

FY 2008 New Project Proposal
Project Number: BUSC-08

Enzymatic Polymerization

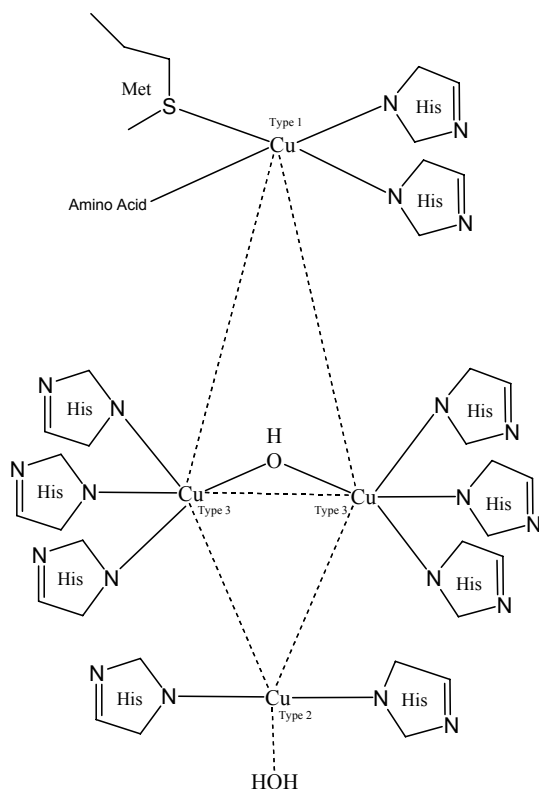
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Objective:

With predictable decrease in petroleum in the very near future and concomitant increase in cost of both manufacturing and market price of polymeric materials, alternative synthesis routes have to be developed that encourage the augmented use of renewable resources and/or agricultural by-products. Organic synthesis of polymers via enzymatic catalysis presents a novel route of green chemistry. Enzymatic processes are energy-efficient and environmentally sound. The proposed project will expand on previous research experience using oxidoreductases to couple low-molecular weight substrates under mild conditions in aqueous solution. Products will be characterized for their molecular weight, their inherent color, surface characteristics and structural features, and related to the redox potential of the enzyme as well as the structure of the starting compound.

State of the Art:

Enzymatic polymerization, a so far inadequately explored route to conventional synthesis, can



be achieved by various types of enzymes, such as transferases and oxidoreductases among others. Polyphenols, polyanilines and vinyl polymers of varying molecular weight could be synthesized by enzymatic means [Uyama, 2003; Karamyshev, 2003]. In the case of oxidoreductases, transfer of electron(s) or hydrogen(s) occurs in the presence of suitable substrates, producing reactive radicals that subsequently couple to first form oligomers, then polymers. In our research group most previous experience is based on laccase catalysis for polymerization of substituted phenols [Kenealy, 2006; Stephen, 2007].

Fig. 1: Active center of laccase according to Mita (2003).

In all reactions, required experimental conditions and product characteristics strongly depended on the origin of the laccase and thus, on its redox potential. In Fig. 1 the active center of a laccase from *B. subtilis* is presented as an example [Mita, 2003]. It has been confirmed that substrate oxidation occurs at the Type 1

copper center which is also responsible for the blue color of these multi-copper proteins due to the absorbance band of the Cu-cysteine bond at 600 nm. The “amino acid” connected to Type 1 Cu varies, depending on the source of the laccase, and influences its redox potential. Types 2 and 3 Cu form a trinuclear cluster and are thought to be responsible for reduction of molecular oxygen and release of water (Fig. 2).

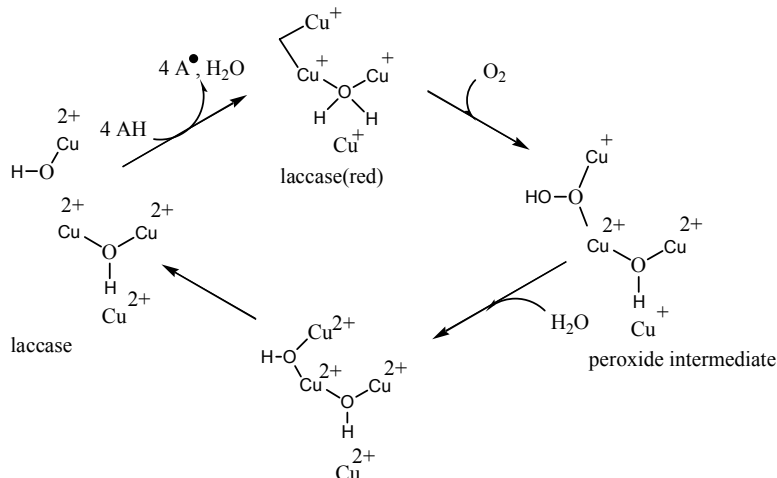


Fig. 2. General reaction mechanism of a laccase

During the reduction of the substrates (substituted phenols, anilines, etc.) reactive radicals (e.g., phenoxy) are formed which further react to form oligomeric and polymeric products with covalent C-C, C-N, and C-O bonds. Some of

these products further proceed via non-enzymatic cross-linking reactions.

