Essay describing the thesis work

Thesis title:

"SYNTHESIS, CHARACTERIZATION AND POSSIBLE APPLICATIONS OF PROTEIN - LIPID AND PROTEIN - GOLD COLLOID BIOCOMPOSITE MATERIALS."

Currently nanotechnology is witnessing tremendous interest due to the unusual physico-chemical properties of nanomaterials useful for the design and development of advanced materials. More specifically, the coupling of biomolecules to nano-entities (the so called area of nanobiotechnology) leads to the formation of biocomposites, which are fundamentally as well as technologically exciting materials. The beautiful chemical/physical interactions within and on the surface of biological cells can be better understood by studying protein-lipid biocomposites. The technological perspective of studying the biomolecule-lipid interaction is towards the realization of efficient immunosensing, biocatalytic and drug-delivery systems. The study of underlying physical chemistry involved in the formation of biocomposites and their utility as efficient immobilized biocatalysts is one of the main objectives of my thesis. Furthermore, understanding nature, how it works, and using the interplay of weak noncovalent bonding interactions, for soft chemistry approaches towards synthesis and rational design of advanced materials, is a topic of tremendous current interest. A part of my thesis also addresses issues towards programmed assembly of bio and nano entities, conjugation of biomolecules with nanoparticles for possible immunosensing and bio-labeling etc.

The motivation for this work came from earlier studies performed by Sastry *et al* on spontaneous organization of thermally evaporated fatty lipid films by an ion-exchange process. The fascinating part of this work was that such ion exchange leads to an organized lamellar film structure similar to *c*-axis oriented Y-type Langmuir Blodgett films. Recognizing the generality of this principle, the approach was extended towards the formation of nanoparticle thin films. The important question was whether this protocol could be successfully extended to intercalation of proteins/enzymes with negligible distortion to their three-dimensional (tertiary) structure, thus enabling them to perform their biological functions without hindrance. What would be the nature of the interactions? Would the entrapped protein be accessible to cofactors and reagents? Would the matrix protect the protein against harsh conditions? A successful attempt towards understanding some such interesting queries has been done and presented in my thesis.

The early breakthrough was achieved when proteolytic enzymes pepsin and fungal protease (F-prot) were first immobilized with negligible distortion to their natural conformation. It was wondered at that time, whether the immobilized enzyme would be biologically active in the entrapped film form. To our excitement, we found that the biocomposite film not only showed excellent biological activity, comparable to the same amount of free enzyme in solution, but also was reusable. The reusability decreased with the number of reaction cycles possibly due to “poisoning” of the enzyme by substrate molecules, and blocking the active sites to new substrate molecules. An interesting result indicated the role of entrapped water in the retention of biological activity as a function of ageing in air. The real challenge was while working with a delicate system such as the endoglucanase-lipid biocomposite. The loss in biological activity upon immobilization of this system, made us to ponder upon the role of local molecules present near the active site of the molecule. We discovered that “protecting” the active site of the enzyme prior to immobilization resulted in retention of the activity and enhancement of thermal and pH stability. Furthermore, it was also interestingly observed that the proteins were intercalated *within the hydrophilic regions* of the lipid bilayers rather than a mere surface adsorption.

Having shown the feasibility of the process, the research was further focused to dig out the intricacies involved in the immobilization process. Role of electrostatics in the entire immobilization process was brought out by demonstrating the formation of biocomposite of two different heme proteins (cytochrome *c* and hemoglobin), with different isoelectric points (pI). It was found that electrostatics to a large extent influences the rate and amount of immobilization. The beauty of using electrostatic interaction was demonstrated by leaching out the immobilized protein, by simple pH variation. This demonstrates the utility of biocomposite films as “off the shelf” kind of protein storage chips, wherein protein could be stored and then “pumped out” when required. Another important observation was the contribution of secondary interactions such as hydrogen bonding and hydrophobic interactions in the immobilization process. Deeper insights into the immobilization process were achieved with the development of 1-D Fickian type diffusion model. The parameters extracted using this model did suggest a significant role of electrostatics in the immobilization process. But some strikingly varied results prompted us to investigate the true influencing factors for the immobilization process. A variety of proteins differing in charges and masses when

analyzed by this model revealed the role of protein charge: mass (e/m) ratio governing the rate of the diffusion process.

Nanotechnology also demands a programmed assembly of entities at desired locations due to the tremendous interest in biochips for multi-analyte bio-assays. An attempt on assembling different proteins and nanoparticles on a single substrate was successfully demonstrated. One important finding was made when we discovered that preordered films could reduce the time required for protein immobilization. This is important especially when delicate industrial enzymes are concerned, which could denature during slow immobilization process. Interestingly, these preordered biocomposite films showed enhanced biological activity possibly due to the preformed channels and pores for efficient transport of analytes. This is important as regards to the famous *mass transport problem* where the biocatalytic activity is limited due to slower diffusion of the substrates to the entrapped enzymes. Furthermore, we synthesized bioconjugates of enzymes with colloidal gold by simple mixing and washing protocol. Such bioconjugates were more stable against temperature and showed excellent biological activity.

According to current trends, interdisciplinary research is the key to advanced materials. In this context my thesis work involving highly interdisciplinary ideas, where synthesis and rational assembly of biologicals using soft chemistry approach (the way Nature works!), ability to control a variety of parameters, enhanced stability and generality of approach are highly advantageous. One could extend this protocol for immobilization of DNA, cells and antibodies and so on. This technique has exciting potential for application in various areas of biocatalytic and biomedical applications and for the design and synthesis of novel advanced materials.

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