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Cholesterol Exchange as a Function of Cholesterol/Phospholipid Mole Ratios

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The activation energy (E_a) for cholesterol exchange between dioleoyl phosphatidylcholine vesicles and erythrocyte 'ghosts' is measured as a function of molar percentage of cholesterol in both donor and acceptor membranes. A sharp increase in E_a occurs (from 39.9 kJ/mol to 84 kJ/mol) when the molar percentage of cholesterol decreases from 30 to 20%.

Despite extensive studies of cholesterol–phospholipid interactions and mechanisms of cholesterol exchange between membranes (for reviews, see Bruckdorfer & Graham, 1976; Demel & de Kruijff, 1976), the nature of these processes in biological tissues remains largely unresolved. Whereas n.m.r. and differential-scanning-calorimetry studies (Ladbrooke *et al.*, 1968; Oldfield & Chapman, 1972; de Kruijff, 1978) have contributed much to our understanding of the effects of cholesterol on the motion and phase states of phospholipid membranes, biological membranes remain far more complex. Cholesterol/phospholipid mole ratios vary from values approaching 1 for plasma membranes to values closer to 0.3 for membranes of intracellular organelles. From studies on model membrane systems (Hinz & Sturtevant, 1972; de Kruijff *et al.*, 1974; van Dijck *et al.*, 1976), it is clear that cholesterol–phospholipid interactions change markedly over this range.

Much attention has been focused on the nature of the packing of cholesterol with various phospholipids. Based on data from a variety of physical techniques, it has been concluded (Rothman & Engelman, 1972; Engelman & Rothman, 1972) that ideal packing occurred at a mole ratio of cholesterol/phospholipid of 0.5 or 33 mol% of cholesterol based on packing of approx. 4 fatty acyl chains/cholesterol molecule. This was based on X-ray diffraction, n.m.r. and differential-scanning-calorimetry data, which indicated that the liquid-crystal-to-gel-phase transition is not present at this value for cholesterol content. Recently, high-sensitivity differential-scanning-calorimetry studies (Mabrey *et al.*, 1978) of mixtures of cholesterol and phospholipid have suggested that the phase behaviour of phospholipid/cholesterol mixtures is more complex. Below 20 mol%, cholesterol/phospholipid mixtures give heat-capacity curves that are resolved into at least two components, one broad and one narrow, suggesting the coexistence of at least two immiscible phases. Between 20 and 50 mol%, only the broad peak is discernible. It appears therefore that the value of 20 mol% of cholesterol is of special significance. Above this value, the broad transition diminishes as more cholesterol is added and the ability of the phospholipid to undergo co-operative phase behaviour decreases. These data are supported by e.s.r. data (Shimshick & McConnell, 1973) and freeze-fracture data (Kleemann & McConnell, 1976), where 20 mol% of cholesterol was postulated to be the upper limit for the coexistence of two distinct phases. Our data, based on the ability of cholesterol to exchange between membranes, support the view that at 20 mol% of cholesterol a distinct change in the nature of the cholesterol–phospholipid interaction occurs.

Although the molecular events associated with cholesterol exchange are not understood, several properties of the exchange processes have been described. The exchange process is non-mediated, but requires that the membranes come into direct contact (Poznansky & Lange, 1978). Net movement of cholesterol can occur from membranes of high cholesterol/phosphilipid mole ratios to those with lower ratios, and the rates of exchange and activation energies are dependent on the fatty acid composition of at least the donor membrane in the exchange process. In the present study, we investigated the exchange process as a function of cholesterol/phospholipid mole ratios under equilibrium conditions, in the absence of any net cholesterol movement where donor and acceptor membranes have similar cholesterol/phospholipid mole ratios.

Materials and Methods

[^{3}H]Cholesterol and [^{14}C]cholesteryl oleate were purchased from New England Nuclear Corp., Boston, MA, U.S.A. Cholesterol was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. and dioleoyl phosphatidylcholine was prepared in our laboratory by the technique of Robles & van den Bergh (1969).

