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KINETICS OF PHOSPHOLIPID EXCHANGE BETWEEN BILAYER MEMBRANES

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Summary

In an accompanying publication by Duckwitz-Peterlein, Eilenberger and Overath ((1977) Biochim. Biophys. Acta 469, 311—325) it is shown that the exchange of lipid molecules between negatively charged vesicles consisting of total phospholipid extracts from *Escherichia coli* occurs by the transfer of single lipid monomers or small micelles through the water. Here a kinetic interpretation is presented in terms of a rate constant, k_{-}, for the escape of lipid molecules from the vesicle bilayer into the water. The evaluated rate constants are $k_{-}^{P} = (0.86 \pm 0.05) \cdot 10^{-5}\ s^{-1}$ and $k_{-}^{E} = (1.09 \pm 0.13) \cdot 10^{-6}\ s^{-1}$ for phospholipid molecules with *trans*-Δ^9-hexadecenoate and *trans*-Δ^9-octadecenoate, respectively, as the predominant acyl chain component. The rate constants are discussed in terms of the acyl chain and polar head group composition of the lipids.

Introduction

As is reported in an accompanying communication, Duckwitz-Peterlein et al. [1] used bilayer vesicles of total phospholipid extracts from *Escherichia coli* to study the mechanism of lipid exchange. The exchange was measured by following the time course of inter-vesicle lipid mixing. It was concluded that the exchange of phospholipids between the negatively charged vesicles in water takes place via the monomeric and/or micellar state. As is shown in this communication, the results of Duckwitz-Peterlein et al. [1] allow a quantitative evaluation of the lipid exchange rate in terms of rate constants for the escape of lipid molecules from the bilayer.

Abbreviations: P- and E-lipids, phospholipid hydrocarbon chains containing *trans*-Δ^9-hexadecenoate and *trans*-Δ^9-octadecenoate, respectively.

Kinetics of molecular lipid exchange

The transition of a lipid molecule between the bilayer state, B, and the monomeric state, M, can be written as

$$M \underset{k_-}{\overset{k_+}{\rightleftharpoons}} B \tag{1}$$

In analogy to a proposal made by Nakagawa [2] for the transition of surfactant molecules between the micellar and monomeric states, it is assumed that the rate at which monomers are captured by the bilayer is proportional to the product of the monomer concentration, $[M]$, and the total bilayer surface area in a unit volume. The latter is proportional to the concentration of lipid molecules in the bilayer state, $[B]$. The rate at which monomers escape from the bilayer is proportional to $[B]$. Then

$$\frac{d[B]}{dt} = k_+[M][B] - k_-[B] \tag{2}$$

To account for surface effects in a non-explicit way Nakagawa [2] introduced an empirical parameter, q', in the first term of Eqn. 2 as $k_+[M][B]^{q'}$. The value of $q' \leqslant 1$ depends on the surfactant considered [2—5]. Since the value of q' is not known for the present case of phospholipid molecules, as an approximation its value is taken as one. This simplification seems to be legitimate in view of the fact that the vesicle surface has a low curvature (average diameter, $\bar{d} = 950$ Å [1]).

The transition of lipid molecules between the bilayer and the monomeric states is considered the mechanism of molecular lipid exchange in the present case. Lipid exchange takes place between two vesicle populations, I and II, which are composed of type P and type E lipids (palmitelaidic and elaidic acid-derived phospholipids, respectively, see ref. 1). The number of lipid molecules of each type in the two vesicle populations in a unit volume of the vesicle mixture is denoted by $[P]_I$, $[E]_I$, $[P]_{II}$ and $[E]_{II}$. The two populations are characterized by their initial lipid composition when population I consists of vesicles containing only P-lipids, population II of vesicles containing only E-lipids. Therefore, at time $t = 0$: $[P]_I = [P]$, $[E]_I = 0$, $[P]_{II} = 0$ and $[E]_{II} = [E]$ where $[P]$ and $[E]$ denote the total concentration of P- and E-lipids in the system, respectively. The concentration of P- and E-lipids which are present as monomers, $[M_P]$ and $[M_E]$, respectively, can be neglected because at all times $[M_P] << [P]$ and $[M_E] << [E]$ by approx. five orders of magnitude for the present lipid system (see Discussion). The ratio, r, is defined as

$$r = \frac{[E]}{[E] + [P]} = \frac{[E]_I + [E]_{II}}{[E]_I + [E]_{II} + [P]_I + [P]_{II}} \tag{3}$$

