Vol. 97, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 16, 1980 Pages 1091-1096

CONCERNING THE MECHANISM OF ACTION OF BOVINE LIVER PHOSPHOLIPID EXCHANGE PROTEIN: EXCHANGE OR NET TRANSFER

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Received October 27,1980

SUMMARY

Bovine liver phospholipid exchange protein catalyzes the transfer of phosphatidylcholine between donor and acceptor populations of single bilayer phospholipid vesicles. In comparing egg and dimyristoylphosphatidylcholine vesicles, larger transfer rates are found for the unsaturated phospholipid. The bidirectional transfer rates measured from donor to acceptor and from acceptor to donor, are equivalent, suggesting that the protein facilitates an exchange rather than a net transfer of phosphatidylcholine.

INTRODUCTION

Present in the cytosol of eukaryotic cells are proteins which catalyze the transfer of phospholipids between membranes. Several phospholipid exchange proteins have been extensively purified and characterized as to molecular properties, phospholipid specificity, and membrane interactions (1,2). Despite little knowledge about their biological function, phospholipid exchange proteins have nonetheless been used as investigative probes of surface topography and transbilayer mobility in natural and artificial membranes (3-5).

Several years ago, a mechanism was proposed for protein-catalyzed phospholipid transfer between membranes (6,7). The exchange protein functioned as a freely diffusable carrier of phospholipid, and upon interaction with a suitable membrane surface protein-bound and membrane-bound phospholipid would be exchanged. Supporting this mechanism of action for bovine liver phospholipid exchange protein were detailed kinetic analyses and substrate specificity studies (6,8). Implicit to the mechanism was the assumption that an

Abbreviations: LacCer, lactosyl-*N*-palmitoyl-DL-dihydroceramide; PtdCho, phosphatidylcholine; $Myr_2PtdCho$, dimyristoylphosphatidylcholine; PtdOH, phosphatidic acid.

0006-291X/80/231091-06$01.00/0

exchange of phospholipid molecules occurred rather than a *net transfer* to one of the participating membranes. In other words, the movement of phospholipid in one direction (donor to acceptor) should be offset by a movement of equivalent magnitude in the opposide (acceptor to donor). To test this assumption, transfers of PtdCho between two populations of single bilayer phospholipid vesicles in the presence of bovine liver phospholipid exchange protein have been investigated. A comparison of the protein's activity toward egg PtdCho and Myr_2PtdCho is also reported.

MATERIALS AND METHODS

The PtdCho-specific phospholipid exchange protein was purified from fresh bovine liver according to a published procedure (9). *Ricinus communis* agglutinin was a generous gift from Dr. L.L. Houston, Department of Biochemistry, University of Kansas, Lawrence, KS. Fatty acid-free bovine albumin and LacCer were products of Miles Laboratories.

The preparation of egg PtdCho, Myr_2PtdCho, and PtdOH (Na^+ salt) has been described (10). [^{3}H]Egg PtdCho was synthesized in the presence of rat liver microsomes from *Crotalus adamanteus* phospholipase A_2-hydrolyzed egg PtdCho and [9,10-^{3}H]oleic acid (New England Nuclear). [^{14}C]Myr_2PtdCho was prepared from *sn*-glycero-3-phosphocholine and the anhydride of [1-^{14}C]myristic acid (Research Products International). Cholesteryl [9,10-^{3}H]oleate was obtained synthetically (11); cholesteryl [1-^{14}C]oleate was purchased from New England Nuclear. All lipids were chromatographically pure and were stored in chloroform:methanol (2:1, by vol) under N_2 at -20°C.

Single bilayer vesicles were prepared by ultrasonic irradiation and quantitated as previously reported (10). Phospholipid transfer was measured between donor and acceptor populations of single bilayer vesicles in the presence and absence of bovine liver phospholipid exchange protein. Donor vesicles contained radiolabelled PtdCho; acceptor vesicles contained a trace amount (0.3 mol%) of radiolabelled cholesteryl oleate which permitted an estimation of vesicle recovery. Separation of the donor and acceptor vesicles was achieved by incorporating 8 mol% LacCer in either vesicle population and precipitating those vesicles upon addition of *Ricinus communis* agglutinin (0.6 mg ml^{-1}) to the reaction mixture at the end of the incubation period (12,13). Details are to be published elsewhere (14). The buffer used in these experiments was 10 mM HEPES, 50 mM NaCl, 1 mM Na_2 EDTA, 0.02% NaN_3 (pH 7.4). Reaction volume was 0.5 ml and included 200 nmol each of donor and acceptor vesicles, the indicated quantity of exchange protein, and 1 mg of fatty acid-free bovine albumin. Following centrifugation of the agglutinated mixtures at 15,600*g* for 3 min, aliquots of the supernate (LacCer-free vesicles) or the pellet (LacCer-rich vesicles) were analyzed for ^{3}H and ^{14}C radioactivity. In the latter case, pellets were redissolved in buffer containing Triton X-100 (1%, by wt) and 10 mM lactose. The reported transfer rates were corrected for those measured in the absence of phospholipid exchange protein.

