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EXCHANGE OF PHOSPHOLIPIDS BETWEEN UNILAMELLAR VESICLES OF 1,2-DIPALMITOYL-*sn*-GLYCERO-3-PHOSPHATIDYLCHOLINE AND PLASMA VERY LOW DENSITY LIPOPROTEINS

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Summary

Purified phosphatidylcholine exchange protein from bovine liver was used to exchange [^{14}C]dipalmitoyl phosphatidylcholine from sonicated vesicles to human plasma very low density lipoproteins (VLDL). The exchange of [^{14}C]-dipalmitoyl phosphatidylcholine for VLDL phospholipids was temperature dependent and linear with respect to time and amount of exchange protein. In the absence of the exchange protein, less than 10% of the [^{14}C]dipalmitoyl phosphatidylcholine was transferred. At an initial weight ratio of [^{14}C]-dipalmitoyl phosphatidylcholine vesicles to VLDL phospholipid (1.2 mg) of 2.2, the exchange protein (14 μg) replaced 55% of the VLDL phospholipids with [^{14}C]dipalmitoyl phosphatidylcholine in 15 min; VLDL protein and cholesterol content were unaltered. From these studies we conclude that the exchange protein is a useful method to alter the phospholipid composition of VLDL under conditions such that there is minimal perturbation of the lipoprotein.

Introduction

Plasma very low density lipoproteins (VLDL) consist of approximately 10% protein, 15% phospholipid, 15% total cholesterol and 60% triglyceride [1]. VLDL are spherical particles of diameters 250—500 Å in which protein, cholesterol and phospholipids form a surface monolayer and surround a central core

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Abbreviations: VLDL, plasma very low density lipoproteins; DPPC, dipalmitoyl phosphatidylcholine.

of triglycerides and cholesteryl esters. During lipolysis, VLDL are degraded by lipoprotein lipase at the capillary endothelium [2,3]; triglycerides are hydrolyzed to free fatty acids and di- and monoglycerides. In addition, there is loss of VLDL protein, cholesterol and phospholipids, the latter being hydrolyzed to lysophosphatides [4,6]. The factors which regulate triglyceride and phospholipid hydrolysis by lipoprotein lipase have been reviewed [7]. It is known that extrahepatic lipoprotein lipase has an activator-cofactor requirement for a VLDL apolipoprotein, designated apo C-II [7]. The role of lipid structure in regulating lipolysis has not been studied in detail since it has not been possible to date to produce human VLDL which contain predominantly fatty acids of a single class.

Recently we have shown [8] by using radiolabeled microsomes and purified bovine liver phospholipid exchange protein, that all of phosphatidylcholine in VLDL is available for exchange. With this information, it was possible to design experiments so as to replace VLDL phospholipids with those of a single fatty acid class. The purpose of the present study was to develop the necessary methods for this replacement using the exchange protein and defined phospholipids.

Materials and Methods

Isolation of lipoproteins. Very low density lipoproteins were obtained from plasma of fasting subjects at the Cincinnati Lipid Research Clinic with endogenous (type IV) hyperlipoproteinemia. VLDL were isolated by ultracentrifugal flotation of plasma in a 50.2 Ti rotor (Beckman Inst.) for 18 h at 48 000 rev./min and 8°C. The VLDL were then layered under a standard buffer (d = 1.006 g/ml) of 10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl, 1 mM EDTA and 0.01% sodium azide, and was refloated a second time. The isolated VLDL were purified further by chromatography on Sepharose 4B (Pharmacia). The column (2.5 × 40 cm) was equilibrated and eluted with standard buffer. The isolated VLDL contained 10.8% protein, 16.2% phospholipid, 15.2% cholesterol (total) and 57.8% triglyceride.

Isolation of phosphatidylcholine exchange protein. Phosphatidylcholine exchange protein was purified from bovine liver (10 000 × g) as described by Kamp et al. [9]. The procedures were exactly as those of Kamp et al. [9] with the exception that CM-Sephadex (Pharmacia) was used in step 5 (Ref. 9) instead of CM-cellulose; the protein was eluted with a citric acid/Na_2HPO_4 buffer as in [9]. The protein was then applied to a column (2.5 × 70 cm) of Sephadex G-50 and eluted with 0.05 M citric acid/0.1 M Na_2HPO_4/0.01 M β-mercaptoethanol, pH 5.0; fractions containing exchange activity were pooled and dialyzed against 50% glycerol. The exchange protein was rechromatographed on the same Sephadex G-50 column, the appropriate fractions pooled, dialyzed against 50% glycerol and stored at −20°C.

