ATP-dependent redistribution of phosphatidylethanolamine in the plasma membrane of an epithelial and a hepatocytic cell line

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ATP-dependent redistribution of phosphatidylethanolamine in the plasma membrane of an epithelial and a hepatocytic cell line

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Abstract. The redistribution of spin-labeled phospholipid analogues of sphingomyelin (SM) and phosphatidylethanolamine (PE) from the outer to the inner leaflet of the plasma membrane of an epithelial (CaCo-2) and a hepatocytic cell line (HepG2) was investigated. The amount of analogues in the outer leaflet was determined by their back-exchange to bovine serum albumin (BSA). For both cell lines a fast ATP-dependent inward movement of spin-labeled PE (SL-PE) was found while SL-SM redistributed only slowly by a passive mechanism. After depletion of intracellular ATP transverse diffusion of SL-PE was similar to that of SL-SM. The data are compatible with the presence of an aminophospholipid translocase in both cell lines.

Key words. CaCo-2 - HepG2 - plasma membrane - aminophospholipid translocase - flip-flop

Introduction

A specific, ATP-dependent inward translocation of aminophospholipids as PE in the plasma membrane of human erythrocytes [1] and several other mammalian cells [2] has been found. The movement of aminophospholipids from the exoplasmic to the cytoplasmic leaflet is fast with respect to passive transverse diffusion ('flip-flop') typically found for choline-containing lipids as SM and PC [3]. The lipid specific movement has been ascribed to the protein aminophospholipid translocase [2]. Phospholipids as PC and SM are not recognized by this protein.

It is not known whether an aminophospholipid translocase activity exists in plasma membranes of epithelial cells as epithelial and hepatocytic cells. Those membranes are morphologically and biochemically divided into specific regions, the basolateral and apical domains. Therefore, in order to elucidate on a quantitative level whether an ATP-dependent transport of aminophospholipids exists in polarized cells, we have measured in a first approach the redistribution of spin-labeled analogues of PE and SM in suspensions of an epithelial cell line (CaCo-2) and a hepatocytic-like cell strain (HepG2).

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Material and Methods.

Monolayer cultures of CaCo-2 (PD7) and HepG2 cells were grown to confluence at 37°C in DMEM medium supplemented with 10% (vol/vol) fetal calf serum, 2mM glutamine, 1mM nonessential amino acids, antibiotics/antimycotics and 10 mM HEPES (CaCo-2) or 1.5 µg/ml insulin (HepG2). For suspending cells, monolayers were rinsed with Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution, harvested by treatment with 0.05% trypsin and 0.02% EDTA in phosphate buffered saline (PBS) for 10 min at 37°C, resuspended in culture medium dispersed by pipetting and filtered to remove cell aggregates. After 30 min on ice and two washing steps cells were resuspended in Dulbecco-PBS with 20 mM glucose, 1 mM sodium pyruvate and 10 mM HEPES to prevent a decline of cellular ATP. ATP depletion was performed by incubating cells for 10 min at 37°C in Dulbecco-PBS containing 5 mM sodium azide and 50 mM 2-deoxyglucose instead of glucose and sodium pyruvate. ATP was determined by the luciferin-luciferase assay.

The outer leaflet of plasma membranes was labeled with spin-labels corresponding to about 0.5 mol% of the total cell phospholipids. Transverse redistribution of both PE and SM was measured by the back-exchange of analogues on the outer half to BSA [3-5] and subsequent measurement of the spin intensity in the presence of 10 mM $K_3Fe(CN)_6$ with an Electron Spin Resonance spectrometer as described [3-5].

Results

The spin-labeled analogues SL-PE and SL-SM with the NO-moiety on the short ß-chain form micelles in aqueous solutions. In the presence of CaCo-2 or HepG2 cells both analogues incorporated rapidly into the outer leaflet of the

plasma membrane as deduced from the ESR spectrum of the label (not shown). Within about 30 sec all probes were inserted into the membrane (T≥10°C). Immediately after cell labeling more than 95% of analogues could be recovered by BSA showing that the label was exclusively localized in the external leaflet. Analogue redistribution was measured at lower temperatures in order to prevent substantial uptake of analogues by endocytosis and to suppress label hydrolysis. While for HepG2 cells 20°C was sufficient for accurate measurements, in case of CaCo-2 cells label internalization was measured at 10°C with respect to the high degree of label hydrolysis.

