Enzyme Catalysis

DOI: 10.1002/anie.200702438

Enzyme-Responsive Materials: Chirality to Program Polymer Reactivity**

*Christopher J. Duxbury, Iris Hilker, Stefaan M. A. de Wildeman, and Andreas Heise**

Enzymatic catalysis has been shown to be a highly powerful tool within chemistry.[1] The in vitro use of enzymes stems from the study and observation of their role in nature, where they catalyze a vast array of different chemical transformations, often with unprecedented levels of enantio- or regioselectivity. Enzymes have also been employed in the synthesis and modification of polymers.[2] However, in most examples enzymes merely replace a chemical catalyst.

One area in which the specific advantages of enzymes are utilized is that of enzyme-responsive materials (ERMs). These substances are seen as a new class of smart materials in which a selective enzymatic action creates a macroscopic change in material properties.[3] According to Ulijn, these materials hold great promise in biomedical applications, as enzyme responsiveness allows for a mutual communication between the material and the biological environment, similar to natural biological materials.[3] A number of approaches to ERMs are described in the literature, most of which comprise relatively complex enzyme-sensitive units, such as amino acid sequences. For example, responsive materials have been reported and discussed for drug-delivery applications.[4]

Chirality, on the other hand, has not yet been utilized in ERMs. This is surprising, because chirality plays a dominant role as a recognition feature in biological interactions. In fact, many enzyme/substrate combinations exhibit exceptionally high biological selectivity, such that an analogy with the binary code of information technology can be drawn. We are interested in exploiting this high selectivity for the design of functional materials that respond to the interaction with an enantioselective enzyme with a property change. Moreover, we are aiming for polymers in which the extent of the property change can be encoded or programmed into the material by its chiral composition. A successful system must address three fundamental requirements: 1) chiral monomers, which can be synthesized in high purity; 2) a suitable enzyme that retains a high stereoselectivity towards the chiral polymer; and 3) an accurate encoding and enzymatic readout of the polymer.

[*] Dr. C. J. Duxbury, Dr. I. Hilker, Dr. S. M. A. de Wildeman, Dr. A. Heise
DSM Research
P.O. Box 18, 6160 MD Geleen (The Netherlands)
Fax: (+31) 46-476-0508
E-mail: andreas.heise@dsm.com

[**] This work was supported by the Marie Curie Action EIF program "Enzymatic Approach to Chiral Multifunctional Materials for Advanced Applications" (EnzyMat; C.J.D. contract no. MEIF-CT-2006-039256).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Herein, we report for the first time the successful synthesis of an ERM based on enantioselectivity. Furthermore, we demonstrate that the system fulfills all of the above requirements and that the polymer can be encoded and read out.

The initial challenge was to address the first requirement and design and synthesize a suitable monomer/polymer system that would be able to contain the programming through chirality. This problem is directly linked to the second requirement of finding an enzyme that retains a high selectivity towards the chiral polymer. A suitable substrate/enzyme pairing was proposed based on previous work: 1-phenylethanol and lipase B from *Candida antarctic*a. Hult et al. showed that this enzyme has a very high selectivity for (*R*)-1-phenylethanol over the *S* form,[5] expressed in a reactivity ratio of 1.3×10^6:1 in the hydrolysis of the corresponding acetates, a selectivity that has been utilized in the synthesis of chiral polyester.[6] Here, we designed a monomer based on the styrene analogue of 1-phenylethanol, which can be polymerized by free-radical polymerization.[7]

The synthesis of this monomer was achieved by the ketone *p*-vinylacetophenone, which can be chemically reduced to obtain the corresponding racemic secondary alcohol. We anticipated that alcohol dehydrogenases (ADHs) might be promising catalysts for this reduction. It has been observed that ADHs, which in nature selectively oxidize alcohols to ketones, can catalyze the reverse reaction under appropriate conditions. Depending on the source of the enzyme, both *R* and *S* selectivity can be found with ADHs. Although their selectivity in the reduction of different aromatic ketones has been demonstrated,[8] they have, to our knowledge, never been employed for the synthesis of a vinyl monomer.