Phosphatidylcholine exchange protein activity was determined using radiolabeled microsomes as a donor and human plasma high density lipoproteins as an acceptor as described previously [8]. The microsomes were prepared from rabbit liver by injection of [*Me*-^{14}C]choline, as described for rat liver microsomes [10]; the isolated microsomes contained 75 900 cpm/μmol phosphatidylcholine. With the microsome high density lipoprotein assay, the puri-

fied phospholipid exchange protein had a specific activity of 1.1 μmol phosphatidylcholine transferred/min per mg protein at 37°C.

Preparation of dipalmitoyl phosphatidylcholine vesicles. Dipalmitoyl phosphatidylcholine was purchased from Sigma and [1-^{14}C]dipalmitoyl phosphatidylcholine (100 Ci/mol) from New England Nuclear; both compounds gave a single species on a thin-layer plate of silica gel (chloroform/methanol/water, 65 : 25 : 4). The unlabeled dipalmitoyl phosphatidylcholine had a fatty acid composition of 98% palmitic acid. Mixtures of dipalmitoyl phosphatidylcholine and radiolabeled dipalmitoyl phosphatidylcholine were prepared in chloroform, evaporated in vacuo and dried 1 h under vacuum. Phospholipid dispersions were then prepared by adding standard buffer to give 10 mg phospholipid/ml. The lipid was suspended in the buffer by mechanical agitation and was sonicated under nitrogen at 42°C for 30 min using a Branson Sonifier. After sonication, large phospholipid structures were removed from the sonicate by ultracentrifugation at 150 000 × *g* for 1 h at 15°C; the vesicles were prepared daily and stored at room temperature.

Other methods. Phospholipid phosphorus was determined by the method of Bartlett [11]. Protein content was determined according to Schacterle and Pollack [12]. Total VLDL cholesterol and triglycerides were determined by Lipid Research Clinics autoanalyzer (AA-II) techniques [13]. VLDL lipids were extracted by the method of Folch et al. [14]. Phospholipid fatty acids were determined by gas chromatography [15].

Experimental procedure. VLDL, [^{14}C]dipalmitoyl phosphatidylcholine and the exchange protein were incubated in 5.0 ml of standard buffer as described in Results. After incubation, solid KBr was added to the mixture to give d = 1.02. VLDL were separated from phospholipid vesicles by ultracentrifugation in a type 50 rotor (Beckman) for 18 h at 48 000 rev./min at 4°C. VLDL were removed by aspiration, dialyzed against standard buffer, and protein and lipid content determined.

Results

Phosphatidylcholine exchange protein was purified from beef liver as described by Kamp and coworkers [9,10]. The purified exchange protein was then used to replace dipalmitoyl phosphatidylcholine for VLDL phospholipids. The effect of temperature on phosphatidylcholine exchange between vesicles and VLDL is shown in Fig. 1. At temperatures of 24°C or less there was essentially no exchange of phospholipid in a 15 min incubation period. At 37°C, exchange was very rapid (Fig. 1).

It is shown in Fig. 2 that the exchange protein catalyzes the transfer of [^{14}C]dipalmitoyl phosphatidylcholine from the vesicles to VLDL as a function of time. With the conditions of the assay, exchange activity was linear for 12 min. In the absence of phospholipid exchange protein there was less than 5% incorporation of dipalmitoyl phosphatidylcholine into VLDL. The assay contained 250 μg VLDL phospholipid (192 μg phosphatidylcholine) and 280 μg dipalmitoyl phosphatidylcholine. Assuming that 60% of the vesicle phospholipid and that all of the VLDL phosphatidylcholine [8] is available for transfer then the maximum ratio for [^{14}C]DPPC/phospholipid (Fig. 2) is 0.45.

