# COMPLETE EXCHANGE OF PHOSPHOLIPIDS BETWEEN MICROSOMES AND PLASMA LIPOPROTEINS MEDIATED BY LIVER PHOSPHOLIPID-EXCHANGE PROTEINS

R. L. JACKSON, J. WESTERMAN+ and K. W. A. WIRTZ+

*Department of Pharmacology and Cell Biophysics, Biological Chemistry and Medicine, University of Cincinnati, College of Medicine, Cincinnati, OH 45267, USA* and *+Biochemistry Laboratory, State University of Utrecht, Transitorium 3, Padualaan 8, 3508 TB Utrecht, The Netherlands*

Received 27 July 1978

## 1. Introduction

It is known that cholesterol and phospholipids are in dynamic equilibrium and spontaneously exchange both in vitro and in vivo between individual classes of plasma lipoproteins [1–4]. Spontaneous transfer of phospholipids has also been shown between lipoproteins and erythrocytes [5], mitochondria [6], liver microsomes [7], liver slices [8] and cells in culture [9]. In these examples of phospholipid exchange it is presumed that transfer occurs upon formation of a collision complex. Evidence for a protein-facilitated transfer of lipoprotein phospholipids in serum is controversial [7,10]. On the other hand, eukaryotic cells contain proteins designated as phospholipid exchange proteins (PLEP) that do facilitate the transfer of phospholipids from one membrane to another [11–13]. A purified PLEP from beef liver shows a highly specific affinity for phosphatidylcholine (PC-PLEP [14]). Two proteins have been isolated from bovine cerebral cortex which stimulate both the transfer of phosphatidylinositol (PI) and PC (PI-PLEP [15]). Recently, exchange proteins have been isolated from rat liver [16] and hepatoma [17] which lack specificity in the transfer of phospholipid.

The purpose of the present study was to determine whether and if so, to what extent, purified PC- and PI-exchange proteins catalyze the transfer of phospholipids between rat liver microsomes and the individual classes of lipoproteins. It will be shown that the protein-mediated transfer of labeled PC and PI from microsomes to very low density lipoproteins (VLDL), LDL and HDL was 5–10-fold higher than without PLEP, and that PC in the various lipoproteins was completely available for exchange. In addition, the present study shows that PC in microsomes is fully exchangeable with lipoproteins, and is consistent with recent reports where liposomes were used as the phospholipid acceptor [18,19].

## 2. Materials and methods

Each of the human lipoprotein classes was isolated from a normal fasting individual by ultracentrifugal flotation of plasma in salt solutions of KBr. A Beckman Model L2-65 centrifuge (Spinco Div., Palo Alto, Calif.) equipped with a type 40 rotor and operated for 22 h at 8°C and 39 000 rev./min was used. The density ranges for the isolation of the lipoproteins were as follows: VLDL, $d<1.006$; LDL, $d$ 1.020–1.050; and HDL $d$ 1.063–1.210; each lipoprotein class was refloated at their respective densities. The isolated lipoproteins were dialyzed against 0.9% NaCl, 0.001 M EDTA, 0.05 M Tris–HCl, pH 7.6, and used immediately. Protein was determined by the method in [20].

Bovine liver PC-PLEP was purified as in [21]. PI-PLEP was isolated from bovine brain as in [22]. Both PC- and PI-PLEP were homogeneous by polyacrylamide gel electrophoresis. Radioactively-labeled microsomes were prepared from rat liver by intravenous injection of [Me-$^{14}$C]choline [21] or in vitro by incubation with [Me-$^{3}$H]inositol [15]. The isolated microsomes contained 530 nmol PC/mg protein and 56 nmol PI/mg protein with spec. act. 77 dpm/nmol and 4200 dpm/nmol, respectively.

 *Elsevier/North-Holland Biomedical Press*

Radioactively labeled microsomes were incubated in total 1 ml with lipoproteins in the absence and presence of purified exchange proteins in a shaking water bath at 37°C. The buffer was 0.05 M Tris–HCl (pH 7.6). For further details, see table legends. The reaction was started by the addition of the exchange proteins and terminated by immersion in an ice bath. After incubation the mixture was transferred quantitatively to a centrifuge tube (total 5 ml), the microsomes were sedimented at 150 000 × $g$ for 90 min (angle rotor no. 50, Spinco) and the supernatant solution containing the lipoproteins removed. Lipids were extracted from the various fractions as in [23] and assayed for radioactivity and phospholipid phosphorus by the method in [24]. Under the conditions of incubation, the microsomal and lipoprotein PC pool accessible to the exchange protein will have equilibrated completely at the end of incubation. Thus, the maximal transfer of [$^{14}$C]PC from microsomes to lipoproteins will equal $(Y/(X + Y))\,a$ cpm, where $X$ and $Y$ are the total microsomal and lipoprotein PC pools, respectively, and $a$ is the microsomal [$^{14}$C]PC radioactivity. We may conclude that the microsomal and lipoprotein PC pools are fully accessible to the exchange protein if the calculated maximal transfer of [$^{14}$C]PC compares with the determined values.

