Study of Exchange Kinetics Between Lipid Vesicles Using Stopped Flow Technique

BENOY B BHOWMIK*, DIPANKAR ROY† & PAPIYA NANDY
Department of Chemistry & Department of Physics, Jadavpur University, Calcutta 700032
Received 14 September 1983; accepted 30 September 1983

The exchange rate, \( k_{ex} \), of an excimer-forming fluorescent probe, pyrenedecanoic acid, between monolayer vesicles of the lipid dipalmitoyllecithin has been measured using stopped flow technique. It is observed that \( k_{ex} \) in the case of monolayer vesicles is about one order of magnitude lower than the value for bilayer vesicles. Further, the lipid exchange between these vesicles is a much slower process compared to the label exchange.

It has been established by several authors that exchange of molecules between bilayer vesicles is a much faster process compared to the actual fusion \(^1\) - \(^4\).

Here we have studied the molecular exchange between monolayer vesicles of dipalmitoyllecithin using stopped flow technique. The excimer-forming fluorescent probe pyrenedecanoic acid has been used as the reporter molecule.

The synthetic lipids, dipalmitoyllecithin and distearoyllecithin were obtained from Fluka, West Germany. Pyrenedecanoic acid was obtained from Molecular Probes, USA.

Monolayer vesicles were prepared by sonication of 80 ml of oxygen-free aqueous (2 mM CsCl) lipid dispersion (40 mg of dipalmitoyllecithin) and 0.2 ml of the organic substance under nitrogen, with the lipid polar head groups facing the aqueous phase \(^5\). The organic substance used here was a mixture of \( n \)-hexane (10 ml) and CCl\(_4\) (5.76 ml). The mixture was optically inert, non-polar in nature, had a density of the order of 1 g/cc and hence distributed in the total volume of the aqueous solution. For probed vesicles, chloroformic solutions of label and lipid were mixed and a thin film was made prior to addition of aqueous solution. The exchange kinetics was studied by stopped flow technique, using a mixing chamber with a round cell of inner diameter 3 mm. The cell was filled with probed vesicles before each new measurement.

Excited dimers were formed in the probed vesicles due to the collision of short living excited monomers and monomers in the ground state. The excimer fluorescent band of intensity \( I' \) at \( \lambda = 485 \) nm and monomer emission peak of intensity \( I_0 \) at \( \lambda = 397 \) nm were well separated \(^6\). The ratio \( I'/I \) was proportional to the concentration of the optical probe per unit area of the lipid matrix \(^7\). This property was utilised to study the exchange kinetics. With the help of a fluorescence spectrometer (Perkin-Elmer spectrofluorimeter, MPF 44B) the values of \( I_0 \) and \( I_0 \) were noted. Then equal amounts of probed and unprobed vesicles were allowed to flow through two tubes and were mixed rapidly by a sudden brief push of the piston in the tube joining the two tubes. Immediately after the simultaneous influx of the probed and unprobed vesicles, \( I' \) increased and \( I \) decreased abruptly, following the exchange of label molecules between the vesicles. The rate of exchange was obtained by recording the change in \( I' \) and \( I \) as a function of time after the rapid mixing of the vesicles. The decrease in the ratio \( I'/I \) gave a direct measure of the extent of exchange. When there was complete randomization of the label molecules, \( I'/I = (I_0/I_0)/2 \). The temperature of the vesicles was kept constant by passing water at a fixed temperature. The temperature dependence of \( k_{ex} \) is shown in Fig. 1a. It is one order of magnitude lower than the value obtained for the bilayer vesicles \(^1\) (Fig. 1b).

In order to find the role of phospholipid exchange, i.e. fusion over the label exchange, a second set of

---

\(^*\)Present address: PTC Hostel, Okhla Industrial Estate, New Delhi 110020.

Fig. 1—Exchange rate \( k_{ex} \) of pyrenedecanoic acid between vesicles of dipalmitoyllecithin, measured as a function of temperature using stopped flow technique. (a) monolayer vesicles, (b) bilayer vesicles \(^1\).
Demonstration of phospholipid exchange between monolayer vesicles using pyrenedecanoic acid as fluorescent probe (the arrow indicates the phase transition temperature of the lipids dipalmitoyllecithin ($T_{tr} = 41 \, ^\circ C$) and distearoyllecithin ($T_{tr} = 51 \, ^\circ C$). Curves (a)-(d) represent time-dependence of transition curves after preserving the mixture of equal volumes of two lipid dispersions at 65 $^\circ C$. The preservation time after mixing is indicated next to each curve. Curve (e) represents transition curve recorded 45 hr after mixing and preserving the mixture at room temperature.

Experiments were performed. Here we took a mixture of equal amounts of monolayer vesicle dispersion of two different lipids, dipalmitoyllecithin and distearoyllecithin with phase transition temperatures $T_{tr}$ = 41.5 and 51.2 $^\circ C$, respectively, both probed with pyrenedecanoic acid. One part of the mixture (A) was preserved at 65 $^\circ C$, a temperature much higher than $T_{tr}$ of both the lipids and the other part (B) was kept at room temperature. Temperature profile of $I'/I$ of the mixtures (A) and (B) were taken separately at different time intervals. Initially, the curves defined the phase transition temperatures, characteristic of both the lipids distinctly and well separated from each other. For mixture (A), the two-phase transition points slowly proceeded to merge to show one-phase transition after fusion of the two types of lipids (Fig. 2, a-d). Noticeable fusion occurred after 10 hr. The lipid randomization was complete after 45 hr (Fig. 2d) and compared well with the transition curve obtained by cosonication of the two lipid vesicles. For the mixture (B), which was kept at room temperature, there was almost no lipid transfer even after 44 hr (Fig. 2e).

The result clearly shows that molecular exchange between different lipid lamellae is not necessarily accompanied by a fusion process. The very slow exchange rate of phospholipids is in complete accord with results obtained by others. A comparison of this result with that reported earlier shows that $k_{ex}$ in the case of monolayer vesicles is about one order of magnitude lower than the value for bilayer vesicles (Fig. 1).

In the living world, the rapid exchange of lipophilic molecules, e.g. hormones, fatty acids between the membranes of subcellular systems like mitochondria, lysosomes, golgi apparatus etc. may provide an important pathway for intercellular communication upon encounters of these systems. Simultaneously, the observed strong attachment of the phospholipids to their membranes helps to maintain the characteristic lipid composition upon these encounters.

We are thankful to Prof E Sackman of University of Ulm, Ulm, West Germany, in whose laboratory a part of this work was undertaken.

References
3 Thilo L. Biochim Biophys Acta, 469 (1977) 326.