

Supporting information

Heavy metals mediated phospholipids scrambling by human phospholipid scramblase 3: a probable role in mitochondrial apoptosis

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Materials and methods

Principle of scramblase assay

Inside labeling: Inside labeled proteoliposomes were generated by reconstituting the NBD labeled lipids along with other phospholipids and protein. Once the proteoliposomes were generated the NBD labeled lipids in the outer leaflet was quenched with the membrane-impermeable dithionite quencher. Therefore in case of inside labeled proteoliposomes, the NBD labeled lipids are present only in the inner leaflet of the vesicle. The hPLSCR3 protein reconstituted in the proteoliposomes will orient in such a way that only 50% of the protein added will be reconstituted with appropriate orientation such that the Ca^{2+} binding motif is exposed for binding. In proteoliposomes incubated with Ca^{2+} , the hPLSCR3 is activated and disrupts the asymmetry of the bilayer by translocation of the phospholipids to the outer leaflet which is then quenched by further addition of dithionite and the residual fluorescence is measured after addition. In Proteoliposomes incubated with EDTA, the hPLSCR3 is not activated and hence there will be no translocation of labeled lipids from inner leaflet. The NBD labeled lipids in the outer leaflet already quenched, the NBD labeled lipids in the inner leaflet contributes for the residual fluorescence and the intensity is higher than that of the Ca^{2+} treated sample (Figure 1). The measure in the difference of residual fluorescence intensity gives the activity of scramblase.

Outside labeling: Proteoliposomes were generated by reconstituting the phospholipids and protein followed by labeling. The NBD labeled phospholipids were added to the proteoliposomes and incubated resulting in outside labeled vesicles. In Proteoliposomes incubated with EDTA, the hPLSCR3 is not activated and translocation of phospholipids does not occur and the NBD labeled lipids are quenched on dithionite addition whereas, in Ca^{2+} treated sample the hPLSCR3 is activated and the translocation of phospholipids happens from outer leaflet to inner leaflet of the liposomes. This sequestration from quencher retains the residual fluorescence in case of Ca^{2+} treated samples. The difference in residual fluorescence intensity gives the scramblase activity (Figure 1).

Figures

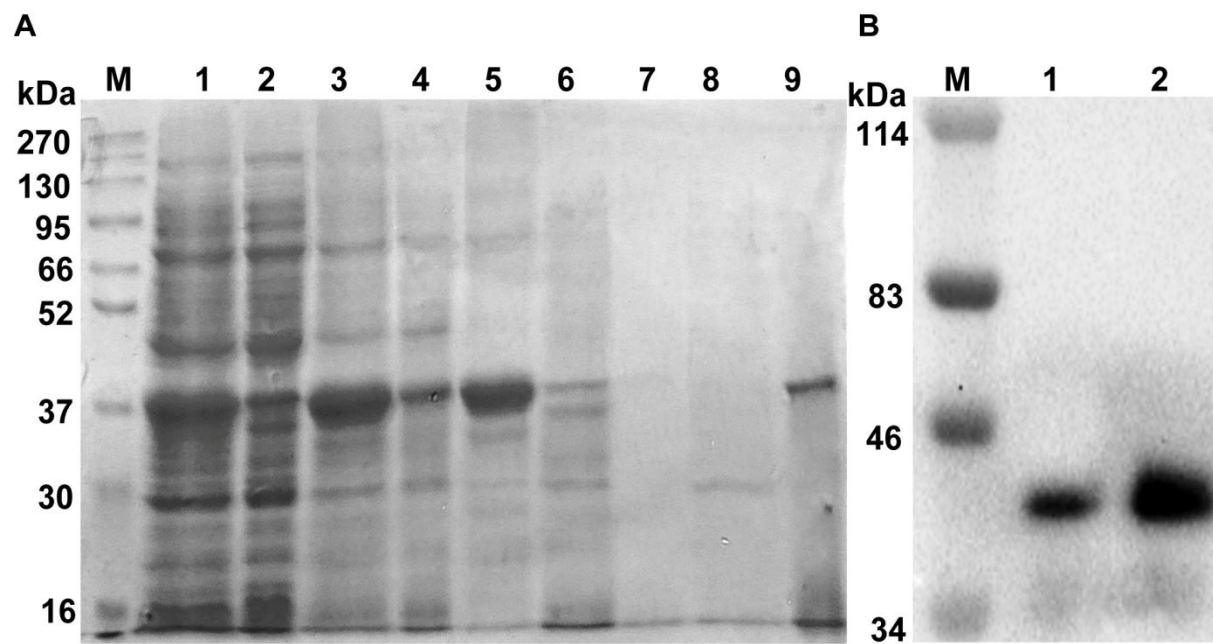


Figure S1. (A) Overexpression and purification of WT-hPLSCR3. Cell-lysate and protein fractions purified by immobilized metal affinity chromatography were visualized in 12% SDS PAGE. *Lane M*-Marker, *Lane 1*- Induced cell lysate, *Lane 2*- Soluble fraction after cell lysis, *Lane 3*- Insoluble fraction after cell lysis (inclusion bodies-IBs), *Lane 4*- Insoluble fraction (after NLS treatment of IBs), *Lane 5*-Soluble fraction (after NLS treatment of IBs), *Lane 6*- Flow-through, *Lane 7*-wash (elution with 20 mM Imidazole from Ni²⁺-NTA column), *Lane 8*-wash (elution with 50 mM Imidazole from Ni²⁺-NTA column) *Lane 9* -Purified WT-hPLSCR3 (elution with 250 mM Imidazole from Ni²⁺-NTA column). (B) Western Blot analysis of purified WT-hPLSCR3. *Lane M*-Marker, *Lane 1*- Induced cell lysate *Lane 2*- purified WT-hPLSCR3.