Interaction analysis of chimeric metal-binding green fluorescent protein and artificial solid-supported lipid membrane by quartz crystal microbalance and atomic force microscopy

Virapong Prachayasittikula, Charchalerm Isarankura Na Ayudhyaab, Lutz Hilterhausb, Andreas Hinzb, Tanawut Tantimongkolwatab, Hans-Joachim Gallab

a Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
b Institute of Biochemistry, Westfälische Wilhelms University, 48149 Muenster, Germany

Received 23 November 2004 / Available online 9 December 2004

Abstract
Non-specific adsorption and specific interaction between a chimeric green fluorescent protein (GFP) carrying metal-binding region and the immobilized zinc ions on artificial solid-supported lipid membranes was investigated using the quartz crystal microbalance technique and the atomic force microscopy (AFM). Supported lipid bilayer, composed of octanethiol and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine/1,2-dioleoyl-sn-glycero-3-[N-(5-amino-1-carboxypentyl iminodiacetic acid)succinyl] (NTADOGS)-Zn2+, was formed on the gold electrode of quartz resonator (5 MHz). Binding of the chimeric GFP to zinc ions resulted in a rapid decrease of resonance frequency. Reversibility of the process was demonstrated via the removal of metal ions by EDTA. Nanoscale structural orientation of the chimeric GFP on the membrane was imaged by AFM. Association constant of the specific binding to metal ions was 2- to 3-fold higher than that of the non-specific adsorption, which was caused by the fluidization effect of the metal-chelating lipid molecules as well as the steric hindrance effect. This infers a possibility for a further development of biofunctionalized membrane. However, maximization is needed in order to attain closer advancement to a membrane-based sensor device.

Keywords: Quartz crystal microbalance; Green fluorescent protein; Atomic force microscopy; Hexahistidine; Metal-chelating lipid; Functionalized membrane; Protein–lipid interaction

Biochemical and Biophysical Research Communications. 2005;327 : 174–182