Abstract

Membrane palmitoylated protein-1 (MPP1) plays an important role in the formation of raft domains in erythrocytes. It was previously established that a lack of palmitoylated MPP1 causes a suppression of membrane-raft formation in the membranes of human erythrocytes, which consequently leads to the loss of normal erythrocyte features. Moreover, the inhibition of MPP1 palmitoylation in normal human erythrocytes results in significant changes in the physicochemical properties of the plasma membrane, namely changes in membrane order. These results, along with other published data, demonstrate that the formation of raft domains in erythrocyte membranes is influenced by MPP1. Despite these observations, still, not a lot is known about the molecular mechanism underlying the association of MPP1 with the plasma membrane.

Previous studies involving MPP1 have mainly focused on its interactions with other membrane proteins, rather than seeking for the possibility of how MPP1 might interact with membrane lipids. So far, the molecular mechanism of the association of MPP1 with membrane lipids has been connected to its ability to link the erythrocyte membrane skeleton to the membrane bilayer by forming a tripartite complex with other membrane proteins, namely, protein 4.1 and glycophorin C or on the other hand with flotillins as major raft marker proteins.

Markedly, the data of this thesis demonstrated that recombinant, bacterially expressed MPP1, as a single protein component, is able to directly interact with membrane lipids. The experimental approach of this thesis involved the study of the interaction of MPP1 with membrane lipids in two membrane model systems, namely, lipid monolayers at the air/buffer interface using the Langmuir-monolayer technique and lipid bilayers using liposome flotation and FRET-based approaches. The results obtained from these studies revealed that MPP1 binds membrane lipids with high affinity, forming the basis for a novel mechanism involving the participation of specific lipid-protein interactions in the association of MPP1 with the plasma membrane.

Although there are many studies suggesting crucial role of palmitoylation in the final targeting of the (raft) proteins to plasma membrane, the experiments involving a mutant of MPP1 mimicking

palmitoylated MPP1 (C242F) revealed that mutated-MPP1 binds lipid mono- and bilayer with an affinity very similar to that obtained for wild-type MPP1.

Interestingly, the results of this study showed a direct effect of cholesterol on the binding activity of MPP1 with membrane lipids, revealed as a marked decrease in the binding affinity of MPP1 to mono- and bilayers prepared from cholesterol-free lipid mixtures, in comparison with those prepared from cholesterol-containing lipid mixtures. Additionally, pre-incubation of MPP1 with cholesterol before its addition to the Langmuir subphase of the monolayer resulted in a dramatic reduction in the membrane insertion/binding of MPP1, suggesting an apparent ability of cholesterol to directly interact with MPP1 molecules. The generalized polarization measurements indicated a change in the lipid mono- and bilayer properties upon the addition of MPP1, i.e changes in fluidity.

Finally, the results obtained from the liposome flotation assay and Langmuir monolayers showed that flotillin-1 and flotillin-2 have the ability to bind membrane lipids as well. However, the presence of flotillins did not affect the binding activity of MPP1 to membrane lipids as both flotillin-1 and flotillin-2 were also detected, with MPP1, at the top fraction of the sucrose gradient of the flotation assay in which all mentioned components had been included.

In conclusion, data in this thesis is strongly consistent with the possibility of the participation of the MPP1-lipid interactions in the association of MPP1 with plasma membrane, and furthermore with the idea that formation of lipid rafts may involve multiple types of interactions involving the interaction of MPP1 with membrane raft proteins as well as with lipids.