RESULTS AND DISCUSSION

Phospholipid exchange protein-catalyzed movements of PtdCho between single bilayer vesicles exhibited a linear dependence on exchange protein (Fig. 1A) as well as on time (Fig. 1B). Such first-order kinetics were observed for transfers not exceeding 20-25% of the initial donor pool of PtdCho, with adjustments for the fact that only PtdCho molecules residing on the outer layer of the vesicle bilayer participate in exchange protein-catalyzed reactions (15).

When the concentration of acceptor vesicle was varied and the concentrations of donor vesicles and exchange protein held constant, the resulting rates of PtdCho transfer followed hyperbolic saturation kinetics. Data were obtained for acceptor vesicles prepared from egg PtdCho and Myr_2PtdCho and were expressed in double reciprocal plots to yield the kinetic parameters V_{max} and K_m (Fig. 2). In comparing the unsaturated egg PtdCho vesicles and the saturated Myr_2PtdCho vesicles at 37°C, there was no difference in the apparent K_m (0.35 mM) and a two-fold difference in V_{max} (105 nmol h^{-1} for egg PtdCho, 53 nmol h^{-1} for Myr_2PtdCho). Under these experimental conditions, both phospho-

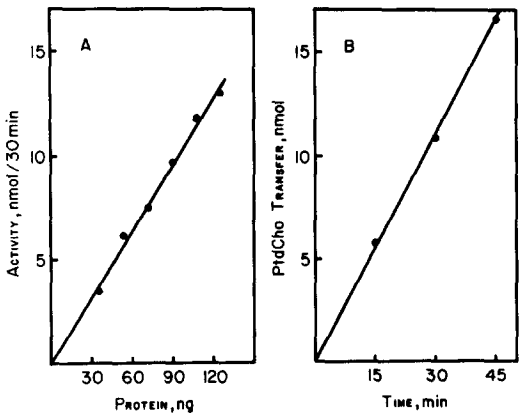

Figure 1. Variation of phospholipid transfer with bovine liver phospholipid exchange protein and time. Donor vesicles were egg PtdCho:PtdOH:LacCer (90:2:8, mol%), and acceptor vesicles were egg PtdCho:PtdOH (95:5, mol%). A. Incubations at 37°C for 30 min contained the indicated amount of exchange protein. B. Incubations at 37°C and the indicated time contained 0.10 µg of exchange protein.

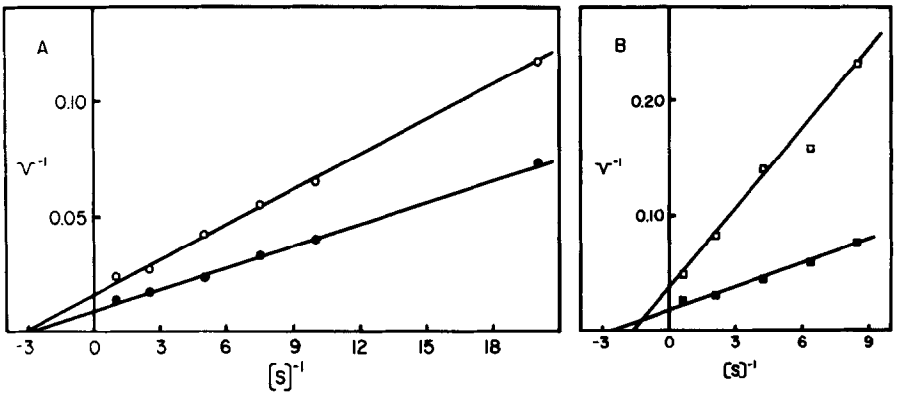

Figure 2. Phospholipid transfer activity as a function of acceptor vesicle concentration. PtdCho transfer was determined from egg PtdCho:PtdOH:LacCer (90:2:8, mol%) donor vesicles to various concentrations of different acceptor vesicles in the presence of 0.10 μg exchange protein. The means of duplicate incubations were expressed in a double reciprocal plot where [S] was total vesicle phospholipid concentration in mM and v was nmol PtdCho transferred per hour. A. Egg PtdCho:PtdOH (95:5, mol%) acceptor vesicles at 23°C (○) and 37°C (●). B. Myr_2PtdCho:PtdOH (95:5, mol%) acceptor vesicles at 23°C (□) and 37°C (■).