Lipid vesicles were prepared by the technique of Huang (1969). Briefly, 8 mg of phospholipid and

various amounts of cholesterol (0–4mg) were co-freeze-dried from benzene in the presence of trace amounts of [^{3}H]cholesterol and [^{14}C]cholesteryl oleate, and then 10ml of KCl/Tris buffer (pH7.4) was added to disperse the lipid. The dispersion was sonicated for 1h under N_2 at 4°C by using a Branson W185 sonicator with a standard titanium probe and a power output of 65W. The sonicate was centrifuged for 30min at 35000*g* to remove titanium particles coming off the probe. As previously indicated (Poznansky & Lange, 1978), sonicated material could be used without further sizing, since results obtained from exchange reactions with erythrocyte 'ghosts' were virtually identical whether or not sizing was carried out. When the vesicles were sized by gel chromatography on Sepharose 4B, over 90% of the lipid in the sonicated material eluted with the 'fraction II' homogeneous vesicles (Huang, 1969).

Human erythrocyte 'ghosts' were prepared by the technique of Dodge *et al.* (1963) with no attempt at resealing. Cholesterol-depleted 'ghosts' were made by incubating 'ghosts' with a 60-fold excess of phosphatidylcholine vesicles for 24h at 37°C in the presence of cholesterol-depleted plasma (Murphy, 1962) and 1μM-streptomycin sulphate. After co-incubation, 'ghosts' and vesicles were easily separated by centrifugation at 12000*g* for 5min. After three washings of the 'ghosts' in a KCl/Tris buffer, no detectable radioactivity from the ^{14}C-non-exchangeable marker ([^{14}C]cholesteryl oleate) could be detected in the 'ghost' fraction. Lipids were extracted by chloroform/methanol from both the vesicle and 'ghost' preparations. Lipid P content was determined by standard procedures (Gomori, 1942) and cholesterol was measured by digitonin precipitation (Parekh & Jung, 1970) or by enzymic determination with cholesterol oxidase (Calbiochem, Palo Alto, CA, U.S.A.).

Exchange of [^{3}H]cholesterol between lipid vesicles and erythrocyte 'ghosts'

After measurement of cholesterol/phospholipid molar ratios in cholesterol-depleted 'ghosts', lipid vesicles were prepared as described to contain identical cholesterol/phospholipid mole ratios. This matching of cholesterol/phospholipid mole ratios was made to ensure that cholesterol exchange was occurring in the absence of any net cholesterol movement (Poznansky & Lange, 1978). Vesicles (100μl containing 0.106μmol of phospholipid and various amounts of cholesterol, 0.5μCi of [^{3}H]-cholesterol and 0.1μCi of [^{14}C]cholesteryl oleate in KCl/Tris buffer, pH7.4) were incubated with 5ml of erythrocyte 'ghosts' (containing 5.6μmol of phospholipid and various amounts of cholesterol) at 37°C in a shaking water bath. At various time intervals, 0.3ml portions were removed and centrifuged for 10min at 12000*g* in an Eppendorf 3200 centrifuge. Supernatant (0.1ml) was counted for [^{3}H]cholesterol and [^{14}C]cholesteryl oleate radioactivity in Bray's solution. The pellet was washed three times in buffer and dispersed and then radioactivity was determined. Only trace amounts of non-exchangeable cholesteryl oleate were ever detected in the 'ghost' pellet, and the disappearance of [^{3}H]cholesterol from the supernatant closely paralleled its appearance in the 'ghost' fraction. Cholesterol exchange was followed for up to 4h as a function of temperature (from 20 to 40°C).

Results and Discussion

In the present paper we describe the energetics of the exchange of cholesterol from phospholipid vesicles to an excess of human erythrocyte 'ghosts' at various cholesterol/phospholipid mole ratios. Details of the calculation of E_a, the energy of activation for the cholesterol-exchange process, have been previously described (Poznansky & Lange, 1978). The initial exchange rate (% of cholesterol exchanged in the first hour) was measured as a function of temperature and the apparent E_a was calculated (Fig. 1). Between 30 and 50mol% of cholesterol, the cholesterol concentration found in normal and moderately depleted erythrocytes, the E_a for the exchange process is consistently between 37.8 and 42kJ/mol (Fig. 2). Between 10 and 20mol% of cholesterol, the E_a for exchange between dioleoyl phosphatidylcholine vesicles and erythrocytes is close to 84kJ/mol. We were not able to deplete erythrocyte 'ghosts' below 10mol% of cholesterol. Between 20 and 33mol% of cholesterol, there was a marked decrease in E_a from 84 to 39.9kJ/mol. Under all these conditions, no net movement either to or from the vesicle population was observed. We have also examined the energetics of the exchange of cholesterol from unilamellar vesicles to multilamellar vesicles as a function of cholesterol/phospholipid mole ratios. Although these two populations are easily separated by centrifugation, total cholesterol and phospholipid in the supernatant (unilamellar vesicles) fraction occasionally increased, supposedly due to break-up of the multilamellar vesicles into smaller particles. Nevertheless, exchange of cholesterol from unilamellar dioleoyl phosphatidylcholine vesicles to multilamellar dioleoyl phosphatidylcholine liposomes exhibited virtually identical apparent E_a values (42kJ/mol at 50mol% of cholesterol and 81.9kJ/mol at 10mol% of cholesterol).

