Vol. 118, No. 3, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1984 Pages 743-746

INTERACTIONS IN THE HYDROGEN BELTS OF MEMBRANES: CHOLESTEROL LEAVING PHOSPHATIDYLCHOLINE BILAYERS

L.S. Ramsammy, V.P.S. Chauhan, L.L. Box and H. Brockerhoff

Institute for Basic Research in Developmental Disabilities
1050 Forest Hill Road, Staten Island, NY 10314

Received January 3, 1984

Cholesterol transfer from sonicated liposomes of phosphatidylcholine containing 10 or 30 mole percent cholesterol was measured with erythrocytes as acceptor. The activation energies of the (rate-limiting) bilayer-cholesterol dissociation were determined. In parallel experiments, phosphatidylcholine was replaced by an analog lacking the carbonyl oxygens, dietherphosphatidylcholine. The activation energies for dissociation of cholesterol from this phospholipid were three Cal/mole smaller than those for cholesterol-phosphatidylcholine dissociation, at both concentrations of cholesterol. These results demonstrate the involvement of the carbonyl oxygen in cholesterol-phospholipid bonding and support the hypothesis of lipid-lipid hydrogen bonding in the hydrogen belts of membranes.

We have postulated the existence of lipid-lipid hydrogen bonding in those regions of a bilayer that we like to call "hydrogen belts" (1,2) because they are neither hydrophobic nor polar but consist of hydrogen bond acceptors (the CO groups of phospho- and sphingolipids) and hydrogen bond donors (the OH of cholesterol, sphingolipids, water). Evidence for cholesterol-phospholipid hydrogen bonding has been obtained from activation energies of permeability (3) and NMR studies (4) with the use of phospholipid analogs in which the hydrogen bonding CO groups had been abolished (etherphospholipids). Here, we measure the activation energy of the desorption of cholesterol from bilayers of diester- and diether-phosphatidylcholine, with erythrocytes as acceptors. It has been shown that the transfer of cholesterol from liposomes to erythrocytes or other acceptors proceeds not by collision but by cholesterol traversing the aqueous phase, with the rate determined by the desorption step (5-9). Thus, the activation energies of transfer can be expected to mirror the forces with which cholesterol is anchored in each membrane.

0006-291X/84 $1.50
Copyright © 1984 by Academic Press, Inc.
All rights of reproduction in any form reserved.

MATERIALS AND METHODS

Phosphatidylcholine (PC), i.e., 1-hexadecanoyl-2-oleoyl-sn-glycerophosphocholine, and dietherphosphatidylcholine (PC*), i.e., 1-hexadecanyl-2-oleyl-sn-glycerophosphocholine, were synthesized (10); ^{14}C-cholesterol was obtained from New England Nuclear. For liposomes, either 10 or 30 mole% labelled cholesterol was added to the phospholipid, the solvent was removed and the lipid sonicated for 1 hr in buffer of 5 mM Tris-HCl, pH 7.4, 0.1 M KCl, 0.06 M NaCl, 10 mM dextrose, and 0.5% NaN_3 (11). The clear solution was centrifuged at 35,000 x g for 1 hr. The concentration of phospholipid was ca. 4 mg in all vesicle preparations. The 30 mole% cholesterol preparations of both PC and PC* were further characterized by Sepharose 4B chromatography. Both contained small amounts, similar in quantity, of larger vesicles and ca. 90% unilamellar vesicles of identical Stokes radius, 14.2 nm. Rat erythrocytes were washed and suspended (50:50 v:v) in the same buffer. For cholesterol transfer measurements, 1 ml each of liposomes and erythrocytes were mixed and incubated for 2 hr with shaking at various temperatures. After centrifugation at 1,600 x g for 10 min, the packed erythrocytes were washed two times, lipids were extracted with cold isopropanol, and radioactivity counted. Transfer was not faster than 5 percent of cholesterol in 2 hours, so that reverse transfer could be neglected. Linearity of transfer during the first four hours was established in separate experiments. It has been reported repeatedly that the rate-limiting step is cholesterol desorption (5-9). For verification, we varied the erythrocyte concentration from 2.5% to 25%; this had no influence on transfer rates.