lipids existed in the liquid crystalline state. At 23°C the substantial difference in V_{max} persisted (61 nmol h^{-1} for egg PtdCho, 26 nmol h^{-1} for Myr_2PtdCho), while the apparent K_m increased dramatically from 0.33 mM for egg PtdCho to 0.61 mM for Myr_2PtdCho. A value of 1.4 mM has been reported for the apparent dissociation constant at 25°C of the complex formed between bovine liver exchange protein and egg PtdCho:PtdOH (95:5, mol%) vesicles (16). The egg PtdCho vesicles were still in the liquid crystalline state at 23°C, but Myr_2PtdCho vesicles had undergone a phase transition to the more highly ordered gel state. For small, single bilayer Myr_2PtdCho vesicles, this transition occurred over the temperature range 12 to 30°C, with a midpoint of 21°C (14, 17). The interaction between phospholipid exchange protein and PtdCho (vesicle or molecule) is, therefore, sensitive not only to the degree of unsaturation of the fatty acyl moieties, but also to the phase behavior of the bilayer structures. A common denominator of these kinetic differences may involve variations in membrane fluidity, similar to those observed for bovine brain phospholipid exchange protein (10).

Table I. Bidirectional Rates of PtdCho Transfer between Single Bilayer Vesicles at 37°C

Composition of Vesicle I[a]	Composition of Vesicle II[b]	Exchange Protein Assayed	PtdCho Transferred I → II	PtdCho Transferred II → I
		μg	*nmol/30 min*	
Egg PtdCho	Egg PtdCho	0.12	38.7 ± 1.4 (4)[c]	36.4 ± 3.7 (7)
Egg PtdCho	Myr_2 PtdCho	0.36	12.7 ± 1.5 (6)	13.1 ± 3.1 (6)
Myr_2 PtdCho	Myr_2 PtdCho	0.59	9.5 ± 2.1 (4)	5.6 ± 1.8 (9)

[a] Vesicles also contained 2 mol% Egg PtdOH and 8 mol% LacCer.

[b] Vesicles also contained 5 mol% Egg PtdOH.

[c] Mean ± S.D. (number of determinations).

For the majority of experimental systems, the initial rates of phospholipid transfer can be readily established for one direction only, e.g., vesicle I to vesicle II. Using two populations of vesicles and interchanging the roles of donor and acceptor, bidirectional rates of protein-catalyzed PtdCho transfer were determined (Table I). For mixtures of egg PtdCho vesicles, the rates in both directions were essentially identical. Likewise, for mixtures of egg PtdCho vesicles and Myr_2PtdCho vesicles, there was no significant difference between the two unidirectional rates. In this case, the transfer of egg PtdCho in one direction (I to II) is balanced by an equivalent transfer of Myr_2PtdCho in the opposite (II to I). Finally, for mixtures of Myr_2PtdCho vesicles, the bidirectional rates of PtdCho transfer were not widely dissimilar. Attempts were made to monitor PtdCho transfer in the heterologous egg PtdCho-Myr_2PtdCho vesicle system at 23°C; these did not succeed because of aggregation of LacCer-containing Myr_2PtdCho vesicles below the phospholipid phase transition temperature.

Under the present experimental conditions there is a preferential exchange of PtdCho molecules between membranes, but no net accumulation of

PtdCho by either membrane. Thus, it is clear that bovine liver phospholipid exchange protein functions as a true exchange catalyst. The likelihood of the protein's dissociating from a membrane surface without a protein-bound phospholipid molecule must be very remote. Nevertheless, as has been recently shown, that probability increases markedly when one of the participating membranes initially lacks PtdCho (18). In this regard, it will be interesting to elucidate further the chemical and physical requirements of membrane structure which dictate a mechanism of exchange or net transfer for phospholipid exchange proteins.

ACKNOWLEDGEMENTS

The author is grateful to A.M. Kasper and C.R. Badger for their expert technical assistance and to the National Institutes of Health for continued financial support (GM 24035).

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