

Monitoring of alternating *ES* complexes formation between cytochrome c_3 and hydrogenase by highly sensitive EQCM technique

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Hydrogenase is an enzyme, catalyzing both hydrogen evolution and hydrogen uptake reactions. A substrate of hydrogenase is cytochrome c_3 , which is a redox protein containing four hemes in one molecule. In the case of hydrogen evolution, the reduced cytochrome c_3 donates electron to hydrogenase. Otherwise, in the case of hydrogen uptake, the oxidized cytochrome c_3 accepts electrons from hydrogenase. The enzyme-substrate complex on the two reactions is probably different from each other. One is the complex between hydrogenase and the reduced cytochrome c_3 and the other is the complex between hydrogenase and the oxidized cytochrome c_3 . The two types of *ES* complexes seem to be different in the docking site of cytochrome c_3 . Methods or techniques to investigate the differences, however, have been unknown and the alternation between the two *ES* complexes is not clarified.

In this study, electrochemical quartz crystal microbalance (EQCM) technique is applied for monitoring the alternating *ES* complex formation. EQCM technique is a simultaneous measurement of electrochemistry and nano-grams mass sensing, so that redox regulated mass change on the electrode surface is investigated. Cytochrome c_3 was immobilized on a quartz crystal electrode and the redox of cytochrome c_3 was under control electrochemically. *ES* complex formation between the cytochrome c_3 immobilized electrode and hydrogenase in solution was investigated. When the cytochrome c_3 on the electrode is reduced electrochemically, the hydrogen evolution complex was formed. Likewise, when the cytochrome c_3 is oxidized, hydrogen uptake complex was formed. Thus, the alternating *ES* complexes were sorted out by potential control on electrode, and kinetics of the *ES* complex formation were investigated. The EQCM technique indicates a new insight for analyzing single molecule kinetics of redox proteins.