## 3. Results

[$^{14}$C]PC-labeled microsomes were incubated with increasing amounts of HDL and excess PC-PLEP to determine what percentage of the microsomal [$^{14}$C]-PC was available for transfer. As shown in table 1, ~90% of the [$^{14}$C]PC was transferred from microsomes to HDL. HDL phospholipid consists of 73% PC [25]. If we assume that the total microsomal and HDL PC pools are involved in the protein-mediated transfer, then the calculated extent of [$^{14}$C]PC transfer is very similar to the determined transfer. This indicates that all PC in the microsomes is accessible to PC-PLEP. This complete protein-mediated exchange is consistent with findings using labeled microsomes and phospholipid vesicles [18,19].

VLDL, LDL and HDL contain 77%, 70% and 73% PC, respectively [25]. These lipoproteins were incubated with [$^{14}$C]PC-labeled microsomes (0.330 μmol PC) and excess PC-PLEP to determine what percentage of lipoprotein PC is accessible to

Table 1
Transfer of [$^{14}$C]phosphatidylcholine (PC) from microsomes to plasma high density lipoproteins (HDL)[a]

| Before incubation | After incubation | | | |
|---|---|---|---|---|
| HDL (μmol phospholipid) | HDL[b] | | Transfer of [$^{14}$C]PC (cpm) | |
| | Phospholipid (μmol) | [$^{14}$C]PC (cpm) | Determined[c] | Calculated[d] |
| 0.0 | n.d. | 562 | – | – |
| 0.796 | 0.666 | 7369 | 8804 | 8473 (78%) |
| 1.592 | 1.322 | 8284 | 9981 | 9526 (88%) |
| 2.388 | 2.036 | 8423 | 9875 | 9938 (91%) |

[a] [$^{14}$C]PC-labeled microsomes (0.3 mg protein; 0.165 μmol PC; 10 879 cpm) were incubated with HDL (73% PC) and PC-PLEP (10 μg protein) in total vol. 1 ml for 45 min at 37°C. For further details see section 2

[b] Amount of HDL phospholipid and [$^{14}$C]PC recovered from supernatant fraction after sedimentation of microsomes

[c] The determined transfer of [$^{14}$C]PC is corrected for the control and for 100% recovery of HDL phospholipid

[d] For calculated transfer see section 2; the numbers in parentheses represent the maximal transfer in % (calculated transfer/10 879 × 100%)

Table 2
Transfer of [$^{14}$C]phosphatidylcholine (PC) from microsomes to plasma lipoproteins[a]

| Before incubation | | After incubation | | | |
|---|---|---|---|---|---|
| PC-PLEP (μg) | Lipoprotein (μmol phospholipid) | Lipoprotein | | Transfer of [$^{14}$C]PC (cpm) | |
| | | Phospholipid (μmol) | [$^{14}$C]PC (cpm) | Determined | Calculated |
| – | – | n.d. | 1232 | – | – |
| 10 | – | n.d. | 1440 | – | – |
| | VLDL | | | | |
| – | 0.280 | 0.280 | 3235 | 1795 | – |
| – | 0.560 | 0.526 | 3454 | 2145 | – |
| 10 | 0.280 | 0.274 | 9475 | 8207 | 8753 (39%) |
| 10 | 0.560 | 0.502 | 12 634 | 12 493 | 12 530 (56%) |
| | LDL | | | | |
| – | 0.343 | 0.318 | 2430 | 1068 | – |
| – | 0.686 | 0.614 | 3004 | 1747 | – |
| 10 | 0.343 | 0.338 | 9931 | 8620 | 9338 (42%) |
| 10 | 0.686 | 0.608 | 13 178 | 13 248 | 13 133 (59%) |
| | HDL | | | | |
| – | 0.318 | 0.338 | 3695 | 2121 | – |
| – | 0.637 | 0.562 | 3034 | 1807 | – |
| 10 | 0.318 | 0.334 | 10 949 | 9056 | 9133 (41%) |
| 10 | 0.637 | 0.556 | 13 716 | 14 062 | 12 941 (58%) |

