PROTEIN ADSORPTION AT SOLID-LIQUID INTERFACES

by

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ABSTRACT

The main aim of this research was to extend our understanding of protein-surface interactions which are important for many biological and non-biological applications. Surface induced protein unfolding and aggregation phenomena are of critical importance and therefore a thorough investigation of the protein-surface interactions is necessary. The overall goal of this work was to measure and characterize protein adsorption and judiciously combine experimental results with theoretical studies performed by other collaborators. Combining a wide range of protein adsorption experiments on alkanethiols and with Monte Carlo simulations of lattice proteins at different concentrations and on surfaces of varying "polarity", we explore the extent and rheological behavior of adsorbed proteins as a function of substrate polarity, "on" rate constants (k_a) and steric parameters ($|A_1|$) from the random sequential adsorption model, and demonstrate a folding to unfolding transition upon adsorption. We show that model globular proteins (hen egg lysozyme, ribonuclease A and insulin dimer) behave similarly with respect to adsorption. Experimentally, above a substrate wettability $\cos\theta > 0.4$ (where θ is the sessile contact angle of water on a substrate in air), the adsorbed mass, rigidity and k_a of the proteins are diminished, while $|A_1|$ is increased, suggesting a lower packing density. We also offer a novel process to render hydrophobic surfaces resistant to relatively small proteins during adsorption. This was accomplished by self-assembly of a well-known natural osmolyte, trimethylamine oxide (TMAO), a small amphiphilic molecule, on a hydrophobic alkanethiol surface. While self-assembled monolayers are excellent model systems to tune surface wettability and study protein adsorption, removing adsorbed protein from metals has significant health and industrial

consequences. A major finding of this work is that, besides protein-metal surface adhesion energy, adsorption of protein-associated water and secondary structural content determines the access and hence ability to remove adsorbed proteins from metal surfaces with a strong alkaline-surfactant solution.

Besides protein adsorption on alkanethiol and metallic surfaces we also studied the stability of covalently attached proteins. The stability of tethered globular proteins under denaturing conditions was interrogated with a hydrophobic probe, since conventional structural methods like circular dichroism (CD) and fluorescence or infrared spectroscopy could not be used due to the presence of an opaque solid substrate and extremely low surface concentrations. The unfolding process for covalently tethered LYS and RNase A was followed, with multi-molecular force spectroscopy (using an atomic force microscope in force-mode), via the adhesion energy between a functionalized self-assembled monolayer (CH_3-SAM) hydrophobic probe and the protein molecules covalently bound to a carboxylic SAM on a gold coated glass cover slip. The adhesion energy passed through a maximum for the tethered proteins during excursions with temperature or chemical denaturants. The initial rise in adhesion energy on increasing the temperature or GuHCl concentration was due to increasing exposure of the unfolded hydrophobic core of the proteins to the CH_3-SAM tip, while the decrease in adhesion energy at high temperature or large concentrations of denaturant is attributed to inter-protein association with nearest neighbors.

Our aim was therefore to judiciously combine experimental results and theoretical studies to study the effect of surface chemistry on protein adsorption, chemical modification of a greasy protein adsorbing surface to render it protein resistant,

adsorption and removal of protein layers on metallic surfaces and to also study the stability of surface tethered proteins.