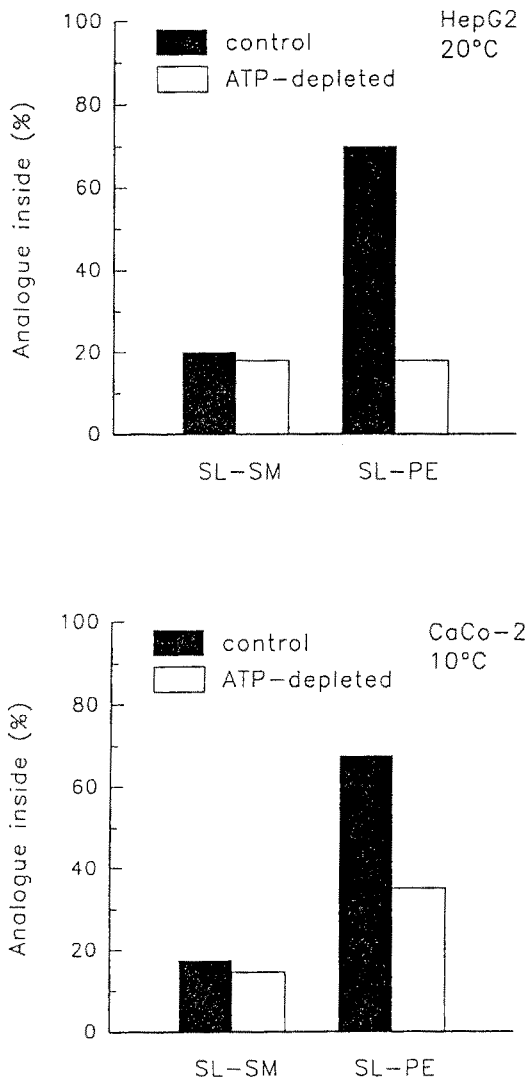

Fig. 1 Redistribution of spin-labeled PE and SM across the plasma membrane of HepG2 (top) and CaCo-2 cells (bottom) at 20°C and 10°C, respectively. The amount of analogues oriented to the cytoplasm 30 min after labeling is shown (average of two independent measurements).

Spin-labeled PE moved rapidy to the inner leaflet of the plasma membrane of both cell lines (Fig. 1). In comparison, the extent of SL-SM redistribution was much lower. E.g., while for HepG2 cells after 30 min about 70% of SL-PE were in-accessible to BSA extraction, only 18% of SL-SM were protected. However, after ATP depletion the fast inward movement of SL-PE was completely abolished and similar to that of SL-SM which was not affected by energy exhaustion. The intracellular ATP level was declined to ≤ 5% of the level of control cells for both cell strains.

Discussion

In the present study we have shown that the inward movement of the aminophospholipid PE is ATP-dependent. Even at suboptimal temperature we found for HepG2 and CaCo-2 cells that more than 70% and 50%, respectively, of the labeled analogue redistributed within 30 min to the cytoplasmic side while only 20% of the SM analogue disappeared from the outer layer. The pronounced internalization of SL-PE cannot be explained by an endocytic activity. First, we have measured at temperatures where endocytosis is low or even inhibited. Secondly, if endocytosis would be the major mechanism of uptake of SL-PE we would not expect such a discrepancy to SL-SM. One may question whether the analogues reflect the behaviour of endogenous phospholipids. However, it has been shown by long chain radioactive lipid phospholipids that those short chain spin-labeled analogues reflect very well the transverse motion and distribution of endogenous lipids (see [2]).

In conclusion, our data indicate that in polarized cells, presumably, an aminophospholipid translocase exists similar to the plasma membrane of other mammalian cells [2]. At the present stage of investigation we do not know whether such an activity is confined to specific regions or present in all domains of the plasma membrane of polarized cells.

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References

1. Seigneuret M, Devaux P (1984) ATP-dependent asymmetric distribution of spin-labelled phospholipids in the erythrocyte membrane: relation to shape change. Proceedings of the National Academy of Sciences, USA, 81, 3751
2. Zachowski A (1993) Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem J. 294: 1
3. Morrot G, Herve P, Zachowski A, Fellmann P, Devaux PF (1989) Aminophospholipid translocase of human erythrocytes: phospholipid substrate specificity and effect of cholesterol. Biochemistry, 28: 3456
4. Calvez J-Y, Zachowski A, Herrmann A, Morrot G, Devaux PF (1988) Asymmetric distribution of phospholipids in spectrin-poor erythrocyte vesicles. Biochemistry 27: 5666
5. Pomorski T, Herrmann A, Zimmermann B, Zachowski A, Müller P (1995) An improved assay for measuring the transverse redistribution of fluorescent phospholipids in plasma membranes. Chemistry and Physics of Lipids, in press