The consideration which leads to Eqn. 2 can now be applied to the mixture of the two vesicle populations in order to express the rate of change of $[P]_I$, $[E]_I$, $[P]_{II}$ and $[E]_{II}$ due to molecular lipid exchange. One can write

$$\frac{d[P]_I}{dt} = k_+^P[M_P]([P]_I + [E]_I) - k_-^P[P]_I \tag{4}$$

$$\frac{d[E]_I}{dt} = k_+^E[M_E]([P]_I + [E]_I) - k_-^E[E]_I \tag{5}$$

$$\frac{d[P]_{II}}{dt} = k_+^P[M_P]([P]_{II} + [E]_{II}) - k_-^P[P]_{II} \tag{6}$$

$$\frac{d[E]_{II}}{dt} = k_+^E[M_E]([P]_{II} + [E]_{II}) - k_-^E[E]_{II} \tag{7}$$

As is indicated by the indexes P and E, the rate constants, k_+^P, k_-^P and k_+^E, k_-^E, are characteristic for the P- and E-lipid molecules, respectively. As an approximation it is assumed that these lipid specific rate constants are independent of the lipid composition of the bilayer by which the lipid molecules are captured or from which they escape. This assumption implies that the interior of the bilayer represents a non-specified hydrophobic environment (see analogous case for micelle interior, p. 38 of ref. 6). The rate constants may contain a "geometry" factor to account for the fact that the inner lipid layer is not directly in contact with the monomer reservoir in the inter-vesicle space (see Discussion). When the P- and E-vesicles are mixed, steady-state monomer concentrations $[M_P]$ and $[M_E]$ will be established. Therefore,

$$\frac{d[P]_I}{dt} + \frac{d[P]_{II}}{dt} = 0 \tag{8}$$

and

$$\frac{d[E]_I}{dt} + \frac{d[E]_{II}}{dt} = 0 \tag{9}$$

Using Eqns. 3—9 one obtains

$$\frac{k_+^P}{k_-^P} = \frac{1-r}{[M_P]} \tag{10}$$

and

$$\frac{k_+^E}{k_-^E} = \frac{r}{[M_E]} \tag{11}$$

The ratios, $k_+^{P,E}/k_-^{P,E}$ in Eqns. 10 and 11 are constants determined by the properties of the specific lipid molecules. They represent the equilibrium constants, $K^{P,E}$ of Reaction 1 for P- and E-lipids, respectively. Eqns. 10 and 11 therefore imply that the monomer concentrations, $[M_P]$ and $[M_E]$ reach steady-state values which depend on r, i.e. on the relative abundance of the other exchange partner. These steady-state monomer concentrations will be retained once they have been established since the rate constants have been assumed to be independent of the vesicle composition.

Evaluation of rate constants, k_-^P and k_-^E

In order to determine the rate constants, k_-^P and k_-^E, from the experimentally

observed time course of the lipid phase transition temperatures, T_{t_I} and $T_{t_{II}}$ [1] this time course must be related to that of $[P]_I$, $[E]_I$, $[P]_{II}$ and $[E]_{II}$ as expressed in Eqns. 4—7. The transition temperatures depend on the lipid composition which can be expressed as the mol fractions, x_I and x_{II}, of E-lipids in vesicle populations I and II, respectively, viz.

$$x_I = \frac{[E]_I}{[P]_I + [E]_I} \tag{12}$$

and

$$x_{II} = \frac{[E]_{II}}{[P]_{II} + [E]_{II}} \tag{13}$$

The transition temperatures are then given by

$$T_{t_I} = x_I(T_{t_E} - T_{t_P}) + T_{t_P} \tag{14}$$

and

$$T_{t_{II}} = x_{II}(T_{t_E} - T_{t_P}) + T_{t_P} \tag{15}$$

where T_{t_P} and T_{t_E} denote the transition temperatures of vesicles consisting of only P- or E-lipids, respectively. The time course of x_I and therefore of T_{t_I} is given by the time derivative of Eqn. 12,

$$\frac{dx_I}{dt} = \frac{1}{([P]_I + [E]_I)} \cdot \left[(1 - x_I)\frac{d[E]_I}{dt} - x_I \frac{d[P]_I}{dt}\right] \tag{16}$$

