DEPENDENCE OF ADSORBED PROTEIN'S MORPHOLOGY AND ELASTICITY ON HYDROPHOBICITY OF SURFACE AND THEIR EFFECT ON AGGREGATION

INDU SHARMA

DEPARTMENT OF CHEMICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY DELHI
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by

Indu Sharma

Department of Chemical Engineering

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Dedicated to my parents, my husband

and my son

Certificate

This is to certify that the thesis entitled ***"Dependence of Adsorbed Protein's Morphology and Elasticity on Hydrophobicity of Surface and their Effect on Aggregation"*** being submitted by **Ms. Indu Sharma** to the Department of Chemical Engineering, **Indian Institute of Technology Delhi**, for the award of the degree of **Doctor of Philosophy,** is a record of the bonafide research work carried out by her 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.

(Dr. Sudip K. Pattanayek)
Associate Professor
Department of Chemical Engineering
Indian Institute of Technology Delhi

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Abstract

The adsorption of protein at the solid surfaces is the fundamental issue in designing the bio-materials, development of biosensors, purification, and storage of therapeutic proteins, etc. Despite many studies, the understanding of adsorption of protein over a surface and their subsequent behavior are far from complete due to the inherently complex nature of an adsorbate protein and the enumerable possibility of an adsorbent surface. There is a possibility of the huge different number of configurations of protein at different external factors such as pH, salt concentration, buffer type, temperature, mechanical stress, etc. Also, the surface can be smooth, rough, hydrophobic, hydrophilic, etc. Even a smooth surface can have molecularly roughness with spikes, which can also control the adsorption of protein.

To understand the effect of surfaces, it is customary to see the adsorption behavior of a protein on a self-assembled monolayer (SAMs) of desired chemical functionality over a substrate. Due to an enumerable number of factors, such as reaction condition, chemical nature of SAMs forming reactants, a number of physical and chemical heterogeneity appears on the surface. The surface energy, which is macroscopic property, depends on the microscopic properties of the surface. The dependence of micro and macroscopic roughness and surface energy of the adsorbing surface on the surface energy of the adsorbed proteins has been studied. Surfaces of three different chemical functionalities namely, amine, hydroxyl, and octyl, were obtained through self-assembled monolayer on silica surfaces and were tested for responses towards adsorption of lysozyme and bovine serum albumin (BSA).

Many times, these SAMs are made with the appropriate reactants at a reaction condition, which is not optimized. We have tested three silane coupling agents (SCA) containing propyl amine with varying groups linked to Si present on it. In addition, we have used a silane coupling

agent to prepare SAM for methyl head group. The approach of these molecules towards the surface depends on the head group and groups linked to Si of the SCA. The morphology of the surfaces is analyzed using power spectral density distribution (PSD), skewness, ellipsometry thickness, and surface energy. It is found from the morphology of the adsorbed insulin over various substrates that a low number of aggregates of big size are formed on the surfaces obtained from a low concentration of SAMs, while a higher number, but of a smaller grain size of aggregates are formed over surfaces obtained from 1% concentration of SAMs modifiers.

We have explored the interrelation among characteristics of substrates with characteristics of adsorbed proteins, namely adsorption isotherm, adsorption kinetics, viscoelastic, properties of the adsorbed proteins and desorption of the adsorbed protein by a surfactant. Quartz crystal microbalance is used to determine the change in mass due to adsorption and the elasticity of the adsorbed proteins. The parameters required to fit the adsorption isotherm, given by the BET equation for a finite number of layers, can be used to explain the elasticity of four proteins of different molecular weights, can be used to explain their elasticity on the surfaces. In addition, the parameters can be used to explain desorption behavior of proteins by using sodium-do-decyl sulfate.

We have investigated the effect of temperature, mechanical stress on the aggregates of BSA in solution and their viscosity. The mechanism of the association of BSA molecules leading to the formation of aggregates is proposed from the analysis of secondary structures of proteins, particle size, and viscosity. We have shown the difference of the continuous shear and interrupted shear on the particle size distribution, secondary structures and viscosity of protein in solution. The interruption of shear gives particles to relax to form a bigger aggregated structure.

