Immobilized Enzyme System for Lignocellulosic Biomass Saccharification

Investigators
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Abstract

The overall objective of this research is to enhance the bioactivity of cellulase enzymes as well as the stability of the enzymes to increase it reusability through immobilization of the enzymes in the microenvironment of porous inorganic-organic hybrid sol-gel polymers. The high cost of cellulase enzymes makes it desirable to be able to reuse the enzymes for the efficient conversion of cellulosic material into fermentable sugars, which is perhaps the most critical step in the bioconversion process. Recently, we have discovered that the microenvironment of these sol-gel polymers markedly increases the bioactivity of immobilized enzymes. Using these polymers for the confinement of the enzymes or as supports to covalently attach them will allow us to explore the effects to these two different approaches to immobilization on the bioactivity and reusability of cellulase enzymes. We have prepared polymers of different porosities in which β-glucosidase, a cellulase enzyme, has been covalently immobilized. At room temperature, the immobilized enzyme in a sol-gel polymer with low porosity was able to partially hydrolyze D-(-)-salicin to saligenin in a 5-minute period, while a room-temperature hydrolysis of salicin with the enzyme did not produce saligenin. The higher porosity polymers with immobilized enzymes are being evaluated.

Objectives

The overall goal of our project is to improve the enzymatic hydrolysis of cellulosic materials through the development of a confined enzyme system. The specific objectives are:

(i) Synthesis of porous polymeric materials for immobilization of cellulase enzymes;
(ii) Development of a fundamental understanding of how confined, immobilized enzymes are able to increase their activity; and
(iii) Bioconversion of cellulosic materials into fermentable sugars.

Our study initially involves the immobilization of β-glucosidase and its bioactivity toward the hydrolysis of the substrate salicin to produce saligenin and glucose.

Background

The most critical step in the cellulose-to-ethanol bioconversion appears to be saccharification, the enzyme hydrolysis of cellulose to produce fermentable sugars that can yield ethanol. The high cost of cellulases makes it desirable to be able to reuse the enzymes and increase their bioactivity. Recently, we have discovered that confined enzymes that have been immobilized in microenvironments have markedly increased bioactivity. Specifically, we have reported that pepsin immobilized in a porous inorganic-organic hybrid sol-gel monolith exhibited a 700-fold increase in bioactivity[1] and similarly encapsulated trypsin was found to have enhanced stability and bioactivity.[2,3] Covalent attachment of trypsin on a similar hybrid sol-gel polymer resulted in a 2000-fold increase in the bioactivity of the enzyme for the hydrolysis of benzoyl-L-arginine ethyl ester, a substrate commonly used to determine the bioactivity of trypsin.[4] These porous hybrid sol-gel polymer monoliths indeed appear to be good candidates

for the confinement of enzymes, resulting in increased enzymatic hydrolysis at room temperature.

Fundamental understanding of how confined, immobilized enzymes are able to increase their activity is presently lacking, although several hypotheses exist. The purpose of this study will be to elucidate this enzymatic enhancement mechanism and to apply it to the pressing need of converting biomass to biofuel.

Results

Our study of confined enzymes starts with β-glucosidase, one of the cellulase enzymes. In the first 5 months of this project, we have prepared immobilized β-glucosidase in a porous sol-gel polymer monolith following a previously developed protocol for enzyme immobilization. The immobilized enzyme-sol-gel polymer system is prepared inside a small-bore fused-silica capillary to allow for on-line chromatographic separation and detection of the hydrolysis products. Figure 1 shows how the polymer is prepared and how the enzyme is immobilized onto the polymer. Different porous sol-gel polymers have been prepared by varying the amounts of porogen and the ratio of acid catalyst to silicate content. Figure 2 is an SEM image of one of these sol-gel polymers, showing its porous nature.