By inserting Eqns. 4 and 5 into Eqn. 16 and using Eqns. 10 and 11 one obtains

$$\frac{dx_I}{dt} = Ax_I^2 + 2Bx_I + C \tag{17}$$

where the constants are given by

$$A = k_-^E - k_-^P \tag{18}$$

$$B = \tfrac{1}{2}[rk_-^P - (1 + r)k_-^E] \tag{19}$$

$$C = rk_-^E \tag{20}$$

An identical differential equation as Eqn. 17 is obtained for x_{II} by taking the time derivative of Eqn. 13 and inserting from Eqns. 6, 7, 10 and 11.

The differential equation can be solved for x_I and x_{II} for the boundary conditions, $x_I = 0$ and $x_{II} = 1$ at $t = 0$. With x_I and x_{II} inserted into Eqns. 14 and 15, respectively, one obtains

$$T_{t_I} = \left[\frac{1 - e^{-\lambda t}}{\frac{1}{r} - \left(1 - \frac{k_-^P}{k_-^E}\right)e^{-\lambda t}}\right](T_{t_E} - T_{t_P}) + T_{t_P} \tag{21}$$

TABLE I

ACYL CHAIN COMPOSITION OF P- AND E-LIPIDS

Data from Duckwitz-Peterlein et al. [1]. Abbreviations used are: $C_{12:0}$, dodecanoic acid; $C_{14:0}$, tetradecanoic acid; *trans*-Δ^9-$C_{16:1}$, *trans*-Δ^9-hexadecenoic acid; $C_{16:0}$, hexadecanoic acid; *trans*-Δ^9-$C_{18:1}$, *trans*-Δ^9-octadecenoic acid. Results are expressed in mol %.

	$C_{12:0}$	$C_{14:0}$	*trans*-Δ^9-$C_{16:1}$	$C_{16:0}$	*trans*-Δ^9-$C_{18:1}$	Mean molecular weight
P-lipids [a]	—	4.3	82.8	12.8	—	760
E-lipids	1.6	14.0	2.6	13.5	68.3	809

[a] Lipids were 82 mol % phosphatidylethanolamine, 11 mol % cardiolipin and 7 mol % phosphatidylglycerol.

and

$$T_{t_{II}} = \left[\frac{(1-r)k^{E}_{-}\, e^{-\lambda t} + rk^{P}_{-}}{(1-r)(k^{E}_{-} - k^{P}_{-})\, e^{-\lambda t} + k^{P}_{-}}\right](T_{t_E} - T_{t_P}) + T_{t_P} \tag{22}$$

where

$$\lambda = rk^{P}_{-} + (1-r)k^{E}_{-} \tag{23}$$

In experiments of Duckwitz-Peterlein et al. [1] values of T_{t_I} and $T_{t_{II}}$ were measured pairwise after various incubation times, t. These values of T_{t_I} and $T_{t_{II}}$ with the corresponding value for t can be inserted into Eqns. 21 and 22. This yields two equations from which k^{P}_{-} and k^{E}_{-} can be evaluated. For each pair of T_{t_I} and $T_{t_{II}}$ a value of k^{P}_{-} and k^{E}_{-} is obtained. Average values for k^{P}_{-} and k^{E}_{-} are given in Table II for experiments using different values of r. These average values can be used in Eqns. 21 and 22 to calculate the time course of T_{t_I} and $T_{t_{II}}$ as is shown in Fig. 1 for the three applied values of r. The asymmetric behavior due to the difference of the two rate constants, k^{P}_{-} and k^{E}_{-}, becomes evident.