We chose two commercially available enantiocomplementary ADHs, that is, *Lactobacillus brevis* (*R* selective) and *Thermoanaerobacter sp.* (*S* selective). Both enzymes depend on nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor, which serves as reduction equivalent (Figure 1). However, both enzymes are also able to oxidize isopropanol for cofactor regeneration, and therefore only catalytic amounts of $NADP^+$ are needed. The reaction equilibrium is driven by using an excess of isopropanol in buffer (20% v/v). Moreover, the high isopropanol content facilitates the solubilization of hydrophobic substrates, such as *p*-vinylacetophenone. Gram-scale reductions of *p*-vinylacetophenone were performed in good yield. The enantiopurity of the resulting chiral alcohols was excellent ($ee > 99\%$ by chiral GC, Figure 1) and the unwanted enantiomer was not detectable in either reaction.

Copolymers of styrene and *p*-vinylphenylethanol were prepared from these monomers over the whole range of compositions from 100% *R* to 100% *S*. These copolymer

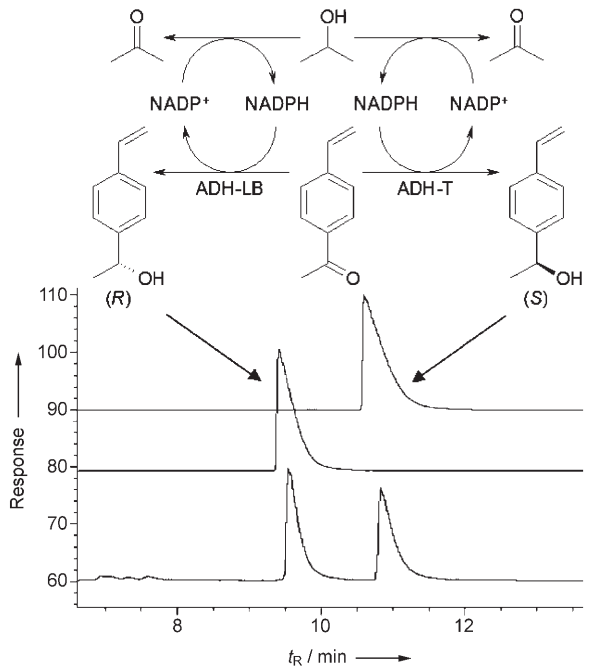
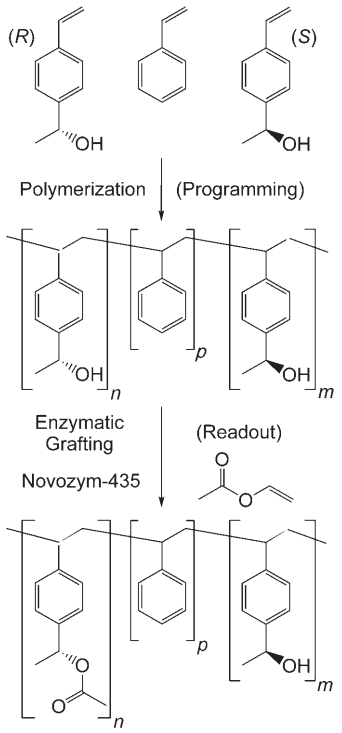


Figure 1. Enzymatic synthesis of enantiomerically pure *p*-vinylphenylethanol and the chromatograms obtained from chiral GC of the resulting *S* and *R* monomers (top and middle traces, respectively) and the chemically synthesized racemic monomer (bottom trace).