RESULTS AND DISCUSSION

Energies of activation (E_a) for desorption of cholesterol from phosphatidylcholine bilayers have been measured repeatedly (6,7,9,10,12-16) with values from 9.0-20.0 Cal/mole reported. Poznansky and Czekanski (13) found that E_a was dependent on the concentration of cholesterol in the bilayer, varying from 20.0 Cal/mole at 10% to 9.5 Cal/mole at 40%. Our results, 19.6 Cal/mole at 10 mole% cholesterol (Fig 2) and 14.2 Cal/mole at 30 mole% cholesterol (Fig 1), confirm this dependence of E_a on cholesterol concentration and are completely compatible with the values reported by Poznansky and Czekanski. The reason for the difference in E_a is not clear, but it is known that a number of other parameters of phosphatidylcholine/cholesterol bilayers change at the 30 mole% cholesterol level (16-18). A change in the packing of the lipids is usually thought to be responsible. Whatever the cause, the concentration-dependent difference also appears in the PC*/cholesterol system (Fig 1 and Fig 2), and therefore is not dependent on the presence or absence of phospholipid CO groups.

The replacement of phosphatidylcholine by dietherphosphatidylcholine reduces, at both cholesterol concentrations, the E_a of cholesterol desorption by ca. 3 Cal/mole (see Fig 1 and Fig 2). Since the structures of the phospholipids

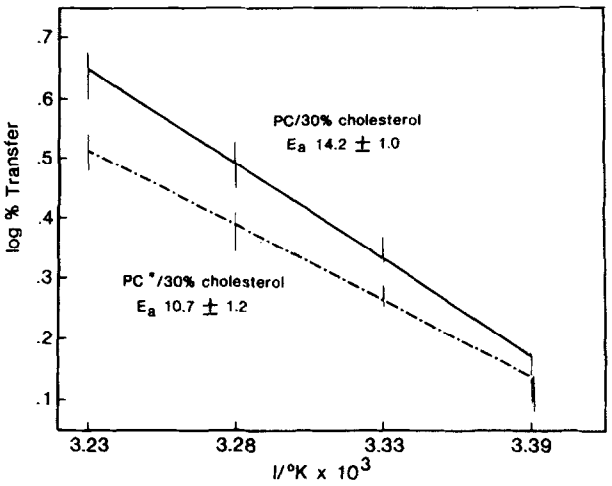

Fig 1. Arrhenius plot of transfer rate, over temperature range 22-37°C, for release of cholesterol from unilamellar liposomes containing 70% phospholipid and 30% cholesterol. —— PC/cholesterol vesicles; -·-·- PC*/cholesterol vesicles. E_a = Cal ± SEM.

are otherwise strictly identical, and the packing of the bilayers the same (as judged by vesicle radius), it must be the presence or absence of the CO groups that is responsible for the difference. If it is argued that PC* is possibly somewhat more hydrophobic, because of the conversion of the fatty acid C-1 esters to ether groups: such increased hydrophobicity (a CH_2 group adds ca.

