

RECONSTITUTION OF FLIPPASE ACTIVITY INTO LIPOSOMES

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Phospholipids are asymmetrically distributed in the plasma membrane of eucaryotic cells, the aminophospholipids being mostly on the cytosolic leaflet, whilst the choline containing phospholipids (phosphatidylcholine and sphingomyelin) are predominantly exposed on the outer leaflet [1]. There is strong evidence in support of the role of specific proteins (“flippases”) that are involved in the transmembrane segregation of phospholipids [1-3]. Among these proteins, some are active transporters. The aminophospholipid translocase is a ubiquitous protein that transports selectively phosphatidylserine (PS) and phosphatidyl-ethanolamine (PE) from the outer to the inner monolayer of the plasma membranes of eucaryotic cells [1]. In the same cells, the P-glycoprotein (P-gp) and several proteins of the ABC family have been reported to transport phospholipids from the inner monolayer to the outer monolayer [2, 4]. Finally a calcium dependent “scramblase” plays a key role in the ATP-independent rapid lipid randomization, which takes place in stimulated platelets or during apoptosis, leading in particular to phosphatidylserine exposure on the outer cell surface [5]. It was reported also that lipid scrambling involves simultaneously an ATPase (ABCA1) that would transport selectively phosphatidylserine from the outer to the inner monolayer [6].

Although the evidence for various flippases activities has been well established, the assignment to specific proteins is still a matter of controversies. The best evidence for the ability of a specific protein to translocate phospholipids through a lipid bilayer is ultimately the study of purified proteins in a well-defined reconstituted system. Very few reports on flippases activities in proteoliposomes appear in the literature [7-9]. In 1994, we showed ATP-dependent aminophospholipid translocase activity of a Mg ATPase purified from human red cell membranes and reinserted into LUVs [7]. These experiments indicated a non-negligible transport of spin-labeled PS or PE that was ATP dependent. More recently, we attempted to measure the transport of spin-labeled or fluorescent lipids analogs by a purified P-gp reinserted into LUVs [8]. However, these experiments indicated only a small diffusion of the probed lipids and failed to reveal any ATP-dependence. The group of Sharom in Canada has reported almost simultaneously a transport of fluorescent lipid analogs by the P-glycoprotein in reconstituted systems. But the extent of lipid transport was small [9]. Furthermore, to date they all are based on the use of partially water soluble lipid probes. Yet, the relevance of phospholipid with either two very short chains

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[3] or one long chain and one short chain bearing a fluorescent or a paramagnetic probe [1,2,5] has been frequently questioned.

A clue for the understanding of the limitations inherent to experiments involving lipid translocation in large unilamellar vesicles (LUVs) is probably that the accumulation of lipids on one leaflet triggers the formation of lateral stress. NMR experiments with asymmetrical LUVs containing on one leaflet 1 to 5% lyso-PC added after the formation of the vesicles indicated an increased surface tension which was detected in both leaflets. This lateral stress is due to the mismatch between the two coupled monolayers. It is able to modulate the activity of mechano-sensitive ion channels [10]. We therefore are considering as a working hypothesis a possible inhibition of phospholipid translocase activity in LUVs that could be due to the lateral stress produced by the accumulation of lipids on one leaflet. In the case of the P-gp the specificity seems to be very broad since it is reported that the P-gp can transport PC, PE and glycolipids [4]. If it does so, it should transport long chain unlabeled PC and PE molecules as well as the NBD or spin-labeled derivatives. Consequently, in LUVs made essentially of PC the vesicle should very quickly experience an increased surface tension. Our hypothesis is therefore that the surface tension generated in 200 nm LUVs can inhibit the translocase activity of a reconstituted P-gp. This could explain the difficulties one encounters to observe a flippase activity with a low selectivity in reconstituted proteoliposomes involving PC vesicles. On the other hand, because the aminophospholipid translocase transports very specifically PS and PE, it can transport a small amount of labeled PS diluted in unlabeled PC [7]. Comparison of the effect of lyso-PC insertion in the outer monolayer of LUVs and into GUVs [11,12] indicate that the former resist to the surface tension while the latter undergo shape change as soon as one monolayer has 1% excess of lipids. Thus, if, instead of investigating flippase activity in LUVs, one is able to fuse small proteoliposomes containing a flippase to GUVs (with a diameter of several tenth of microns) one should expect a shape change even if only a very small percentage of lipids is translocated by a flippase. In this case there is no need to use labeled lipids nor short chain lipids. Experiments are in progress in our laboratory to trigger shape change in GUVs after fusion of red cell ghosts containing the aminophospholipid translocase.

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