TABLE II

RATE CONSTANTS k^{P}_{-} AND k^{E}_{-}

$r = \frac{[E]}{[E]+[P]}$	$k^{P}_{-} \times 10^5$ s^{-1}	$k^{E}_{-} \times 10^6$ s^{-1}	$\frac{k^{P}_{-}}{k^{E}_{-}}$
1/3 [a]	0.59 ± 0.11	1.16 ± 0.49	5.1 ± 2.4
1/2 [a]	0.86 ± 0.14	1.68 ± 0.60	5.1 ± 2.0
2/3 [a]	1.02 ± 0.44	0.42 ± 0.23	24 ± 17
Weighted mean [b]	0.86 ± 0.05	1.09 ± 0.13	8 ± 2

[a] Errors are mean standard deviations ($n \approx 10$).
[b] Errors are standard deviations of the mean.

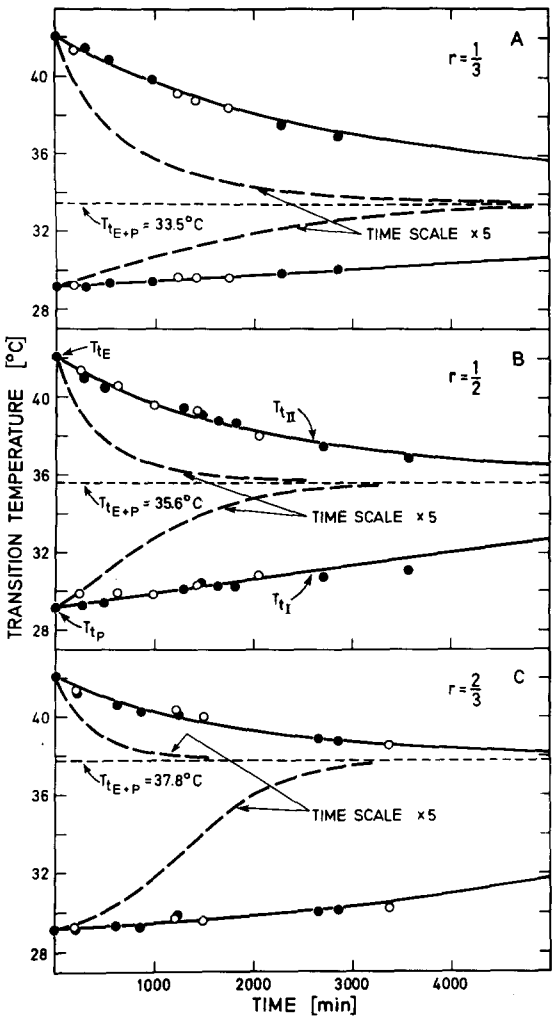

Fig. 1. The time course of lipid mixing was followed by measuring changes in the lipid phase transition temperatures due to lipid exchange between two vesicle populations, I and II. The experimental points are those of Fig. 6 from Duckwitz-Peterlein et al. [1] and were used pairwise to calculate values for the rate constants, $k^{\underline{P}}$ and $k^{\underline{E}}$. Averages of these values for experiments with different lipid ratios, r, as indicated in the figure are summarized in Table II. The average values were then used in Eqns. 21 and 22 to calculate the time course of T_{t_I} and $T_{t_{II}}$ as indicated by the curves in the figure. The broken lines differ from the corresponding solid lines in that the time scale is contracted by a factor of 5 to show the extrapolations to longer exchange periods.

Discussion

Eqns. 4—7 do not explicitly account for the fact that the lipid molecules are arranged as a bilayer. The constants, k_+ in these equations, contain a factor of about 0.5 which takes account for the fact that the inner layer does not contribute to the surface area through which the lipid exchange takes place. With regard to the terms of Eqns. 4—7 which contain the rate constants k_-, no additional factor is necessary as long as the mixing of lipid molecules between the

outer and inner layer is fast compared to the inter-vesicle lipid exchange. Then, molecules in both layers can be considered as being able to escape through the outer layer into the inter-vesicle space. As is discussed by Duckwitz-Peterlein et al. [1] their results require that the equilibrium between the two layers (flip-flop) is fast compared to the inter-vesicle exchange. However, it is possible to interpret the rate constants, k_-, as containing a factor of between 0.5 and 1 to take account for the bilayer arrangement. The values for k_-^P and k_-^E could then be larger than the values given in Table II by a factor of up to two.

