

Maintenance of membrane phospholipid asymmetry

Lipid-cytoskeletal interactions or lipid pump?

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Two models for the mechanism of maintenance of lipid asymmetry in erythrocytes are considered: binding of internal lipids to cytoskeletal proteins, and pumping of internal lipids from the outside to the inside of the cell. Analysis of the kinetics of lipid internalization suggests that the first model is more likely, and that the apparent pumping of lipids represents the activity of an ATP-dependent lipid flip/flop catalyst.

Phospholipid asymmetry; Lipid flip/flop; Lipid pump; Spectrin; Cellular adherence; (Erythrocyte, Blood cell)

1. INTRODUCTION

The phospholipid species in the plasma membranes of erythrocytes are not uniformly distributed between the two sides of the membrane. Phosphatidylserine (PS), and to a lesser extent phosphatidylethanolamine (PE), are concentrated in the inner leaflet of the bilayer, while sphingomyelin and phosphatidylcholine (PC) are preferentially localized in the external leaflet [1,2]. This differential distribution is not a result of lipid-lipid interactions, since purified lipids do not assume this distribution spontaneously [3]. Neither is it maintained as a kinetically trapped state, as is the case for membrane proteins, since the half-times of lipid flip/flop (see below) are considerably shorter than the average lifespan of the erythrocyte itself (120 days in humans). An explanation for the maintenance of phospholipid asymmetry must therefore lie in some mechanism supplied by the cell.

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Two alternatives have been proposed for this mechanism. The older view derives from observations from several laboratories that disruption or loss of the cytomembrane network of spectrin, actin, and associated proteins is uniformly correlated with a loss of phospholipid asymmetry [4–11]. A simple interpretation of these observations is that the equilibrium distribution of PS, and to a lesser extent PE, is shifted by binding of the lipids, either directly to spectrin, or to a protein or proteins associated with spectrin. An important, and sometimes unappreciated, correlate of this model is that lipid distribution would in this view be insensitive to the rate of lipid flip/flop between the two leaflets; this rate would only affect the rate at which the lipid distribution would relax to any new distribution as a result of changes in the interaction between lipids and cytoskeleton.

Recently, an entirely different mechanism has been proposed, based on the remarkable observations of Devaux and colleagues, who introduced spin-labelled phospholipids into the external leaflet of the erythrocyte membrane [12,13]; these probes were transferred to the interior of the cell by an

ATP-dependent mechanism at a rate which was dependent on lipid headgroup, with PS > PE > PC [12]. Based on these findings, it was suggested that the erythrocyte membrane contains a headgroup-selective, ATP-dependent phospholipid pump which moves PS, and at a slower rate, PE to the internal leaflet; passive backdiffusion opposes this inward movement. In contrast, PC transfers only by passive diffusion, at similar rates in both directions. In this view, the difference in rate between active and passive movement establishes the observed transbilayer distribution of phospholipids. Additional evidence for selective lipid transfer has been provided by Daleke and Huestis [14], who monitored rates of lipid internalization by assessing changes in erythrocyte morphology.

Herrman and Muller [15] have pointed out that these kinetic data can be analyzed using a simple model involving a rate of internalization (k_i) and a rate of externalization (k_o). We present here a somewhat more general version of this formalism. When applied to the kinetic data of Devaux and colleagues, the results suggest that the lipid 'pump', rather than operating unidirectionally, is actually a bidirectional ATP-dependent 'flip/flop' enzyme (flippase). Such an activity could not maintain lipid asymmetry; however, it could play an essential role in the expression of shifts in equilibrium distribution controlled by some other headgroup-specific mechanism such as internal binding.

2. MODELS

The distribution of lipids between the outer and inner leaflets of the membrane may be described by the general model:



where N_o is the number of lipid molecules in the outer leaflet; N_{if} and N_{ib} are the number free and bound lipids in the inner leaflet; $K_b = N_{ib}/N_{if}$; k_i and k_o are the rate constants for internalization and externalization of phospholipids.

If the rates of the binding reactions are fast compared to the rate of flip/flop, then the kinetics for

internalization of a probe molecule introduced into the outer leaflet are described by:

$$I(t) = Q*[1 - \exp(- (k_i/Q)*t)] \quad (2)$$

where $I(t)$ is the internal fraction of the probe as a function of time (t), and

$$Q = [k_i*(1 + K_b)]/[k_o + k_i*(1 + K_b)] \quad (3)$$

2.1. Pump model

In the pump model of Devaux and colleagues the asymmetric distribution of phospholipids is maintained by the differential affinity of the pump for different species of lipids. The binding constant K_b is 0 and eqn 3 reduces to the expression given by Herrman and Mueller:

$$Q = k_i/(k_i + k_o) \quad (4)$$

2.2. Flippase/cytoskeletal binding model

In this model, the asymmetric distribution of phospholipids is maintained by differential binding of different species of lipids to internal cytoskeletal components. In this view, the inward and outward rate constants are equal and can be considered as a rate constant k_f for the flippase, in which case eqn 3 reduces to

$$Q = k_f/(k_f + [k_f/(1 + K_b)]) \quad (5)$$

The form of the fitting equation for these two views is thus identical. The difference between them is that the numerator in the pump model, k_i , is simply the inward pumping rate, while in the flippase model, it is the bidirectional flipping rate, k_f . Similarly, in the denominator, k_o in the pump model represents passive diffusion, while the homologous constant in the flip/flop model represents the flipping rate constant k_f reduced by the sequestration of internal lipid. For purposes of neutrality, these constants will be referred to as k_x and k_y where model-specific interpretations are not being discussed, so that

$$Q = k_x/(k_x + k_y) \quad (6)$$

3. METHODS

Data were fitted by nonlinear regression to the expression [15]:

$$I(t) = Q*[1 - \exp(-k_x*t/Q)] \quad (7)$$

Fits were carried out using the RS1 data analysis package by Bolt, Beranek and Newman, running on a Digital Equipment Corporation Vax 11/780. The statistical significance of the fits was <0.001, and the standard errors were <10%, unless otherwise indicated. Analysis of the error as a function of Q and k_x indicated the absence of multiple local minima.

4. RESULTS

The fits of eqn 7 to the data of Seignuret and Devaux [12] for uptake of PS, PE, and PC derivatives into erythrocytes are shown in fig.1. Because there was no approach to a plateau in the experimental data for PC, a fitted value of Q could not be obtained; instead, a Q value of 0.4 was taken from measurements of the rate of internalization of lyso-PC by Bergmann et al. [16] (see also Herrman and Mueller [15]). The values of k_x from these fits, and the values of k_y calculated from the fits using eqn 6, are presented in table 1. The k_y values for PS and PE are higher than for PC, suggesting that movement of these lipids from the internal to the external surface may be occurring by a process other than simple diffusion.

A critical and distinguishing feature of the two models is their prediction of the dependence of k_y on ATP concentration. In the pump model, k_y is simply k_o , the rate of passive outward diffusion, and should therefore be ATP-independent. In the flippase/binding model, k_y is the flipping rate constant k_f divided by $(1 + K_b)$ and should therefore display the same dependence on the concentration of ATP as k_i . In table 2 are shown k_y values obtained from rates of PS internalization measured at various ATP concentrations (fig.5 of [12]). As seen, k_y does change as a function of ATP concentration, contrary to the predictions of the pump model. Moreover, when the values obtained for k_x (k_i or k_f) and k_y are plotted as a function of ATP concentration (fig.2), it is clear that the dependence of k_y on ATP concentration resembles remarkably that observed for k_x , suggesting that their ATP sensitivity derives from a common source. These results provide strong evidence that the lipid pump whose activity was monitored by Devaux and colleagues is in fact a catalyst of both

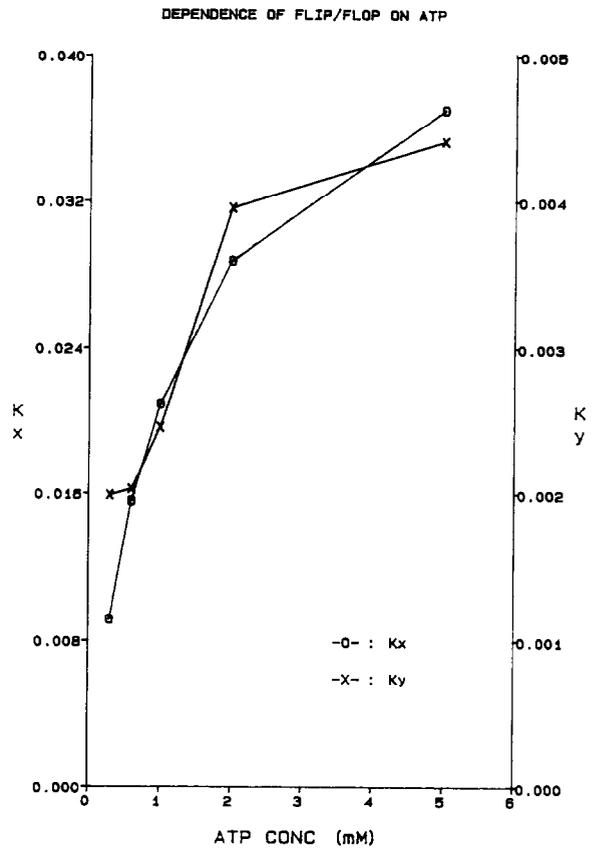


Fig.1. Inward and outward translocation rates of PS as a function of ATP concentration. Calculated values for k_x , the inward apparent translocation rate, and k_y , the outward apparent translocation rates, are plotted as a function of ATP concentration. The data are taken from columns 3 and 4 of table 2. (×—×) Inward rate; (○—○) outward rate.