Subsequently, we have investigated the effect of surface and mechanical stress on the aggregation of the Insulin in solution at 40°C. The interrupted shear leads to the formation of bigger particle size of HI with a more proportion of intermolecular β-sheet in solution. There is evidence of desorption of adsorbed insulin from the surface to the solution during the shaking period in its folded state. The deformed proteins lead to subsequent aggregation process. The particle size of insulin in the presence of surfaces is independent of the surfaces used, with an exception for PTMS surface. The unstructured PTMS surface leads to the higher particle size with more random coils in its secondary structure.

सार

ठोस सतहों पर प्रोटीन का सोखना जैव-सामग्री, बायोसेंसर, शुद्धि और चिकित्सीय प्रोटीन आदि के भंडारण को डिजाइन करने में मौलिक मुद्दा है। कई अध्ययनों के बावजूद, सतह पर प्रोटीन के सोखना और उनके बाद के व्यवहार सोखना एक प्रोटीन की अंतर्निहित जटिल प्रकृति के कारण और एक सोसाइटी सतह की अनगिनत संभावना के कारण पूरी तरह से दूर हैं। पीएच, नमक एकाग्रता, बफर प्रकार, तापमान, मैकेनिकल तनाव, आदि जैसे विभिन्न बाह्य कारकों पर प्रोटीन के बहुत से अलग-अलग कॉन्फिगरेशन की संभावना है। इसके अलावा, सतह चिकनी, मोटा, हाइड्रोफोबिक, हाइड्रोफिलिक आदि हो सकती है। यहां तक कि एक चिकनी सतह पर अणुओं का मोटापा हो सकता है जो कि प्रोटीन के सोखना को नियंत्रित कर सकते हैं।

सतहों के प्रभाव को समझने के लिए, सब्सट्रेट पर वांछित रासायनिक कार्यक्षमता के आत्म-इकट्ठे हुए मोनोलायर (एसएएम) पर एक प्रोटीन के सोखना व्यवहार को देखने के लिए प्रथा है. अभिकर्मक बनाने वाले सैम के रासायनिक प्रकृति, जैसे कि प्रतिक्रिया की स्थिति, कारकों की संख्या की एक संख्या के कारण सतह पर कई भौतिक और रासायनिक विविधताएं दिखाई देती हैं।. सतह ऊर्जा, जो मैक्रोस्कोपिक संपत्ति है, सतह के सूक्ष्म गुणों पर निर्भर करती है।. सूखा प्रोटीन की सतह ऊर्जा पर सूक्ष्म और मैक्रोस्कोपिक खुरदरापन और सोखना सतह की सतह ऊर्जा पर निर्भरता का अध्ययन किया गया है। तीन अलग-अलग रासायनिक क्रियात्मकताएं, अमाइन, हाइड्रॉक्सिल और ऑक्टील, के सतहों को सिलिका सतहों पर स्व-इकट्ठे हुए मोनोलायर के माध्यम से प्राप्त किया गया था और लाइसोसिम और गोजातीय सीरम एल्बूमिन के सोखना के प्रति प्रतिक्रियाओं के लिए परीक्षण किया गया था।

कई बार, इन एसएएम को प्रतिक्रिया की स्थिति में उपयुक्त रिएक्टरों के साथ बनाया जाता है, जो कि अनुकूलित नहीं है। हमने तीन सिलेन युग्मन एजेंट्स (एससीए) का परीक्षण किया है जिसमें प्रोलीन एमाइन शामिल हैं, जो इस पर मौजूद सी से जुड़े विभिन्न समूहों के साथ हैं। इसके अलावा, हमने मिथाइल सिर समूह के लिए एसएएम तैयार करने के लिए एक सिलेन युग्मन एजेंट का इस्तेमाल किया है। सतह के प्रति इन अणुओं का दृष्टिकोण सिर समूह और एससीए के सी से जुड़े समूहों पर निर्भर करता है। सतहों के आकृति विज्ञान का विश्लेषण शक्ति वर्णक्रमीय घनत्व वितरण, तिरछा, दीर्घवृत्ताकार मोटाई, और सतह ऊर्जा का उपयोग किया जाता है। यह विभिन्न

