389e Proteolytic Degradation of Immobilized Proteins at the Solid/Liquid Interface: Implications for Detergency

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Proteolysis at the solid/liquid interface of an immobilized protein substrate is examined using ellipsometry to measure kinetics of surface-bound protein degradation and Optical Waveguide Lightmode Spectroscopy (OWLS) to study enzyme adsorption. To study the kinetics and mechanism of proteolysis at the solid/liquid interface for detergency applications, it is necessary to create a well-defined, stable, multilayer protein surface. Thick, covalently bound protein films that cannot be removed by aqueous buffer or surfactant alone can be produced on a silicon wafer surface. This is accomplished by functionalizing the silica on the wafer surface with 3-aminopropyltriethoxysilane (APTES), and subsequent covalent binding and crosslinking of the protein to the surface with glutaraldehyde. The serine protease subtilisin Carlsberg removes the bound protein layer. The thickness of the protein films (~100 nm) permits use of ellipsometry to measure protein layer thickness as a function of time, as the enzyme adsorbs on the substrate and degrades it.

Atomic force microscopy (AFM) has shown that the morphology of the adsorbed protein layer is uniform and reproducible. Cleavage rates of the protein film are linear in time, illustrating that, in addition to surface uniformity, the substrate protein is also homogeneous in height. We find that at low bulk enzyme concentrations pertinent to detergency applications, the substrate degradation rate is linear in enzyme concentration, and in the limit of high aqueous enzyme concentration, becomes zeroth order in enzyme concentration. The degree of substrate crosslinking may be tuned by decreased exposure time to the glutaraldehyde, leading to faster proteolysis. A strong temperature dependence of the substrate cleavage rate, as well as sensitivity to detergency-relevant additives such as anionic and nonionic surfactants, has been observed.

We describe the observed enzyme reaction kinetics by a Langmuir-Michaelis-Menten model resulting from reversible enzyme adsorption at the protein/water interface and surface proteolysis. The Langmuir equilibrium parameters used in the model are based on adsorption experiments conducted using OWLS with varying protease concentrations. The subtilisin turnover number was found to be several orders of magnitude smaller than typical values for aqueous proteolysis, possibly due to the highly crosslinked nature of the protein substrate. A TNBS assay (2,4,6-trinitrobenzene sulfonic acid) for detection of free amino groups in solution was used to corroborate kinetic results from the modeling and ellipsometry experiments.