LYSENIN DISTINGUISHES TWO POOLS OF SPHINGOMYELIN IN THE PLASMA MEMBRANE

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Microdomains (rafts) of the plasma membrane are assemblies of sphingomyelin (SM), glycolipids and cholesterol. In an attempt to obtain a probe for SM, we prepared recombinant wt lysenin-GST, a toxin originating from the earthworm *Eisenia foetida*, which specifically recognizes SM and evokes cytolysis. To interfere with the lytic activity, W20A point mutation was introduced (W20A lysenin-GST). The protein-lipid overlay assay revealed that both proteins at 3 μg/ml detected as little as 5 pmoles of SM. They both labeled the surface of fixed erythrocytes with similar intensity and the staining was reduced by bacterial sphingomyelinase. To investigate the lytic activity of the protein, an efflux of lactic dehydrogenase from U937 monocytes was measured. In contrast to wt lysenin-GST, W20A lysenin-GST did not display the lytic activity nor caused efflux of potassium ions form the cells. SDS-PAGE analysis revealed that the lytic activity of wt lysenin-GST was correlated with protein oligomerization during interaction with SM-containing membranes and the amount of oligomers was increased with elevation of SM/lysenin ratio. After isolation of raft fraction from monocytes pretreated with wt lysenin-GST, it was found that the protein bound and oligomerized exclusively in the raft region. In contrast, W20A lysenin-GST failed to oligomerize and the vast majority of the protein was bound to SM outside rafts. Taken together, the data indicate that the ability of lysenin to oligomerize is correlated with its lytic activity. Wt lysenin-GST and W20A lysenin-GST are able to distinguish SM concentrated in rafts from that dispersed in glycerophospholipid-enriched regions of the plasma membrane converting the proteins into a useful tool to study organization and function of SM in the plasma membrane.