EFFECT OF SOLID SURFACE WITH SELF ASSEMBLED MONOLAYERS ON ADSORPTION OF PROTEINS

by

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Certificate

This is to certify that the thesis entitled ***"Effect of Solid Surface with Self Assembled Monolayers on Adsorption of Proteins "*** being submitted by Mr. Lalit Mohan Pandey to the Department of Chemical Engineering, Indian Institute of Technology, Delhi is the record of the bonafide research work carried out by him under my supervision and guidance. The work presented in this thesis have not been submitted either in part or in full to any other university or institute for the award of any degree or diploma.

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Abstract

Understanding of protein-surface interaction is important in various biomedical applications such as medical implants, biosensors, tissue engineering and drug delivery etc. Irreversible adsorption of protein leads to change in its native conformation, which in turn leads to bio-fouling and failure of the implants. To resist the fouling, reversible adsorption of protein at the surface is essential. Many researchers have been trying to understand the protein adsorption for the last three decades. However, the phenomena is far from clear understanding due to (a)complexity of the protein, which consists of hydrophobic, charged (positively and negatively charged) and polar moieties and (b)chemical and physical nature of substrate. We do not have control over protein's conformation within the body fluid, but we can modify the surface according to our requirement. The surfaces with different hydrophobicities can be formed by self-assembled monolayers (SAMs). Over the last two decades, various researchers have used different type of mono SAMs, mixed SAMs. Mixed SAMs can give good control of hydrophobicity of surfaces; but it produces phase-separated surface morphology due to strong attraction between like molecules and strong repulsion between dissimilar molecules.

We have studied kinetics of adsorption and properties of adsorbed proteins (two model proteins: bovine serum albumin and fibrinogen) on newly synthesized hybrid SAMs, which has short chain hydrophobic and hydrophilic groups on the same molecule and compared the results with various mono SAMs and mixed SAMs by using quartz crystal microbalance (QCM). QCM measures change in frequency (ΔF) and dissipation energy (ΔD) simultaneously to high precision in real time. We have proposed a kinetic model of protein adsorption and fitted it to data, ΔF vs time obtained from QCM. The physical phenomena occurred during adsorption are explained through the estimated modeled parameters. It is found that the adsorption of BSA and FB follow

Langmuir adsorption isotherm at low bulk protein concentration. The elasticities determined by the QCM data suggest that proteins adsorbed on hybrid surface are more viscous. The adsorbed layer of BSA is softer than that of Fb. The viscoelastic properties, such as relaxation times of adsorbed proteins are the slowest on the octyl surface, while those are the fastest on the hybrid surface.

We have analyzed the change in secondary structures of adsorbed proteins using ATR-FTIR and found that the proportions of secondary structures of proteins on hybrid surface are similar to that of the proteins in solution. We found that surface controls the proportions of secondary structures of proteins in adsorbed state.

We have studied the adsorption of proteins from their mixed solution. The mass and viscoelasticity of adsorbed proteins depend on the total concentration of proteins in solution. Vroman effect was found to appear on all the surfaces. The mixed protein adsorption found to follow co-operative adsorption on mixed surface.

Hydrophobicity of surface and adsorbing proteins/polymer molecule controls adsorption behavior and hence affects wettability of surfaces with different hydrophobicites with solution of polymer or protein. We have classified polymer/protein to two groups depending on their polar to dispersive component of surface energy. The contact angle, wetting tension of the solutions is characteristics of the polar to dispersive ratio. It is found that measurement of wetting effect can lead to exact measurement of adsorbed amount.

Kinetics of aggregation of insulin is characteristic of a nucleation controlled mechanism and follows a sigmoid shape curves with lag phase, exponential growth phase and plateau phase.

Lag time varies with surface functional groups, which regulates the adsorption and secondary structures of insulin.

Stability tests in saline and albumin solutions suggest that the hybrid SAMs is more stable than other SAMs on the silica surface. These properties make the hybrid surface more promising than the mixed SAMs for biomedical applications.

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