

National Textile Center

FY 2003 (Year 12) Continuing Project Proposal

Project No. M02-CD05

Competency: **Materials**

Functional Fibers for Immobilization of Biomolecules

Project Team:

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Objective:

The goal of this project is to generate novel fibrous supports for encapsulation/immobilization of biomolecules. The focus is to exploit surface chemistry principles for creating reactive fibrous materials that are capable of binding with biomolecules. Several synthesis and processing methods are being explored for the development of fibers capable of containing biomolecules, specifically enzymes. These solid-supported enzymes will be tested for preservation of enzymatic activities. This research pioneers the concept development of using fibrous supports for the conservation, recovery and reuse of specialty enzymes.

Progress Statement:

Synopsis of First-Year Progress: This research is to immobilize enzymes onto fibrous supports to serve as reusable and removable catalysts which have potentially improved storage and operational stability. A hydrolyzing enzyme, triacylglycerol ester hydrolases (EC 3.1.1.3), has been identified as the model biomolecule. Methods to assay the activities of this enzyme in its soluble form as well as in immobilized solids have been developed. Several chemical approaches have been exploited to activate fiber surfaces and to incorporate enzyme molecules. One specific linking system using chemical bonding mechanism to bind this enzyme to fibrous materials has been successfully developed. Work has begun in assessing and comparing effectiveness of the activation mechanisms, efficiency of enzyme incorporation and the activities of the immobilized enzymes. The activities of these bonded-enzymes are being compared with free enzymes as well as physically encapsulated enzymes.

In our first year, we have focused on exploring chemical and physical means for the purpose of immobilizing enzymes. Specifically, covalent bonding to solids using compounds such as crosslinking and multi-functional reagents and surface active functional reactants, have been investigated. Among these methods, chemical covalent bonds that offer the strongest links and yield the most stable enzyme-solid complexes, have been targeted. To chemically bond enzymes to a solid, the structures and functions of both the enzymes and the solids should be considered. Of functional groups on enzyme proteins that can be efficiently covalently bonded, we have targeted the amino $-NH_2$ group or the ϵ -aminolysine of the enzyme. Work on reactions of enzymes has shown that physico-chemical properties of enzymes modified at ϵ -aminolysine are only slightly altered, even in markedly modified proteins, suggesting that ϵ -aminolysine residues are non-essential for catalytic activity.

With regard to solids, we have explored and evaluated methods to incorporate enzymes in several fibers. While surveying and developing chemical strategies to offer various linkers or spacers with functional end groups that can further react with enzyme molecules, we have specifically selected one versatile linker system to begin. Specifically, we have devised methods to 1) incorporate enzymes and proteins in fibers, 2) to activate fiber surfaces and to introduce chemical links that can react with enzyme proteins, and 3) to devise methods to evaluate enzyme

activities. Several material characteristics, such as surface areas, wetting, and diffusion properties have been examined.

We have selected a hydrolyzing lipase enzyme, i.e., lipase (triacylglycerol ester hydrolyses, EC 3.1.1.3), as the model biomolecule to be attached to fiber surfaces. The primary focus of the first year has been placed on the introduction of functional groups on fiber surfaces to regulate surface architecture, the chemical characteristics of the spacers (length and structure) and functional groups. These primary functional groups are then extended to include spacers or converted to specific active functional groups. To react with ϵ -aminolysine, potential functional groups have been identified and include those containing primary functional groups of hydroxyl -OH, carboxylic -COOH, and aldehyde -C(O)H. Since few polymers contain these primary functional groups, reactive functional groups must be introduced to the surfaces of these to fibers. We have selected compounds with acylating end groups to react with the highly reactive ϵ -aminolysine to form peptide bonds.