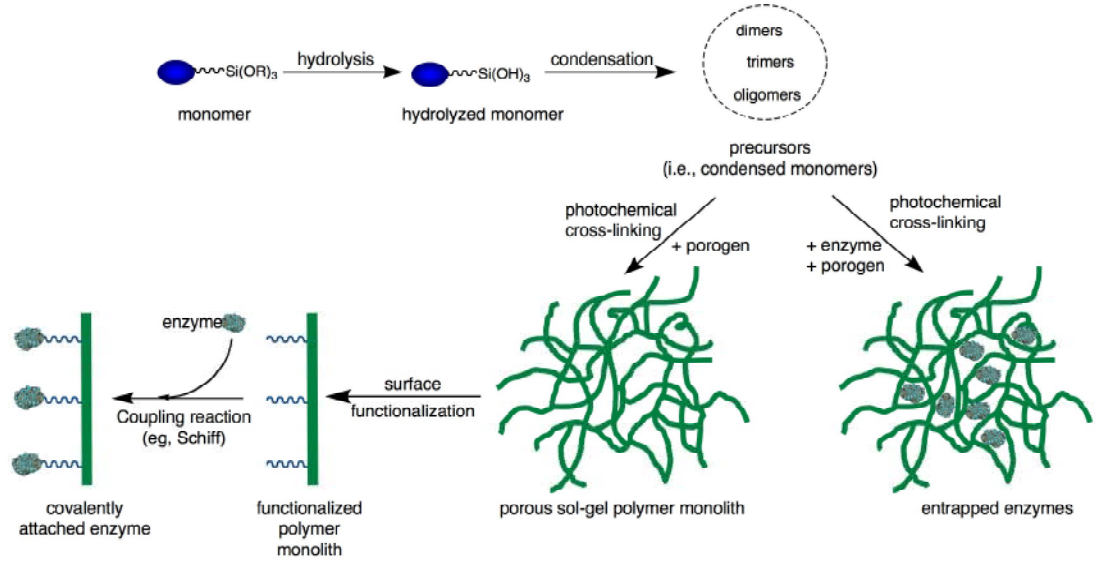

Figure 1. Schematic diagram of porous inorganic-organic sol-gel polymer preparation and immobilization of enzyme by covalent attachment and entrapment. ⬬ is a photoactive group and R is an alkane chain.

The first generation immobilized-β-glucosidase-sol-gel polymer was evaluated. At room temperature, hydrolysis of salicin was observed by monitoring the formation of saligenin by UV absorbance (Figure 3). An increase in the residence time of salicin in the enzyme-sol-gel polymer by 5 minutes led to a 38% increase in the absorbance of saligenin. This result is remarkable given that saligenin was not observed when the hydrolysis was conducted in free solution at room temperature with a higher concentration of the enzyme. (β-Glucosidase is typically used at 37°C.)

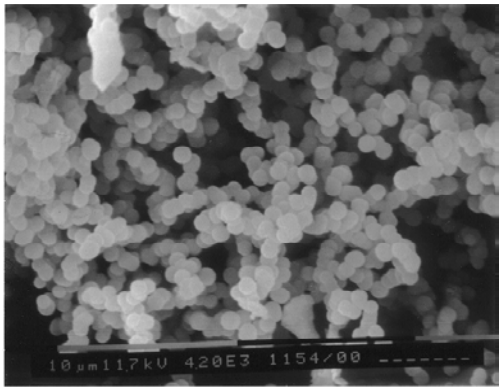

Figure 2. Scanning electron micrograph of a porous sol-gel polymer monolith (without enzyme).

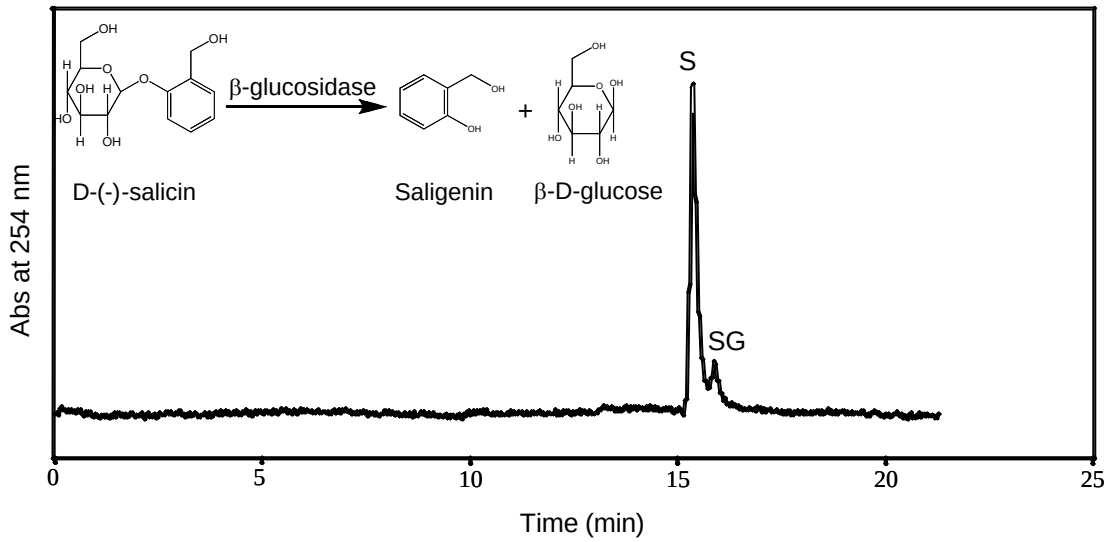

Figure 3. Separation of salicin (S) and saligenin (SG) after hydrolysis of salicin on a β-glucosidase enzyme immobilized in a sol-gel polymer monolith. Saligenin was formed after a 5-minute reaction with the immobilized enzyme.

Three separately prepared first generation immobilized enzyme systems were evaluated. It was found that all three first generation systems had similar bioactivities up to 30 uses.

Future Plans

Future plans are focused on the evaluation of immobilized cellulase enzymes in sol-gel polymers of different porosities to determine what physical structure of the polymer will provide optimal conversion of substrate to products. It will follow that a comparison of enzyme stability under optimal conditions for cellulase bioconversion and kinetics will be made between entrapped cellulase enzyme, covalently attached enzyme, and free enzyme. The kinetics of enzyme hydrolysis will be monitored by the Michaelis constant (K_m) and the maximum velocity (V_{max}) as a function of substrate concentration using immobilized and free cellulase enzyme. The operational stability of the immobilized cellulase enzymes at higher temperatures for increased rate of reaction will be an important aspect of our future plans.

References

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