[a] See legend to table 1. Incubation contained 0.61 mg of [$^{14}$C]PC-labeled microsomal protein (0.330 μmol of PC; 22 125 cpm)

the exchange protein. Table 2 shows that PC-PLEP gives rise to an extensive transfer of [$^{14}$C]PC to each type of lipoprotein. By comparison of the determined and calculated transfer of [$^{14}$C]PC, we conclude that all PC in VLDL, LDL and HDL is involved in the protein-mediated exchange. In contrast to the experiment described in table 1, the total lipoprotein PC-pools were in the same range as the microsomal PC pool. Under conditions where the microsomal and lipoprotein PC pool would be equal, complete equilibration of PC amounts to 50% transfer. Non-facilitated PC-transfer was also measured; exchange, however, was 5–10 fold enhanced by addition of PC-PLEP.

VLDL, LDL and HDL contain small but significant amounts of PI [25]. The interaction of PI with lipoprotein proteins is not known. To investigate whether the various lipoprotein classes can accept PI from rat liver microsomes, PI-PLEP was incubated with [$^{3}$H]-PI-microsomes and lipoproteins. As shown in table 3, PI-PLEP (1.3 μg proteins) transfers approximately 30% of the microsomal [$^{3}$H]PI in 20 min. Since PI-PLEP transfers both PI and PC [15,22] we cannot be certain whether the lipoproteins have become enriched in PI or whether there has been exchange of PI. These data indicate that there is very little restriction on the part of the lipoprotein proteins for the insertion of PI. Furthermore, the non-facilitated transport of [$^{3}$H]PI was 6–10-fold slower than that observed with PI-PLEP.

## 4. Discussion

As seen in table 1, with the addition of a large excess of HDL to microsomes there was complete transfer of [$^{14}$C]PC to the lipoproteins indicating that all of the PC in the microsome was available for protein-mediated transfer. With this information, it was then possible to determine to what extent lipoprotein PC was available for transfer. In these experiments (table 2), approximately an equivalent amount

Table 3
Transfer of [$^3$H]phosphatidylinositol (PI) from microsomes to plasma lipoproteins[a]

| Before incubation | | After incubation | |
|---|---|---|---|
| PI-PLEP (μg) | Lipoprotein (μmol phospholipid) | Lipoprotein | |
| | | [$^3$H]PI (cpm) | [$^3$H]PI transferred[b] (%) |
| | VLDL | | |
| – | 0.857 | 3679 | 6.3 |
| 1.3 | 0.857 | 21 579 | 36.7 |
| | LDL | | |
| – | 0.514 | 2325 | 4.0 |
| 1.3 | 0.514 | 18 739 | 31.8 |
| | HDL | | |
| – | 0.478 | 2027 | 3.4 |
| 1.3 | 0.478 | 18 093 | 30.7 |

[a] [$^3$H]PI-labeled microsomes (1.25 mg protein; 0.07 μmol PI; 58 846 cpm) were incubated with VLDL, LDL and HDL in total vol. 1 ml for 20 min at 37°C. For further details in section 2

[b] [$^3$H]PI transferred to lipoproteins in the supernatant fraction was corrected for 100% recovery of lipoprotein phospholipid

of lipoprotein phospholipids was added to [$^{14}$C]-PC-labeled microsomes. With the assay conditions, there was 10% non-protein facilitated transfer. In the presence of PC-PLEP, the transfer was 8–10-fold greater. The most significant finding in this study was that all of the PC in VLDL, LDL and HDL had equilibrated with the microsomal PC. This result indicates that PC-PLEP does not discriminate between fatty acid chain length or degree of saturation–unsaturation but only requires the phosphatidylcholine moiety [14,26]. In addition, these findings suggest that proteins and other lipids in the lipoprotein do not effect the accessibility of PC for the exchange proteins.

In the experiments of tables 1 and 2 we used an excess of PC-PLEP to assure complete equilibration of the PC pools. When less exchange protein was used, rates of PC-transfer between the various lipoprotein classes and microsomes were similar. This suggests that different lipid–protein ratios in the lipoproteins do not affect exposure of PC at the interface. While these results would argue against strong electrostatic interactions between the apoproteins and the polar head group of PC as suggested [27], caution must be taken since phospholipids in the lipoprotein are in a dynamic state.