It was observed that polymeric reactions with laccases function much faster and to a higher yield when lignin was present [Kenealy, 2006, Felby, 2002], a fact that is not surprising considering that laccases are involved in both lignin biosynthesis as well as delignification reactions. A fairly large number of phenolic substrates with different substituents has been polymerized on pulp [Kenealy, 2006; Buschle-Diller, 2003] leaving a soft hydrophobic coating which sometimes developed a distinct color. The products were not isolated from the pulp fiber material but used to improve fiber/fiber adhesion properties.

In most recent research, the impact of free and bound lignin was investigated in connection with the enzymatic synthesis of colorants [Stephen, 2007]. The focus of this research had been to enzymatically couple lignin monomers with the potential to yield colored compounds. The products could be applied as pigments or dyes to fibrous (e.g., hair) or polymeric materials. Resorcinol, guaiacol and vanillic acid were reacted under enzymatic catalysis to result in orange, brown and dark-purple colorants, respectively. Only resorcinol-based products were water-soluble. Neither free nor sulfonated lignin enhanced the formation of colored compounds. However, lignin bound to unbleached linen fibers clearly increased the color yield.

Approach:

Based on the experience gained described above, a systematic approach to enzymatic polymerization will be performed. Guaiacol will be the foundation from which to build as a substrate since the products did not only polymerize easily but also showed some discernable color. Vanillic acid will also be included since for both guaiacol and vanillic acid already research results exist to build on. Some of the proposed starting compounds are shown in Fig. 3.

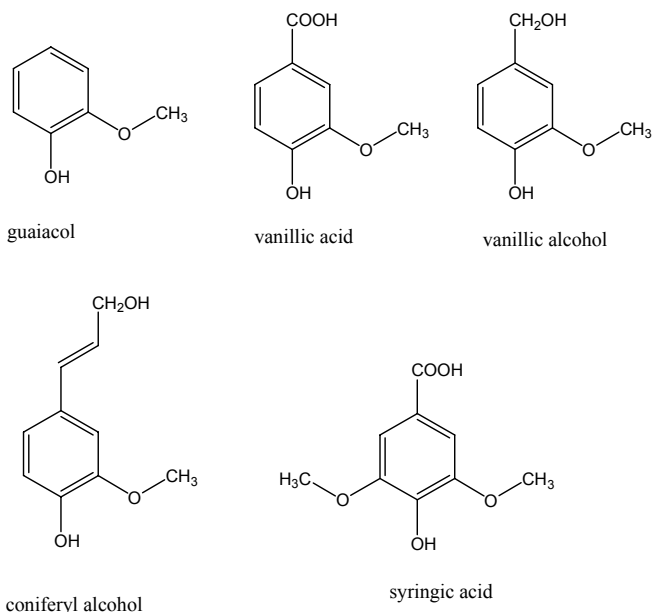


Fig. 3: Potential monomers for enzymatic polymerization.

Laccase will be used as a primary catalyst, in either free or immobilized form. The effect of an additional biocatalyst (e.g., horseradish peroxidase or tyrosinase) will be evaluated as well as the use of mediators, in regard to improved yield and/or rate of the reaction.

Natural products, such as lignin, tannin or gallic acid will be used as auxiliaries and their effect studied.

Products will be isolated and purified if possible; tools for characterization will

include FT-IR-spectroscopy, DSC, MS-GC, and light-scattering. In case the products show color, they will be incorporated in films formed from natural or biodegradable films. Structure and observed properties will be correlated with the reaction conditions and optimized where possible.

Specific Goals:

- Use of oxidoreductases as biocatalysts for polymerization reactions
- Effectiveness of immobilization of enzymes on carrier materials for repeated use and thus preparation of scale-up of the process
- Systematic selection and coupling reactions of starting monomers based on their specific structural features
- Characterization of products with potential end-use in mind (e.g., coatings, coloration of films, etc.)

Relevance to Industry - Outreach:

Theoretically, the U.S. industry has all necessary intellectual and technological tools to hold a leadership position in the area of green chemistry and engineering in the 21st Century. With petroleum resources becoming increasingly scarce and expensive, the traditional polymer manufacturing industry must start thinking outside the box. The proposed approach offers an alternative route to create materials for an array of applications. Both the polymer manufacturing as well as the enzyme producing industry (Novozymes, Franklinton, NC, and Davis, CA) have expressed their strong interest in this project.

Resources Requested:

Funds (\$6600) will be allocated as contribution towards the purchase of light-scattering instrumentation. Support for a graduate student and a postdoc are also requested.

References

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