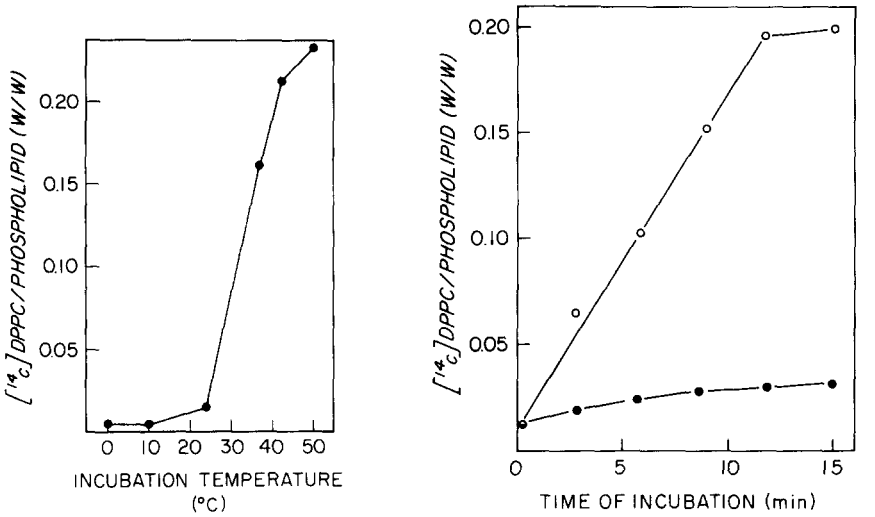


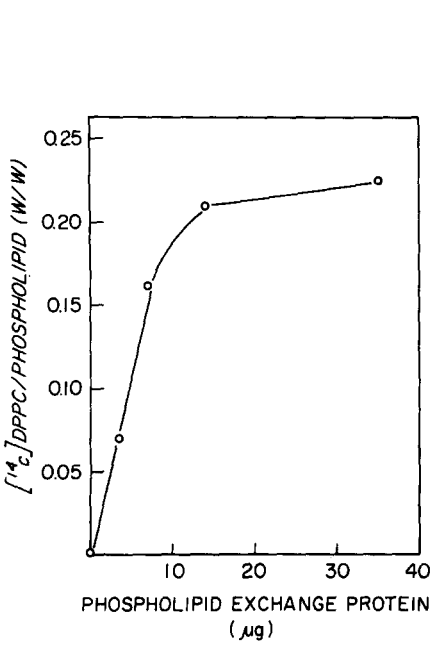
Fig. 1. Effect of temperature on the transfer of [^{14}C]DPPC to VLDL. The incubation mixtures contained VLDL (250 μg phospholipid), [^{14}C]DPPC vesicles (280 μg) and phosphatidylcholine exchange protein (14 μg) in a total volume of 5.0 ml of 10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl, 1 mM EDTA and 0.01% sodium azide. Incubation mixtures containing no exchange proteins were used in order to determine the non-facilitated transfer of phospholipid, and the values were subtracted from the facilitated transfer values. After 15 min incubation at the indicated temperatures, VLDL were isolated by ultracentrifugation at d = 1.02, and the ratio of [^{14}C]DPPC/total VLDL phospholipid determined.

Fig. 2. Effect of time of incubation on the transfer of [^{14}C]DPPC to VLDL. The incubation mixtures contained VLDL (250 μg phospholipid), [^{14}C]DPPC vesicles (280 μg) and plus (○———○) or minus (●———●) phosphatidylcholine exchange protein (14 μg) in a total volume of 5.0 ml of 10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl, 1 mM EDTA and 0.01% sodium azide. The samples were placed at 42°C and at the indicated times, tubes were removed and placed at 4°C.

Thus, in 15 min approximately 50% of the exchangeable phospholipid is transferred.

In Fig. 3 it is seen that the transfer of [^{14}C]dipalmitoyl phosphatidylcholine increases with the amount of exchange protein. With the conditions of the assay (Fig. 3), the exchange was linear up to 9 μg of protein. The linear relationship between time and exchange protein concentration and transfer of phosphatidylcholine indicated that the assay system could be utilized to alter the phospholipid compositions of VLDL.