सतहों पर सूखा इंसुलिन के आकारिकी से पाया जाता है कि एसएएम की कम एकाग्रता से प्राप्त सतह पर बड़े आकार के समुच्चय की कम संख्या का गठन होता है, जबकि उच्च संख्या में, लेकिन छोटे अनाज का आकार समुच्चय का गठन होता है एसएएम संशोधक के 1% एकाग्रता से प्राप्त सतहों पर. हमने सब्सट्रेट्स की विशेषताओं के साथ-साथ सूखा प्रोटीन, सोखना इज़ोटेर्म, सोखना कैनेटिक्स, विस्कोइलिस्टिक, सूखा प्रोटीन की गुणधर्म और एक सर्फटेन्ट द्वारा सूखा प्रोटीन की वपस जान के लक्षणों के साथ आपसी संबंध का पता लगाया है। क्वार्ट्ज क्रिस्टल सूक्ष्मता का उपयोग सोखना और सूखा प्रोटीन की लोच के कारण बड़े पैमाने पर परिवर्तन को निर्धारित करने के लिए किया जाता है। सोखना इस्तोमेट फिट करने के लिए आवश्यक पैरामीटर, परतों की एक सीमित संख्या के लिए बीईटी समीकरण द्वारा दिए गए, विभिन्न आणविक भार के चार प्रोटीन की लोच को समझाने के लिए इस्तेमाल किया जा सकता है, सतहों पर उनकी लोच को समझाने के लिए इस्तेमाल किया जा सकता है. इसके अलावा, सोडियम-डी-डीसीएल सल्फेट का उपयोग करके प्रोटीन के वपस जान व्यवहार को समझाने के लिए पैरामीटर का उपयोग किया जा सकता है।

हमने तापमान के प्रभाव, बीएसए के समुच्चय पर यांत्रिक तनाव और उनकी चिपचिपाहट की जांच की है। समुच्चय बनाने के लिए अग्रणी बीएसए अणुओं के संघ के तंत्र को प्रोटीन, कण आकार, और चिपचिपापन के माध्यमिक संरचनाओं के विश्लेषण से प्रस्तावित किया गया है। हम कण आकार वितरण, माध्यमिक संरचनाओं और समाधान में प्रोटीन की चिपचिपाहट पर सतत कतरनी और बाधित कतरनी का अंतर दिखा चुके हैं। कतरनी का रुकावट एक बड़ा एकत्रित संरचना बनाने के लिए कणों को आराम करने के लिए कण देता है।

इसके बाद, हमने सतह के प्रभाव और 40°सी तापमान पर समाधान में इंसुलिन के एकत्रीकरण पर यांत्रिक तनाव की जांच की है। बाधित कतरनी, हाई के बड़े कण आकार के गठन के साथ-साथ समाधान में इंटरमॉलिक्यूलर बीटा-शीट के अधिक अनुपात के साथ होता है। सतह से घिसाए हुए अवस्था के दौरान सीलबंद इंसुलिन का समाधान करने के साक्ष्य हैं। विकृत प्रोटीन बाद में एकत्रीकरण प्रक्रिया को जन्म देते हैं। सतहों की उपस्थिति में इंसुलिन का कण आकार पीटीएमएस सतह के लिए एक अपवाद के साथ उपयोग की जाने वाली सतहों से स्वतंत्र है। अनस्ट्रक्टेड पीटीएमएस सतह इसकी माध्यमिक संरचना में अधिक यादृच्छिक कॉइल के साथ उच्च कण आकार की ओर ले जाती है।

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Abbreviations and Notations

SCA	Silane Coupling Agents
SAMs	Self-Assembled Monolayers
APTMS	3-Aminopropyl tri methoxy silane
APTES	3-Aminopropyl triethoxysilane
APDMES	Aminopropyldimethylethoxy silane
PTMS	Propyltrimethoxy silane
OTS	Octyl tri methoxy silane
OTCS	Octadecyltrichloro silane
GPTMS	3-Glycidyloxypropyltrimethoxy silane
AFM	Atomic Force Microscopy
ATR-FTIR	Attenuated Total Reflection Fourier Transform Infrared Spectroscopy
STM	Scanning Tunneling Microscopy
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
PM-IRRAS	Polarization Modulation-Infrared Reflectance Absorbance Spectroscopy
QCM-D	Quartz Crystal Microbalance-Dissipation
PBS	Phosphate-Buffered Saline
TBS	Tris Buffer Saline
BSA	Bovine Serum Albumin
LSZ	Lysozyme
β-gal	β-galactosidase
Mb	Myoglobin
HI	Human Insulin
MI	Di-Iodomethane

EG	Ethylene Glycol
SE	Surface Energy (mJ/m^2)
E_d	Ellipsometry Thickness (nm)
γ	Surface Energy(mJ/m^2)
Γ	Adsorbed Mass (mg/m^2)