Similar to the experiments of Duckwitz-Peterlein et al. [1] the exchange of lipid molecules was measured by Martin and MacDonald [7] in a mixture of dimyristoyl phosphatidylcholine (DMPC) and dipalmitoyl phosphatidylcholine (DPPC) vesicles. Within the sensitivity of their experiments, they found a completely unidirectional exchange, i.e. no dipalmitoyl phosphatidylcholine molecules seemed to take part in the exchange. By using their time-dependent values of T_t in Eqn. 22 with $k_-^{DPPC} = 0$ for k_-^E and k_-^{DMPC} for k_-^P, one finds with $r = 1/2$ that $k_-^{DMPC} = (5.5 \pm 0.8) \cdot 10^{-5}\ s^{-1}$ (error: mean standard deviation, $n = 10$).

In the absence of other estimates for k_-^P, k_-^E and k_-^{DMPC}, their relative values can be compared with theoretical predictions based on the difference in acyl chain and polar head group composition of these three phospholipids. The change in free energy, ΔG, upon transfer of a lipid molecule from the monomeric state to the bilayer is a linear function of the effective acyl chain length in terms of the number of methyl groups, n_c, viz.

$$\Delta G = \text{constant} - mn_c \tag{24}$$

The slope as given by m, can be determined by measuring the change in critical micelle concentration for lipids with different values of n_c (see ref. 6 for detailed examples). The value of m was found to be about 700 cal/mol for zwitterionic molecules and 400 cal/mol for negatively charged molecules in water without salt (ref. 6, Chapter 7). Therefore, for the polar head group composition of the lipids used by Duckwitz-Peterlein et al. [1] a suitable average value for m would be $0.82 \times 700 + 0.\ 18 \times 400 = 646$ cal/mol (vesicles in water; see footnote a to Table I). As is discussed by Tanford [6] on the basis of experimental results, n_c for molecules with two acyl chains is given by the sum of the CH_2 groups of the longer chain and about 60% of the CH_2 groups of the shorter chain. For example, in the case of dimyristoyl phosphatidylcholine as used by Martin and MacDonald [7], $n_c = 13 + 0.6 \times 13 = 20.8$. For the P- and E-lipids of Table I n_c was calculated by taking $C_{16:1}$ and $C_{18:1}$ chains in position 2 of the P- and E-lipids, respectively, as the longer chain and taking the remaining $C_{16:1}$ and $C_{18:1}$ chains together with the saturated chains in position 1 as the shorter chain. The effective value of n_c so obtained is 26.0 and 23.9 for the E- and P-lipids, respectively. The difference in ΔG for the E- and P-lipids is then 2.1×646 cal/mol which at 318°K implies a ratio $[M_P]/[M_E] = 8.6$. This ratio has about the same value as the ratio $k_-^P/k_-^E = 8 \pm 2$ in Table II. For the dipalmitoyl phosphatidylcholine-dimyristoyl phosphatidylcholine system one obtains a difference in ΔG of 3.2×700 cal/mol, i.e. $[M_{DMPC}]/[M_{DPPC}] = 35$. This large difference between the monomer concentrations of the dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine molecules explains the experimental observation of an apparently unidirectional lipid

exchange. A similar calculation shows that $[M_{DMPC}]/[M_P] = 4$ as compared to the experimentally observed ratio of the rate constants, $k_-^{DMPC}/k_-^P = 5.5 \cdot 10^{-5}/0.86 \cdot 10^{-5} = 6.4$. As can be seen from Eqns. 10 and 11 with $r = 0$ and $r = 1$ for the pure P- and E-lipids, respectively, the equilibrium monomer concentration is given by the ratio k_-/k_+. If then, as shown above, $k_-^P/k_-^E \approx [M_P]/[M_E]$, it follows that $k_+^P \approx k_+^E$. Also, $k_+^{DMPC} \approx k_+^P$ within a factor of two. The rate constant, k_+, is therefore independent of the type of lipid molecule considered and the monomer concentration is governed by a rate-limiting step involving a rate constant, k_-, which depends on the properties of the lipid molecule under given conditions.

