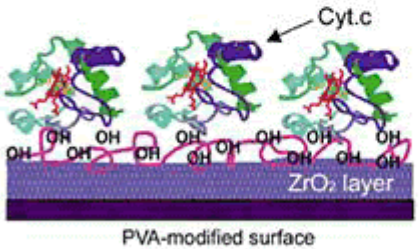
Mild Immobilization of Proteins

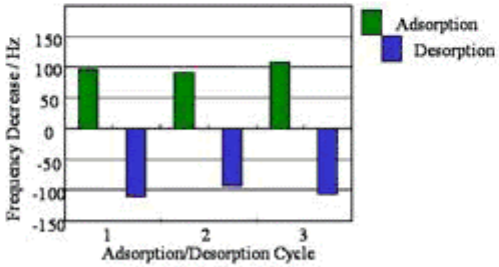
The adsorption of water-soluble proteins on solid substrates has attracted research interest along with rapid advances of biotechnology. Increasing demands on understanding of the adsorption mechanism are noted in the research of biocompatible materials, biosensors, and separation devices.

Our group is engaged in research on new technologies which will enable us to immobilize proteins on the surface of inorganic materials while minimizing protein denaturation. The protein cytochrome c, which plays a key role in electron transfer in living organisms, is charged positively in neutral condition, and therefore is strongly adsorbed on the surface of negatively-charged metal oxides. However, because the folding structure is prone to collapse on the metal oxide surface, it is adsorbed as an insoluble denatured protein. We discovered that this denaturation can be prevented by coating the surface of the metal oxide with an ultrathin organic layer. Using a technique called "Surface Sol-Gel Process", a thin film of ZrO_2 with a thickness of 3.0-4.0 nm was deposited on a metal substrate, and a thin layer (1.6 nm) of polyvinyl alcohol (PVA) was then coated on this oxide film. Because the surface of the ZrO_2 is weakly charged negatively in neutral condition, it adsorbs cytochrome c. The uniform PVA layer prevents denaturation of the protein. We also found that proteins immobilized by this method completely desorbed in alkaline solution. The adsorption/desorption rate was constant during repeated experiments.

The surface sol-gel process provides a convenient method to immobilize water-soluble proteins through moderate electrostatic attraction. The PVA-modified surface is not only an excellent substrate for protein immobilization but also useful to evaluate the conformational stability.



Schematic illustration of cytochrome c on a metal surface modified with nanometer-thick ZrO_2 and PVA layers.



QCM frequency changes during repeated adsorption and desorption of cytochrome c. The protein completely desorbs in alkaline solution.