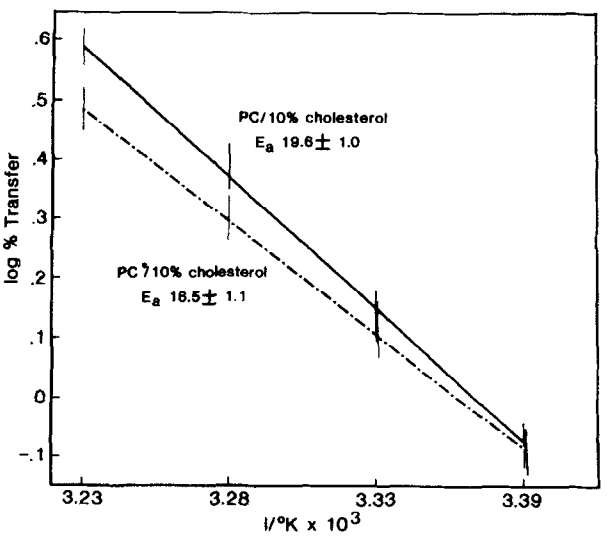

Fig 2. Arrhenius plot of transfer rate, over temperature range 22-37°C for release of cholesterol from unilamellar liposomes containing 90% phospholipid and 10% cholesterol. —— PC/cholesterol vesicles; -·-·- PC*/cholesterol vesicles. E_a = Cal ± SEM.

-0.7 Cal free energy to hydrophobic bonding) (19) should elevate rather than reduce E_a; thus, our argument is strengthened. No other conclusion can be drawn than that the CO group is directly responsible for 3 Cal/mole activation energy of cholesterol desorption. This value is in the range, 2-6 Cal/mole, expected for the free energy of a hydrogen bond. The result provides evidence for lipid-lipid hydrogen bonding, either directly or with mediation by water, in the hydrogen belt of the phosphatidylcholine/cholesterol bilayer.

ACKNOWLEDGEMENT

This work was supported by research grant GM21875 from the National Institute of General Medical Sciences, U.S. Public Health Service.

REFERENCES

1. Brockerhoff, H. (1974) Lipids 9, 645-650.
2. Brockerhoff, H. (1977) in: Bioorganic Chemistry (van Tamelan, E.E., ed.), Vol. III, pp. 1-20, Academic Press, New York.
3. Tirri, L.J., Ayengar, N.K.N., Lipton, L.C., Chatterjie, N. and Brockerhoff, H. (1978) Lipids 13, 267-269.
4. Ramsammy, L.S., Volwerk, H. and Brockerhoff, H. (1983) Chem. Phys. Lipids 32, 83-89.
5. Bacher, J.M. and Dawidowicz, E.A. (1981) Biochemistry 20, 3805-3810.
6. McLean, L.R. and Phillips, M.C. (1981) Biochemistry 20, 2893-2900.
7. Rothblat, G.H. and Phillips, M.C. (1982) J. Biol. Chem. 257, 4775-4782.
8. Lange, Y., Molinaro, A.L., Chauncey, T.R. and Steck, T.L. (1983) J. Biol. Chem. 258, 6920-6926.
9. Slotte, J.P. and Lundberg, B. (1983) Biochim. Biophys. Acta 750, 434-439.
10. Brockerhoff, H. and Ayengar, N.K.N. (1979) Lipids 14, 88-89.
11. Parkes, J.G., Watson, H.R., Joyce, A., Phadke, R.S. and Smith, I.C.P. (1982) Biochim. Biophys. Acta 691, 24-29.
12. Poznansky, M.J. and Lange, Y. (1978) Biochim. Biophys. Acta 506, 256-264.
13. Poznansky, M.J. and Czekanski, S. (1979) Biochem. J. 177, 989-991.
14. Jonas, A. and Maine, G.T. (1979) Biochemistry 18, 1722-1728.
15. Philips, M.C., Stoudt, G.W. and Rothblat, G.H. (1980) Atherosclerosis 36, 409-422.
16. DeKruyff, B., Van Dijck, P.W.M., Damel, R.A., Schuijff, A., Brants, F. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 356, 1-7.
17. Mabrey, S., Mateo, P.L. and Sturtevant, J.M. (1978) Biochemistry 17, 2464-2468.
18. Estep, T.N., Mountcastle, D.B., Barenholz, Y., Biltonen, R.L. and Thompson, T.E. (1979) Biochemistry 18, 2112-2117.
19. Tanford, C. (1962) J. Amer. Chem. Soc. 84, 4240-4247.