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Enzyme-Catalyzed Regioselective Acylations and Synthesis of Sugar-Containing Monomers and Linear Polymers

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Enzymes are now well known to catalyze polymer synthesis (Gross et al., 1998). Indeed, a wide range of enzymatic polymerization reactions have been demonstrated including the synthesis of polyphenols (Dordick *et al.*, 1987), aliphatic polyesters (Geresh and Gilboa, 1990), polycarbonates (Abramowicz and Keese, 1989; Rodney *et al.*, 1998), polysaccharides (Kobayashi *et al.*, 1996), and polyamines (Liu and Dordick, 1999). In nearly all cases, the advantage of enzymatic catalysis over synthetic techniques is the high degree of reaction selectivity (e.g., stereo-, regio-, and chemoselectivity) and the use of mild reaction conditions (ambient temperature and pressure) that enable exquisite control of polymer properties.

The vast majority of polymers are prepared from rather simple monomers. While polymer structure involves complex secondary and tertiary structures, the structural diversity is inherently limited by the simplicity of common monomers. Unlike chemical techniques, highly selective enzymatic catalysis can allow the incorporation of structurally complex monomers into polymers. In particular, enzymes allow the controlled incorporation of polyfunctional monomers into macromolecules, and one need not look any further than the complex macromolecules found in nature to see evidence of the power of enzyme-catalyzed polymer synthesis.

Sugars are a particularly interesting class of polyfunctional compounds (Haines, 1981). They are biologically relevant and contain a large number of hydroxyl groups that are nearly indistinguishable chemically, yet differentiable enzymatically. Their incorporation into

polymers is a natural event, leading to a tremendously diverse universe of polysaccharides. Sugars have also been modified enzymatically and have been incorporated into polymers quite distinct from polysaccharides. In our previous work, we have used chemoenzymatic strategies to incorporate sugars into polyacrylates (Martin *et al.*, 1992; Chen *et al.*, 1994), polyamides (Patil *et al.*, 1991b), and polyamines (Liu and Dordick, 1999). These strategies resulted in rapid synthesis and polymers with high molecular weights to provide materials that have been used as highly swelling hydrogels, viscosity enhancers, and drug delivery matrices. We have also used a wholly enzymatic strategy to incorporate sucrose into polyesters (Patil *et al.*, 1991a), thereby yielding biodegradable materials. Specifically, in the presence of bis(2,2,2-trifluoroethyl)adipate or vinyl adipate, an alkaline protease from a *Bacillus* species catalyzed the synthesis of poly(sucrose adipate) in anydrous pyridine. Unfortunately, the M_w of the polymer was only 2,100 and the polymerization time was unacceptably long. Nonetheless, this was the first reported case of using enzymes to incorporate a sugar into a polyester backbone. In our continuing work described herein, we have improved the productivity and polymer size of sugar-containing polyesters using a completely enzymatic strategy.

Linear polyesters are formed via the polycondensation of equimolar concentrations of a diol and a diacid (or diester). Acid- or base-catalyzed polyester synthesis is fast and efficient but proceeds with essentially no selectivity on multifuntional monomers, such as sugars. In the present work, as part of our on-going interest in sugar-based polymers, we have taken advantage of the exquisite regioselectivity of lipase- and protease-catalyzed acylation reactions in organic solvents to yield sugar esters that can then undergo subsequent polycondensation reactions.

Commercially available proteases and lipases were screened for their ability to acylate regioselectively sucrose and trehalose with divinyladipic acid ester. Opticlean M375 (subtilisin from *Bacillus licheniformis*) was observed to form sucrose 1'-O-adipate and trehalose 6-O-adipate in anhydrous pyridine. Novozym-435 (lipase B from *Candida antarctica*) catalyzed the synthesis of sucrose 6, 6'-O-divinyladipate and trehalose 6, 6'-O-divinyladipate in acetone.

These diesters were then employed as monomers in polycondensation reactions with various diols (aliphatic and aromatic) catalyzed by Novozym-435 in organic solvents to yield linear polyesters with M_w's up to 22,000 Da. Spectroscopic analysis confirmed that only the vinyl end groups of sugar esters reacted in the enzymatic polymerization with the diol, and not the internal sugar-adipate linkages.

Novozym-435 is an effective catalyst to synthesize linear polyesters from sucrose and trehalose. The same enzyme could catalyze subsequent polycondensation reactions between SDVA and various diols in acetone. Polymer M_w of 22,000 Da could be obtained in a two step process. These two-step polymer synthesis reactions utilize the exquisite regioselectivity of the *C. antarctica* lipase to produce hydrophilic and polyfunctionalized materials. Moreover, because the polymer is comprised of simple ester linkages, these materials are expected to be biodegradable. Thus, Novozym-435 is capable of catalyzing the synthesis of poly(sugar adipates) in organic solvents and catalyzing the degradation of the polymers in water.

The components of these polymers include sugars, a dicarboxylic acid (or related esters), and various diols. By employing different combinations of these components, e.g., in a combinatorial strategy, one would expect to obtain a wide range of different properties that could be screened to yield desired physicochemical properties (Michels *et al.*, 1998; Mozhaev *et al.*, 1998).

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