Functional Studies Of Human Phospholipid Scramblase 1

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Phospholipid scramblases (PLSCRs) constitute a group of homologous ATP-independent bidirectional lipid translocators that are conserved in all eukaryotic organisms, from yeasts to humans. They are involved in the calcium-dependent transbilayer movement of phospholipids, which tends to eliminate their asymmetric distribution across the membranes (Basse et al. 1996).

Human phospholipid scramblase 1 (hPLSCR1) is a palmitoylated type II endofacial membrane protein, widely expressed in most human tissues. The increase in cytosolic calcium (1000-fold the basal level) in response to cell injury, coagulation, cell activation or apoptosis, activates the protein. This activation induces a rapid transbilayer movement of PE and PS from the inner to the outer membrane leaflet destroying the bilayer asymmetry (Zhou et al., 1997; Zhao et al., 1998).

We have constructed a truncated mutant, hPLSCR1ΔC290 (MutIV) that lacks the putative transmembrane C-terminal α-helix. MutIV was overexpressed with a N-terminal histidine tag and purified under 6 M urea denaturing conditions. Langmuir balance studies at the air-water interface showed the same protein adsorption behavior in the presence and absence of calcium. In lipid monolayers the protein insertion was higher in the presence of calcium, by about 6 mN/m. In contrast with the wild type, the reconstituted mutant in liposomes (PC:PS, labeled with pyrene-PC) did not show any scramblase activity.

We conclude that the transmembrane helix is crucial for the protein scramblase activity, but not for calcium binding nor for the insertion into lipid monolayers.