Nanoscale orientation and lateral organization of chimeric metal-binding green fluorescent protein on lipid membrane determined by epifluorescence and atomic force microscopy

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Abstract

Epifluorescence microscopy as well as atomic force microscopy was successfully applied to explore the orientation and lateral organization of a group of chimeric green fluorescent proteins (GFPs) on lipid membrane. Incorporation of the chimeric GFP carrying Cd-binding region (His\textsuperscript{6}CdBP\textsubscript{4}GFP) to the fluid phase of DPPC monolayer resulted in a strong fluorescence intensity at the air–water interface. Meanwhile, non-specific adsorption of the GFP having hexahistidine (His\textsuperscript{6}GFP) led to the perturbation of the protein structure in which very low fluorescence was observed. Specific binding of both of the chimeric GFPs to immobilized zinc ions underneath the metal-chelating lipid membrane was revealed. This specific binding could be reversibly controlled by addition of metal ions or metal chelator. Binding of the chimeric GFPs to the metal-chelating lipid membrane was proven to be the end-on orientation while the side-on adsorption was contrarily noted in the absence of metal ions. Increase of lateral mobility owing to the fluidization effect on the chelating lipid membrane subsequently facilitated crystal formation. All these findings have opened up a potential approach for a specific orientation of immobilization of protein at the membrane interface. This could have accounted for a better opportunity of sensor development.

Keywords: Chimeric green fluorescent protein; Epifluorescence microscopy; Atomic force microscopy; Metal-binding peptide; Metal-chelating lipid

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