Table 1

Transverse diffusion of PS, PE and PC in erythrocytes

Lipid	Q (× 10)	k_x (min ⁻¹ × 1000)	k_y (min ⁻¹ × 1000)
PS	9.10	19.2	1.83
PE	6.55	3.80	2.00
PC	4.00	0.242	0.36

The transverse diffusion rates of spin labelled phospholipid analogues in intact erythrocytes were calculated from fits to probe internalization data taken from fig.2 of [12]. Curves were fitted to the equation $I(t) = Q*(1 - \exp[-(k_x*t)/Q])$ as described in section 3

Table 2

Dependence of transverse diffusion rates of PS on the ATP concentration in resealed erythrocyte ghosts

ATP conc.	Q ($\times 10$)	k_x ($\text{min}^{-1} \times 1000$)	k_y ($\text{min}^{-1} \times 1000$)
0.3	8.21	9.14	1.99
0.6	8.85	15.6	2.03
1.0	8.95	21.0	2.46
2.0	8.79	28.8	3.95
5.0	8.94	37.0	4.41

Transverse diffusion rates were calculated from fits to data taken from fig.5 of [12] for internalization of spin labelled PS analogue in erythrocyte ghosts resealed in the presence of ATP

internally and externally directed lipid movement analogous to the PC-specific activity discovered by Bell and co-workers in the endoplasmic reticulum [17].

4.1. Sick cell anemia

This analysis was extended to erythrocytes from patients with sickle cell anemia where apparently abnormal lipid movements are observed [18]. As seen in table 3, both k_x and k_y for PS are altered in sickle cells (SS or SS/ISC) with a 30–50% reduction in k_x and a 150–300% increase in k_y when compared with normal (AA), sickle trait (AS), or mixed sickle/hemoglobin C (SC) patients. The apparent changes in k_y are entirely consistent with a hypothesis of weaker internal binding of PS and PE, causing a corresponding rise in k_y . The fall in k_x would not be easily explained, however, had Devaux and colleagues not carried out similar experiments with cells from an otherwise normal patient with high levels of reticulocytes, similar to those seen in sickle cell samples. Although the data themselves were not presented, fits to an equation of the same form as that used here were carried out, and the results of those fits given [18]. When those data are adjusted to the units used here (table 3), it is clear that the reduction in k_x observed for sickle cells is a consequence of elevated reticulocyte levels, and that k_y is actually increased 6–7-fold when compared to normal cells at the same stage of development. These results suggest that the rate of lipid movement is normal in sickle cells, and that the only changes observed are best explained

Table 3

Transverse diffusion rates of phosphatidylserine in various normal and sickle cells

Geno type	Q ($\times 10$)	k_x ($\text{min}^{-1} \times 1000$)	k_y ($\text{min}^{-1} \times 1000$)
AA	8.83	9.07	1.20
SC	8.69	8.97	1.35
AS	8.42	7.36	1.38
SS	5.70	4.44	3.36
SS/ISC	5.32	4.52	3.98
RETICS (AA)	8.80	5.00	0.68

Transverse diffusion rates were calculated from fits to data taken from figs 1 and 2 of [18]. Values for PS diffusion in normal (AA) reticulocytes were taken from table 2 of the same paper

as results of changes in binding of membrane phospholipids to internal components [19].

5. IMPLICATIONS

When leukocytes are activated, changes in plasma membrane lipid organization occur that are consistent with a transbilayer redistribution of phospholipids ([20,21], review [22]). These activation-dependent changes take place within minutes for monocytes, neutrophils and platelets and several hours for lymphocytes, suggesting that a flippase may be necessary to account for the rate of observed lipid rearrangements in a variety of blood cells. Indeed, the discovery that lymphocytes have a plasma membrane lipid transport activity [23] in addition to a spectrin-based membrane cytoskeletal system [22] suggests that the mechanisms for regulating lipid organization are similar in leukocytes and erythrocytes.

Disruption of phospholipid asymmetry in erythrocytes increases their binding to cells of the reticuloendothelial system [24–27]. Interestingly, the activation events just described in leukocytes are accompanied by a similar increase in adherence [22]. These results suggest that the activity of the flippase acting in concert with changes in lipid-cytoskeletal interactions may play a critical role in controlling cellular interactions associated with leukocyte activation.

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