The equilibrium relationship $k_-/k_+ = [M]$ allows the evaluation of k_+ if k_- and $[M]$ is known. The value of $m = 646$ cal/mol can be used to make an estimate of the monomer concentration of the P- and E-lipids. By using Eqn. 24 with the "constant" term as 2514 cal/mol (see ref. 6, Eqns. 7—9, for *N*-alkyl betaines), $m = 646$ cal/mol as above, ΔG can be calculated with $n_c = 26.0$ and $n_c = 23.9$ for the E- and P-lipids, respectively. Since $\Delta G = RT \ln X$, the mol fractions, X^E and X^P of E- and P-lipids in water at 318°K are $X^E = 1.4 \cdot 10^{-10}$ and $X^P = 1.2 \cdot 10^{-9}$. The monomer concentration is then $7.7 \cdot 10^{-9}$ and $6.6 \cdot 10^{-8}$ mol/l for E- and P-lipids, respectively. Using the values of k_- as in Table II, one finds that $k_+ \approx 10^2$ l · mol^{-1} · s^{-1}. This value is much smaller than the values between 10^7 and 10^8 l · mol^{-1} · s^{-1} for diffusion-controlled reactions between ionic particles [3—5,8]. For comparison, Folger et al. [3] found a value of $k_+ = 2 \cdot 10^6$ l · mol^{-1} · s^{-1} for the insertion of tenside molecules into charged micelles. It must be concluded that the reaction step involving k_+ is not diffusion controlled but that the lipid molecules upon encounter with the bilayer surface experience some hindrance with regard to the integration into the bilayer. In the case of micelles from ionic surfactants, a decrease of k_+ due to tighter packing with increasing micelle size is diccused by Hoffmann et al. [12].

The experimental evidence in the foregoing paper [1] cannot exclude the possibility that lipid exchange takes place by the transfer of micelles which are small compared to the average vesicle size. If such a micelle consists of *n* lipid molecules, the rate constant for the separation of a micelle from the bilayer (fission) would be $k'_- = k_-/n$. The dependence of k'_- on the acyl chain and polar head group composition of the lipids will be quantitatively different from that discussed for k_- above.

The values of k_- as obtained for the three different values of *r* as shown in Table II, seem to increase (k_-^P) or decrease (k_-^E) with increasing *r*. As far as this tendency is significant in view of the average standard deviations occurring with fixed *r*, it must be ascribed to effects outside the scope of the approximations made in the present treatment. Such effects could be: (i) insufficiently fast bilayer equilibration (flip-flop), (ii) partial lipid transfer by micelles, (iii) dependence of k_- and k_+ on vesicle composition and (iv) on vesicle size, (v) $q' < 1$, see ref. 2, and (vi) inhomogeneity of the polar head group composition of the lipids.

A rate constant, $k_- \approx 10^{-5}$ s^{-1} means that lipid mixing via the monomeric state occurs with a half-life in the order of approx. 1 day. It therefore is a slow process when compared to intra-membrane lipid mixing by means of lateral

diffusion over distances in the order of μm within seconds, as described by a constant of diffusion, $D \approx 10^{-8}$ cm^2/s [9—11]. The observed rate of lipid transfer can be compared with protein-mediated lipid transfer rates. Van den Besselaar et al. [13] measured the rate of phosphatidylcholine transfer between vesicles of this lipid in the presence of the phosphatidylcholine exchange protein from beef liver. Dissociation of a lipid-carrying protein from the membrane surface. The protein-coupled escape of egg yolk phosphatidylcholine in branesurface. The protein-coupled escape of egg yolk phosphatidylcholine in this case, therefore occurs 10^5—10^6 times faster than the protein-free escape of *trans*-Δ^9-$C_{16:1}$-derived lipids of *E. coli*. Although lipid exchange via the monomeric state seems to be a comparatively slow process it cannot be neglected in the case of biological structures with a doubling time in the order of days as far as such structures need to have different lipid compositions in spatially separated domains.

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