These results verify earlier n.m.r., e.s.r. and calorimetric studies describing unique changes in phospholipid–cholesterol interactions occurring between 20 and 25mol% of cholesterol. Our data agree more closely with the recent high-sensitivity-scanning-calorimetry studies of Mabrey *et al.* (1978).

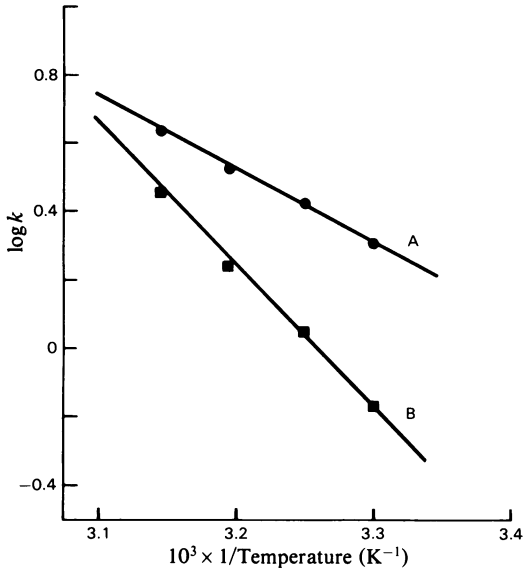

Fig. 1. *Arrhenius plots for cholesterol exchange between dioleoyl phosphatidylcholine vesicles and human erythrocyte 'ghosts' at molar percentages of cholesterol of* 48% *(A) and* 18% *(B)*

Donor vesicles and acceptor 'ghosts' are matched (by cholesterol depletion of 'ghosts') to have identical cholesterol/phospholipid mole ratios. No net movement of cholesterol occurs in these experiments. The logarithm of the exchange rate is plotted as a function of the inverse of the temperature and the activation energy derived from a form of the Arrhenius equation: $\log k_2/k_1 = E/2.303\,R\,(T_2 - T_1)/(T_1\,T_2)$.

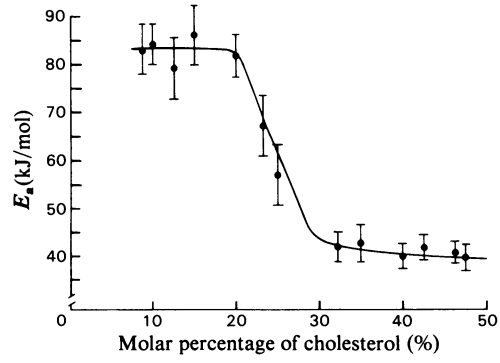

Fig. 2. *E_a values for [^3H]cholesterol exchange between dioleoyl phosphatidylcholine vesicles and a* 50*-fold excess of human erythrocyte 'ghosts' as a function of the mole percentage of cholesterol*

The cholesterol/phospholipid mole ratio is constant in both donor and acceptor membrane. Erythrocyte 'ghosts' were cholesterol-depleted by incubation with cholesterol-free vesicles and cholesterol-free lipoproteins. The error bars represent standard deviations for a minimum of three experiments.

One can speculate on the physiological consequences of these large differences in activation energies for the exchange process depending on the cholesterol/phospholipid mole ratio. The higher cholesterol content of plasma membranes may allow for more ready movement of cholesterol beween cells and between cells and plasma lipoproteins, perhaps bearing on cholesterol distribution and accumulation.

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