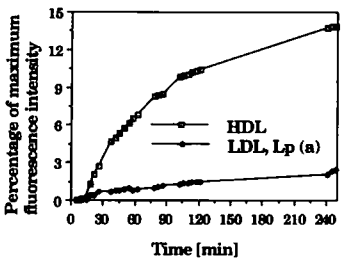

Fig. 2. Transfer of pyrene-diether PC to lipoproteins: lipid transfer from self-quenched donor vesicles containing pyrene-diether-PC (0.1 μM) to unlabeled lipoprotein (10 μM phospholipid) was determined from the time-dependent increase in pyrene monomer fluorescence intensities at 400 nm. Maximum fluorescence intensities were obtained after the addition of sodium cholate.

ferred to lipoproteins and thus diluted by unlabeled lipoprotein lipid.

Spontaneous incorporation of pyrene dialkyl-PC from vesicles into isolated low-density lipoprotein (LDL) and lipoprotein(a) Lp(a) proceeds at very slow rates compared to high-density lipoprotein (HDL) (Fig. 2). In contrast, transfer of fluorescent diether-PC from vesicles to these lipoproteins is highly facilitated in the presence of lipoprotein-deficient serum. In this case, transfer rates observed with Lp(a) as an acceptor were lower compared to LDL. This is probably due to the higher rigidity of surface phospholipids in Lp(a) [4].

After density gradient centrifugation of serum preincubated with pyrene-diether-PC for 3 h, a distinct fluorescence pattern was observed which was identical to that of a control sample stained with Coomassie blue for proteins. The fluorescence spectra showed only pyrene monomer emission of the label in LDL and HDL but monomer and excimer fluorescence for labeled Lp(a). Thus, pyrene-diether-PC must be highly concentrated and/or clustered in the latter lipoprotein, whereas it is more uniformly distributed and highly diluted in LDL. A similar conclusion has already been reached on the basis of time-resolved fluorescence studies [4].

We speculate that enrichment of pyrene-diether-PC in the Lp(a) fraction of native serum may be due to the higher affinity of apoproteins for PC in Lp(a) [4] rather than facilitated phospholipid transfer to this lipoprotein. As already mentioned, the labeled diether phospholipid is transferred more slowly to Lp(a) than to LDL. The only difference between LDL and Lp(a) is the presence of apolipoprotein(a) [apo (a)] [5] in Lp(a), and any changes in lipid–protein association in Lp(a) relative to LDL must be due to this apoprotein. However, it has to be left open whether the large extent of pyrene-diether-PC uptake and clustering in Lp(a) is due to apo(a)–lipid interactions or an effect of apo(a) on the conformation of apoB, which finally interacts with the choline glycerophospholipid.

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REFERENCES

1. A. R. Tall (1986) *J. Lipid Res.* **27**, 361–367.
2. H. J. Pownall, D. L. M. Hickson, A. J. Gotto, and J. B. Massey (1984) *Biochem. Biophys. Res. Comm.* **119**, 452–457.
3. A. Hermetter and F. Paltauf (1983) in H. K. Mangold and F. Paltauf (Eds.), *Ether Lipids*, Academic Press, New York, pp. 389–420.
4. A. Sommer, E. Prenner, R. Gorges, H. Stuetz, H. Grillhofer, G. M. Kostner, F. Paltauf, and A. Hermetter (1992) *J. Biol. Chem.* **267**, 24217–24222.
5. A. M. Scanu (1991) *Curr. Opin. Lipidol.* **2**, 253–258.