In the next experiment, the ratio between [^{14}C]dipalmitoyl phosphatidylcholine vesicles and VLDL phospholipid was varied between zero and ten. As shown in Fig. 4, with increasing concentrations of [^{14}C]dipalmitoyl phosphatidylcholine added to the reaction mixture, the ratio between [^{14}C]-dipalmitoyl phosphatidylcholine and total phospholipid in the isolated VLDL increased proportionally. At a ratio of 2.2 (Fig. 4), 55% of the total phospholipid in VLDL was dipalmitoyl phosphatidylcholine. As shown in Fig. 4, the VLDL which were isolated up to an initial ratio of 2.2 for [^{14}C]dipalmitoyl phosphatidylcholine to VLDL phospholipid had similar protein, phospholipid and cholesterol compositions. However, at greater ratios, there was a loss of both protein and cholesterol. The recovery of phospholipid (Fig. 4) and of triglyceride (not shown) were approximately identical at all ratios studied and



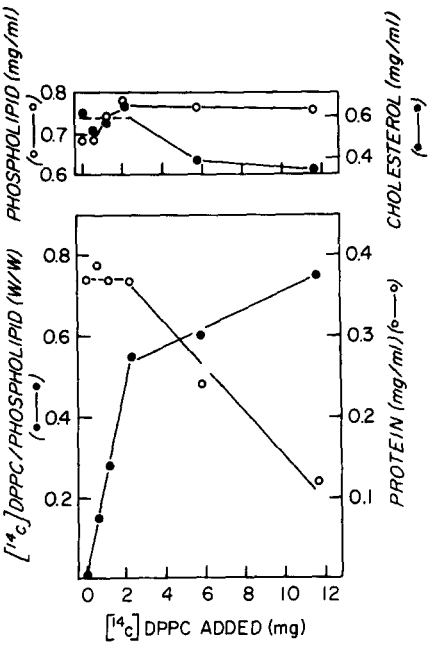


Fig. 3. Effect of amount of phosphatidylcholine exchange protein on transfer of [^{14}C]DPPC to VLDL. The incubation mixtures contained VLDL (250 μg phospholipid), [^{14}C]DPPC vesicles (280 μg) and the indicated amount of phosphatidylcholine exchange protein in 5 ml of 10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl, 1 mM EDTA and 0.01% sodium azide. Incubation mixtures containing no exchange protein were used to determine non-facilitated transfer and the values were subtracted from the plus exchange protein values. After 15 min incubation at 42° C, VLDL were isolated and phospholipid determined.

Fig. 4. Effect of amount of [^{14}C]DPPC on the transfer of [^{14}C]DPPC to VLDL. The incubation mixtures contained VLDL (1.2 mg phospholipid), phosphatidylcholine exchange protein (50 μg) and increasing amounts of [^{14}C]DPPC as indicated in a total volume of 5.0 ml of 10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl, 1 mM EDTA and 0.01% sodium azide. After incubation for 15 min at 42° C, VLDL were isolated by ultracentrifugation.

corresponded to 75—80% of the lipid added to the incubation mixture. The loss of protein and cholesterol observed at high [^{14}C]dipalmitoyl phosphatidylcholine to VLDL ratios occurred even in the absence of the exchange protein. However, at these high ratios, the amount of [^{14}C]dipalmitoyl phosphatidyl-

TABLE I

FATTY ACID COMPOSITION OF VERY LOW DENSITY LIPOPROTEIN (VLDL) PHOSPHOLIPIDS BEFORE AND AFTER PHOSPHOLIPID EXCHANGE

VLDL containing 22% [^{14}C]dipalmitoyl phosphatidylcholine (DPPC) was prepared as described in the text using [^{14}C]DPPC vesicles and phosphatidylcholine exchange protein. Phospholipid fatty acids were determined by gas chromatography. Only the four major fatty acids are shown; the minor ones amounted to less than 5% of the total.

Lipoprotein	Fatty acid (percent of total)			
	16:0	18:0	18:1	18:2
VLDL	32.9	22.7	14.5	29.9
VLDL containing [^{14}C]DPPC	55.2	16.3	9.4	19.1
Percent change	+67%	—28%	—35%	—36%