Scheme 1. Copolymerization of the chiral monomers with styrene to form the chiral copolymers used for subsequent *R*-selective enzymatic grafting with vinyl acetate.

backbones were designed to contain a total of approximately 45% of the secondary alcohol monomer (either *R*, *S*, or a mixture)—the programming that would subsequently determine the extent of the enzyme response. All polymers were synthesized by free-radical polymerization to give products with number-average molecular weight $M_n = 5000$–$6000\ g\,mol^{-1}$ and polydispersity = 1.7–2.1. Differential scanning calorimetry (DSC) analysis was carried out on all of the polymer backbones. A similar glass transition temperature T_g of approximately 115 °C was found for all polymers, irrespective of their chiral composition, which shows that the enantiomeric composition (that is, the programming) has no effect on their thermal properties. Optical rotation measurements of the polymers increased linearly from −20 to +20°, which indicates that the *R* and *S* monomers had been incorporated in the final polymers as expected.[7]

Once the chiral polymers had been prepared, the next step was to determine whether this system fulfills the second requirement and prove that the lipase retains a high selectivity for the secondary alcohols, even when they are distributed along a polymer chain. An immobilized form of lipase B from *C. antarctica* (Novozym-435) was used to catalyze the esterification of the alcohol groups on the polymer backbone with vinyl acetate in toluene (Scheme 1). The extent of the grafting from the secondary alcohol groups was quantified by ^1H NMR spectroscopy, where a clear shift could be seen from $\delta = 4.8$ to 5.8 ppm on esterification of the alcohol.[7]

When a backbone containing 100% *S* groups (styrene copolymer containing approximately 45% alcohol monomer) was used for the enzymatic grafting of vinyl acetate, no grafting was detected over a period of 24 h (Figure 2). Even when the reaction was allowed to proceed for 48 h, there was

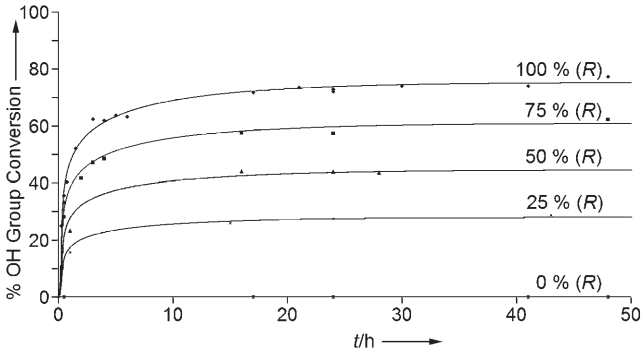


Figure 2. Progression of the selective enzymatic grafting of vinyl acetate for polymer backbones with a range of enantiomeric compositions.

still no evidence of any grafting occurring from the backbone. In contrast, when a backbone containing 100% *R* groups was used, the enzymatic esterification of vinyl acetate occurred from 75% of the alcohol groups within 24 h. This was determined to be the maximum, as even after greatly extended reaction times and the addition of fresh enzyme and vinyl acetate, the conversion of secondary alcohol groups could not be significantly increased, thus indicating that there is some limiting factor in determining the maximum level of grafting. This may be a result of some form of steric hindrance

caused by the alcohol groups being located on a polymer chain.[9]

Comparison of the grafting that occurs with the 100% *R* backbone and the 100% *S* backbone shows that the extremely high selectivity of the lipase towards the 1-phenylethanol moiety is retained, even in analogous polymer reactions. This has never previously been shown. Moreover, the binary reactivity of this system now allows for the design of polymers with predetermined reactivity by copolymerization of the two enantiomers.

To further investigate the programming that could be encoded in the polymer, esterification reactions were carried out on polymer backbones containing mixtures of *R* and *S* alcohol groups. When the enzymatic esterification was carried out on a backbone containing a racemic mixture, vinyl acetate reacted with approximately 45% of the initiating groups, in agreement with the previous levels of grafting. Correspondingly, the use of a polymer backbone containing 25% *R* and 75% *S* groups led to reaction of less than 30% of the secondary alcohol groups, whilst increasing the backbone *R* content to 75% produced an appropriate increase in the level of esterification to more than 55%.

DSC analysis of the polymers showed that the grafting of vinyl acetate caused a decrease in the T_g of the polymers—the higher the level of grafting, the larger the decrease in T_g (Figure 3). The results of NMR and thermal analysis address the third fundamental requirement described earlier. NMR analysis shows that the enzyme can accurately read out the information encoded in the polymer backbone, whilst the DSC results demonstrate that this reading of the encoded information also produces a change in the thermal properties of the polymer.