The finding that all of the PC in lipoproteins is available for exchange and that PI can be also inserted into the lipoproteins (table 3) gives potential application for exchange proteins. By using synthetic phospholipid liposomes as the donor phospholipid and the exchange proteins, it is now possible to alter the phospholipid composition of lipoproteins in a controlled manner. This allows us to determine what effects changes in lipid composition have on lipoprotein catabolism, interaction with cells and fluidity. Exchange proteins may also prove useful in substituting specifically labeled phospho [$^{13}$C]lipids into lipoproteins under physiologic conditions.

## Acknowledgements

These studies were performed during the stay of R.L.J. as a visiting professor at the State University of Utrecht. The author wishes to thank Professor Gerard de Haas and Professor Laurens van Deenen for their hospitality and helpful discussions. This work was done under HL-22169 and a grant from the American Heart Association.

## References


[1] Bruckdorfer, K. R. and Graham, J. M. (1976) in: Biological Membranes (Chapman, D. and Wallach, D. F. H. eds) pp. 103–153, Academic Press, New York.

[2] Kunkel, H. G. and Bearn, A. G. (1954) Proc. Soc. Exptl. Biol. Med. 86, 887–891.

[3] Illingworth, D. R. and Portman, O. W. (1972) J. Lipid Res. 13, 220–227.

[4] Illingworth, D. R. and Portman, O. W. (1972) Biochim. Biophys. Acta 280, 281–289.

[5] Reed, C. F., Murphy, M. and Roberts, G. (1968) J. Clin. Invest. 47, 749–760.

[6] Wirtz, K. W. A. and Zilversmit, D. B. (1969) Biochim. Biophys. Acta 193, 105–116.

[7] Zilversmit, D. B. (1971) J. Lipid Res. 12, 36–42.

[8] Kook, A. I. and Rubinstein, D. (1970) Biochim. Biophys. Acta 202, 396–398.

[9] Peterson, J. A. and Rubin, H. (1969) Exp. Cell Res. 58, 365–378.

[10] Brewster, M. E., Ihm, J., Brainard, J. R. and Harmony, J. A. K. (1978) Biochim. Biophys. Acta 529, 147–159.

[11] Wirtz, K. W. A. (1974) Biochim. Biophys. Acta 344, 95–117.

[12] Zilversmit, D. B. and Hughes, M. E. (1976) in: Methods in Membrane Biology (Korn, E. D. ed) vol. 7, pp. 211–259, Plenum Press, New York.

[13] Kader, J. C. (1977) in: Dynamic Aspects of Cell Surface Organization (Poste, G. and Nicolson, G. L. eds) pp. 127–204, Elsevier/North-Holland, Amsterdam, New York.

[14] Kamp, H. H., Wirtz, K. W. A., Baer, P. R., Slotboom, A. J., Rosenthal, A. F., Paltauf, and Van Deenen, L. L. M. (1977) Biochemistry 16, 1310–1316.

[15] Helmkamp, G. M., Harvey, M. S., Wirtz, K. W. A. and Van Deenen, L. L. M. (1974) J. Biol. Chem. 249, 6382–6389.

[16] Bloj, B. and Zilversmit, D. B. (1977) J. Biol. Chem. 252, 1613–1619.

[17] Dyatlovitskaya, E. V., Timofeeva, N. G. and Bergelson, L. D. (1978) Eur. J. Biochem. 82, 463–471.

[18] Zilversmit, D. B. and Hughes, M. E. (1977) Biochim. Biophys. Acta 469, 99–110.

[19] Van den Besselaar, A. M. H. P., De Kruijff, B., Van den Bosch, J. and Van Deenen, L. L. M. (1978) Biochim. Biophys. Acta 510, 242–255.

[20] Schacterle, G. R. and Pollack, R. L. (1973) Anal. Biochem. 51, 654–655.

[21] Kamp, H. H. and Wirtz, K. W. A. (1974) Meth. Enzymol. 32, 140–146.

[22] Demel, R. A., Kalsbeek, R., Wirtz, K. W. A. and Van Deenen, L. L. M. (1977) Biochim. Biophys. Acta 466, 10–22.

[23] Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917.

[24] Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468.

[25] Skipski, V. P., in: Blood Lipids and Lipoproteins (Nelson, G. I. ed) pp. 471–583, Wiley Interscience, New York.

[26] Wirtz, K. W. A., Van Golde, L. M. G. and Van Deenen, L. L. M. (1970) Biochim. Biophys. Acta 218, 176–179.

[27] Segrest, J. P., Jackson, R. L., Morrisett, J. D. and Gotto, A. M. (1974) FEBS Lett. 38, 247–253.