The method to determine the catalytic activities of the lipase enzyme was also devised. A triglyceride emulsion was prepared by emulsifying 5 g olive oil in 95 ml NaCl (0.89%) solution using gum arabic as an emulsion reagent for 10 min. The incubation mixture was prepared by mixing olive suspension, 10 mM deoxycholate and 1 M triethanolamine buffer (pH 8.5) at the volume ratio of 50:5:45, final concentration being 30mM, 0.5 mM and 0.5 M, respectively. Lipase activity was measured at 30°C and pH 8.5. Immobilized lipase was added to 1.0ml incubation mixture, incubated in a 30°C bath equipped with shaker and denatured by heating for 10 min at 90°C. 5.0 ml chloroform and 2.5 ml copper reagent are added and mixed in a shaker. The mixture was centrifuged for 5 min to separate the phases and the aqueous phase was removed. To assay the enzyme, 2.0 ml chloroform layer was mixed with 0.25 ml of 11 mM diethyldithiocarbamate. Photometric determination is performed at 440 or Hg 436 nm at ambient temperature against the corresponding sample blank that can be prepared in the same procedure except that enzyme is not activated before the assay.

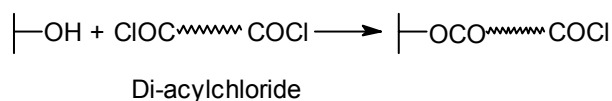
In sum, we have accomplished 1) the selection of an enzyme, 2) the modification and activation of fiber surfaces which are reactive toward the ϵ -aminolysine of the enzyme, 3) the development of an assay to evaluate enzyme activities, and 4) the applications of these toward binding enzyme on selected fibrous materials.

Next Year's Goals:

The goal in the second and third years is to explore and evaluate a broader range of surface activation mechanisms of model fibrous materials and their binding potential with enzymes. The model fibers will be expanded to include those of both natural and synthetic polymers. Additional enzymes will be sought for as model enzyme in addition to the one hydrolyzing enzyme that has been identified. The effectiveness of the activation mechanisms, efficiency of enzyme incorporation, and enzyme activities will be accessed.

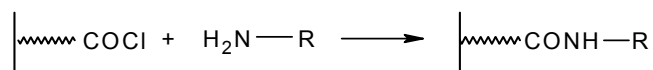
Approach:

The most fundamentally significant aspects of this research are methods of activating the fibers and coupling with the enzymes. The following reaction schemes exemplify the conversion of carboxylic group to acyl chloride of a linker:



The nature of the linker depends on its structure that can be either hydrophilic or hydrophobic. The linkers can be found to hydroxyl containing groups on the fiber surfaces to either reactive acid/acyl chloride end groups or a crosslinked structure. We have been able to optimize the reactive product by controlling reaction conditions.

The surface modified fibers with either acid or acylchloride end groups can then react with the amine of the ϵ -aminolysine in enzyme protein to form amide bonds.



The efficiency of this enzyme coupling reaction was investigated in terms of temperature, pH, catalyst and time. The effects of the length and structure of linker will be examined.

Outreach to Industry:

These investigators seek collaboration with other researchers from academia as well as industry to develop fibrous supports of various polymer compositions and to identify specific enzymes. Collaboration with the government is anticipated. Researchers at US Army Soldier Biological & Chemical Command centers and Medical Command have been investigating methods towards the development of wipes, sponges, and clothing for the decontamination of equipment and personnel upon exposure to toxic chemicals and biological agents. These groups have expressed interest to collaborate with us to transition our results to military for decontamination as well as other applications. In the long run, industrial partners from both the polymer/fiber/ textile industry as well as the biotechnology sector will be sought for further development of the concept and transferring of the developed technology to all affiliated industries, linking university and industrial partners.

New Resources Required:

A major portion of the proposed budget is for supporting graduate students and research consultants, acquisition of chemicals and supplies, recharge for advanced instrument use, and domestic travel. To expand the scope of fibers, linking mechanisms, and possibly enzymes, resources to support the increased research activities are expected to increase for the second and third years. In the second year, a rheometer for determining the critical visco-elastic properties of the polymer solutions and a Raman spectrophotometer for structural characterization are needed.