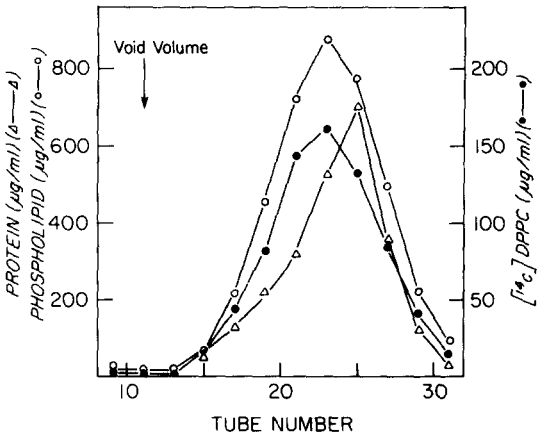


Fig. 5. Chromatography of VLDL containing [^{14}C]DPPC on Sepharose 4B. [^{14}C]DPPC was exchanged into VLDL to the extent of 22% [^{14}C]DPPC of VLDL phospholipids. The sample (20 mg VLDL protein) was subjected to chromatography on a column (2.5 × 30 cm) of Sepharose 4B at 4° C. The column was equilibrated and eluted with 10 mM Tris-HCl, pH 7.4, containing 0.9% NaCl, 1 mM EDTA and 0.01% sodium azide. The flow rate was 20 ml/h and 3 ml fractions were collected.

choline in the VLDL in the absence of the exchange protein was less than 10% of that added to the incubation mixture.

To show that the phospholipid-fatty acid composition of the VLDL was altered after phospholipid exchange, a VLDL preparation which contained a [^{14}C]dipalmitoyl phosphatidylcholine to VLDL phospholipid ratio of 0.22 was extracted with chloroform/methanol and the phospholipid fraction isolated by thin-layer chromatography. The fatty acid composition of the phospholipid was determined and compared to unmodified VLDL phospholipids. Table I shows that the percentage of palmitic acid increased 67% whereas stearic, oleic and linoleic acid all decreased approximately 35%.

To show that [^{14}C]dipalmitoyl phosphatidylcholine cochromatographed with VLDL phospholipids, VLDL containing 22% [^{14}C]dipalmitoyl phosphatidylcholine was subjected to gel filtration chromatography on Sepharose 4B. As shown in Fig. 5, [^{14}C]dipalmitoyl phosphatidylcholine and total VLDL phospholipid chromatographed at a constant ratio. However, VLDL protein and phospholipid did not cochromatograph (Fig. 5) indicating that smaller VLDL particles contain a greater percentage of protein.

Discussion

To probe membrane structure, phospholipid exchange proteins have recently been used by a number of laboratories as a method to alter the phospholipid composition of membranes [16—18]. The advantage of the bovine liver phosphatidylcholine exchange protein in altering plasma and membrane lipoprotein phospholipid composition lies in the fact that the exchange protein is specific for the phosphorylcholine moiety [19]. In addition, transfer is independent of the fatty acyl chain length, and occurs under physiologic conditions such that there is minimal perturbation of the lipoprotein. We have previously used ^{14}C-labeled microsomes and the exchange protein to show that all of the phosphatidylcholine in plasma lipoproteins is available for exchange [8]. In the present study, we have utilized sonicated [^{14}C]dipalmitoyl phosphatidyl-

choline vesicles and the exchange protein in order to replace the phospholipids of VLDL with a single class of phospholipid fatty acids. With the methods described, it was demonstrated that [^{14}C]dipalmitoyl phosphatidylcholine was rapidly exchanged by the exchange protein between vesicles and VLDL. In the absence of the exchange protein, there was less than 5% exchange indicating that the vesicle by itself did not alter the phospholipid composition of the VLDL. Tall et al. [20] have reported that the addition of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine multilamellar liposomes to human plasma high density lipoproteins does alter their structure. In addition, Chobanian et al. [21] have also shown that 20% of egg yolk phosphatidylcholine vesicles is transferred to VLDL in 1 h at 37°C. However, with the conditions of exchange used in the present study (42°C, 15 min), there was less than 10% non-facilitated transfer of dipalmitoyl phosphatidylcholine to VLDL. Furthermore with these conditions we found no evidence for marked alteration of VLDL protein or lipid so long as the initial ratio of [^{14}C]dipalmitoyl phosphatidylcholine to VLDL phospholipid was 2 or less. At higher ratios, there was a loss of both protein and cholesterol and is consistent with the fact that these VLDL constituents are rapidly exchangeable [1]. By using synthetic phospholipid vesicles as the donor phospholipid and the exchange protein, it is now possible to alter the phospholipid composition of VLDL in a defined manner and to determine what effect this change has on VLDL structure and catabolism.

Acknowledgements

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