Lipid-Membrane Affinity of Chimeric Metal-binding Green Fluorescent Protein

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Abstract.
The Green Fluorescent Protein (GFP) is a useful marker to trace the expression of cellular proteins. However, little is known about changes in protein interaction properties after fusion to GFP. In this study, we present evidence for a binding affinity of chimeric cadmium-binding green fluorescent proteins to lipid membrane. This affinity has been observed in both cellular membranes and artificial lipid monolayers and bilayers. At the cellular level, the presence of Cd-binding peptide promoted the association of the chimeric GFP onto the lipid membrane, which declined the fluorescence emission of the engineered cells. Binding affinity to lipid membranes was further investigated using artificial lipid bilayers and monolayers. Small amounts of the chimeric GFP were found to incorporate into the lipid vesicles due to the high surface pressure of bilayer lipids. At low interfacial pressure of the lipid monolayer, incorporation of the chimeric Cd-binding GFP onto the lipid monolayer was revealed. From the measured lipid isotherms, we conclude that Cd-binding GFP mediates an increase in membrane fluidity and an expansion of the surface area of the lipid bilayer. This evidence was strongly supported by epifluorescence microscopy, showing that the chimeric Cd-binding GFP preferentially binds to fluid-phase areas and defect parts of the lipid monolayer. All these findings demonstrate the hydrophobicity of the GFP constructs is mainly influenced by the fusion partner. Thus, the example of a metal-binding unit used here shines new light on the biophysical properties of GFP constructs.

Key words: Green fluorescent protein, Lipid binding, Cd-binding peptide, Lipid bilayers, Lipid monolayers