Abstract

Numerous natural proteins that emit fluorescence have been discovered from various organisms, and their genetically engineered variants have been generated in the last several decades. A variety of physicochemical phenomena exhibited by fluorescence proteins have been utilized to develop many key technologies in life science over the last few decades. Accordingly, the physicochemical properties of fluorescence proteins have been deeply investigated in the bulk solution that mimics the cellular environment, but those at the less common environment such as surface and interface have not been deeply investigated. In this research work, it was found that the fluorescence protein exhibits voltage-dependent photoluminescence after its immobilization on the metal-solution interface. Upon the blue light photoexcitation, a yellow-emitting, version of green fluorescence protein (GFP), called Venus was immobilized on the gold electrode surface, robust enhancement or decrease of fluorescence was induced by applying negative or positive bias, respectively. This previously unappreciated phenomenon was then implemented as a protein-based microdisplay. Several experiments have been done to solve the mechanism for cathodic enhancement utilizing the characteristic optical properties in the three different fluorescence proteins. From the simultaneous electrochemical and fluorescence measurements in Venus, a solid correlation was found between the modulation of fluorescence and current reflecting cathodic hydrogen evolution, which led to a hypothesis that shift in the protonation-deprotonation equilibrium of the chromophore driven by hydrogen evolution at the metal surface underlies the phenomena. The hypothesis predicted that voltage dependency should be also found in the photoconversion from green to red of fluorescence protein which is known as a protonation-dependent process. The hypothesis was verified by observing clear voltage dependency for the photoconversion in Kikume Green - Red (KikGR), an engineered photoconvertible fluorescence protein, at the interface. Later experiments have been done to address how the shift in protonation equilibrium is driven by hydrogen evolution. The analysis using iR-pHluorin, a fluorescence protein variant with the inverse pH-sensitivity revealed that there exists an interface-specific mode of protonation-deprotonation reaction, and where the protonation equilibrium is directly coupled to the cathodic hydrogen evolution.

The interface-specific mode is distinct from that conventionally seen in protein in the bulk solution and there the protonation patterns of the constituent titratable residues are determined through the acid-base equilibrium of the local environment. Interface-specific mechanism-based possible applications are then discussed, including monitoring of hydrogen evolution reactions at near neutral conditions.

Keywords:

Fluorescence protein, Photoluminescence, Imaging, Protonation-deprotonation, Hydrogen Evolution Reaction (HER).