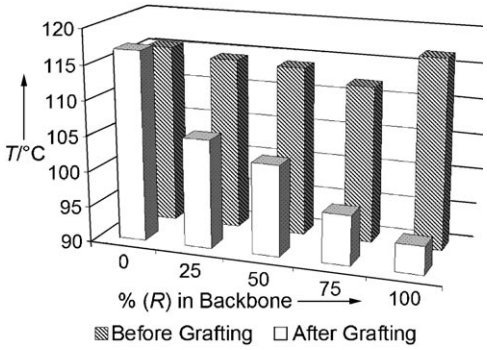


Figure 3. DSC T_g analysis of the chiral polymer backbone before (gray) and after (white) selective enzymatic grafting of vinyl acetate (for 24 h) as a function of chiral composition.

We have demonstrated a novel, simple route to the synthesis of enantiomerically pure monomers that can be used to form polymers containing information encoded in their chiral composition. By using the selectivity of an enzyme, this information can be accurately read out, which leads to a change in the thermal properties of the polymer.

Received: June 5, 2007
Published online: October 5, 2007

Keywords: chirality · enantioselectivity · enzyme catalysis · enzyme-responsive materials · polymerization

[1] K. Faber, *Biotransformations in Organic Chemistry*, Springer, Berlin, **2000**.
[2] a) S. Kobayashi, H. Uyama, S. Kimura, *Chem. Rev.* **2001**, *101*, 3793–3818; b) R. A. Gross, A. Kumar, B. Kalra, *Chem. Rev.* **2001**, *101*, 2097–2124; c) I. K. Varma, A. C. Albertsson, R. Rajkhowa, R. K. Srivastava, *Prog. Polym. Sci.* **2005**, *30*, 949–981.
[3] R. V. Ulijn, *J. Mater. Chem.* **2006**, *16*, 2217–2225.
[4] a) Z. Yang, H. Gu, D. Fu, P. Gao, J. K. Lam, B. Xu, *Adv. Mater.* **2004**, *16*, 1440–1444; b) P. D. Thornton, R. J. Mart, R. V. Ulijn, *Adv. Mater.* **2007**, *19*, 1252–1256.
[5] A. O. Magnusson, M. Takwa, A. Hamburg, K. Hult, *Angew. Chem.* **2005**, *117*, 4658–4661; *Angew. Chem. Int. Ed.* **2005**, *44*, 4582–4585.
[6] a) I. Hilker, G. Rabani, G. K. M. Verzijl, A. R. A. Palmans, A. Heise, *Angew. Chem.* **2006**, *118*, 2184–2186; *Angew. Chem. Int. Ed.* **2006**, *45*, 2130–2132; b) J. van Buijtenen, B. A. C. van As, J. Meuldijk, A. R. A. Palmans, J. A. J. M. Vekemans, L. A. Hulshof, E. W. Meijer, *Chem. Commun.* **2006**, 3169–3171; c) B. A. C. van As, J. van Buijtenen, A. Heise, Q. B. Broxterman, G. K. M. Verzijl, A. R. A. Palmans, E. W. Meijer, *J. Am. Chem. Soc.* **2005**, *127*, 9964–9965.
[7] Supporting Information available: experimental procedures, NMR spectrum, and optical rotation data.
[8] a) W. Hummel, *Adv. Biochem. Eng. Biotechnol.* **1997**, *58*, 145–184; b) K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, *Tetrahedron: Asymmetry* **2003**, *14*, 2659–2681; c) W. Kroutil, H. Mang, K. Edegger, K. Faber, *Curr. Opin. Chem. Biol.* **2004**, *8*, 120–126.
[9] a) C. J. Duxbury, D. Cummins, A. Heise, *Macromol. Rapid Commun.* **2007**, *28*, 235–240; b) M. Hans, P. Gasteier, H. Keul, M. Moeller, *Macromolecules* **2006**, *39*, 3184–3193.

