Immobilization of Enzymes on Nanoparticles for Catalysis in Non-aqueous Media

Barry, J., Carver, J., Sanford, L., Vertegel, A.
Clemson University, Clemson, SC, USA

Statement of Purpose: Enzymes are highly functional biomolecular catalysts that have extremely high specificity and very fast reaction rates. The properties and structure of an enzyme can be to a large extent affected by its environment. Reaction medium, salt content, temperature and many other variables play important roles in the activity of an enzyme. Enzymes have been shown to have activity in non-aqueous media, though considerably lower than the activity in native aqueous conditions. The reason for such decreased activity is enzyme unfolding in the presence of a non-aqueous solvent. It has also been shown that enzymes attached to nanoparticles are stabilized in conditions that would normally negatively affect the activity of an enzyme. We hypothesized that activity in non-aqueous media would be higher for enzyme attached to nanoparticles than for free enzyme. One application of such a system would be in the purification of fossil fuels. Our ultimate goal is to use enzymes stabilized on nanoparticles in organic solvents to remove sulfur compounds from fuels by oxidative desulfurization.

Methods: Chloroperoxidase (CPO) is the enzyme of choice for these experiments because it has been shown to exhibit oxidative desulfurization in non-aqueous media. Latex nanoparticles with a diameter of ~40 nm surface modified with chloromethyl reactive groups were used in the experiments. Attachment of CPO to nanoparticles was performed in phosphate buffer saline overnight at room temperature. CPO-nanoparticle conjugates were separated by centrifugation. A Micro BCA protein assay (Pierce) was used to quantitatively evaluate enzyme attachment to nanoparticles in five different initial mixtures: 400%, 200%, 100%, 50% and 25% of a theoretical monolayer. The nanoparticle conjugates were then lyophilized. Enzyme desorption from the nanoparticle surface was studied using fluorescently labeled chloroperoxidase. Enzyme activity assays were performed both in aqueous and non-aqueous media. Aqueous activity assays were performed in deionized water with 20 mM N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) and 2 mM hydrogen peroxide as the enzyme's oxygen source. Activity was measured by following the oxidation of TMPD and monitoring the change in absorbance spectrophotometrically at 562 nm. Non-aqueous activity assays were performed in 99.5% ethanol. Activity in ethanol was measured by using the same substrate that was employed for aqueous media. Free lyophilized enzyme was used as control; concentrations of free and immobilized enzyme were kept identical in all kinetic experiments. The stabilization effect of immobilization was determined by comparing the activity of free enzyme and conjugated enzyme after different aging periods.

Results: Amine groups of chloroperoxidase react with chloromethyl groups on the nanoparticles resulting in covalent attachement of the enzyme to nanoparticles. After overnight incubation of chloroperoxidase to nanoparticles, there was nearly a monolayer in the two higher concentrations with steps down in each dilution thereafter. Results from the leaching study revealed that there was a loss of ~4% of the immobilized enzyme over the 72 h incubation in an aqueous buffer.

Activity assays were performed on the enzyme-nanoparticle conjugates as well as free enzyme to see the effect of immobilization on nanoparticles on enzyme activity. We observed enzyme activity in both the free enzyme and the enzyme-nanoparticle conjugates in 99.5% ethanol. The comparison of enzyme activity in aqueous and non-aqueous conditions proved that the free enzyme was much more active in water than the conjugate, but our results indicate that the free enzyme activity drops a quarter below the conjugates level of activity after two days of storage. Non-aqueous results indicate similar initial rates in the enzyme-nanoparticle sample when compared to free enzyme. These findings support the hypothesis that the enzyme will be stabilized when attached to the nanoparticle. We expect to find further evidence that the half-life of the enzyme increases in the conjugate as compared to the free enzyme. These favorable outcomes indicate that this method of immobilization may result in higher enzyme activity and stability in harsh environments.

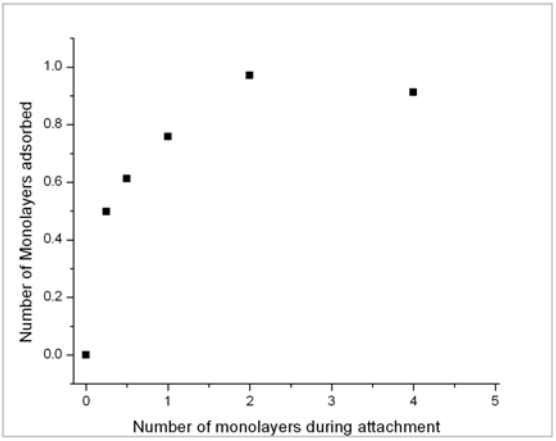

Figure 1. Attachment data for CPO on chloromethyl latex reaches a maximum at a monolayer.

Conclusions: We demonstrated that reaction in non-aqueous media occurs for both free enzyme and enzyme immobilized on nanoparticles. The data also indicates that there is an increase in enzyme stability and activity in non-aqueous media when attached to nanoparticles. Additional steps are necessary to optimize the reaction conditions in non-aqueous media. In addition, nanoparticle conjugates are much easier to retrieve from the reaction mixture than free enzyme. The future steps include a pilot study on oxidation of thiophene by chloroperoxidase-nanoparticle conjugates suspended in diesel fuel.