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Communications

Enzyme-Catalyzed Enantioconvergent Polymerization of β-Hydroxyglutarate in Organic Solvents

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Summary: A strategy has been developed for the enantioconvergent polymerization of symmetrical hydroxy diesters and dihydroxy monoesters that exploits the prochiral stereospecificity of enzymes in organic solvents. This approach has significant potential for the asymmetric synthesis of chiral polyesters from achiral monomers possessing σ-symmetry.

Sir: It is now well established that hydrolytic enzymes are highly stable in organic solvents and can be used for certain types of transformations that are difficult or impossible to do in water.[1] The most common reactions are lipase-catalyzed esterifications and transesterifications, which were used extensively to prepare chiral acids and alcohols.[2] Klibanov[3] and very recently Morrow[4] reported the preparation of chiral polyesters by enzyme-catalyzed transesterification between a racemic diester and an achiral diol, or a racemic diol and an achiral diester. Recently, we have investigated the behavior of ω-hydroxy esters with lipases in organic solvents and found that while substituted γ- and δ-hydroxy esters undergo lactonization to chiral γ- and δ-lactones,[5,6] the β-hydroxy, ϵ-hydroxy, and longer chain hydroxy esters react in an *intermolecular* fashion to give linear polymers.[7] Using this approach, we have prepared unsubstituted achiral as well as substituted chiral polyesters, which in some cases contained more than 100 monomer units (MW > 10 000).[8]

All of these polymerization experiments exploit the enzyme's enantioselectivity, i.e., its ability to discriminate between enantiomers of a racemic mixture. However, since the unfavored enantiomer usually also reacts, all be it at a slower rate, it is practically impossible to obtain a long chain macromolecule without any of the wrong enantiomer being incorporated, and the further the reaction proceeds, the greater the likelihood of incorporation of the unfavored enantiomer. An attempt to use a 2-fold excess of the racemic diester over the achiral dialcohol partially solved the problem of incorporation of the wrong enantiomer, but it severely limited the molecular weight of the polymer and required a separation between the polyester and unreacted monomer to be carried out.[4]

In the present work we demonstrate for the first time a strategy for enzymatic asymmetric synthesis of chiral polymers by exploiting the enzyme's prochiral stereospecificity, i.e., their ability to discriminate between enantiotopic groups of a prochiral molecule. We have shown earlier that β-hydroxy esters undergo polymerization.[7] In principle, the enzymatic polymerization of prochiral symmetrical β-hydroxy dicarboxylates (**1** and **2**) or of dihydroxy monocarboxylates (**3**) will be enantioconvergent

(1) For reviews on enzymatic reactions in organic solvents, see: (a) Klibanov, A. M. *CHEMTECH* **1986**, *16*, 354. (b) Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194. (c) Chen, C. S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695.

(2) (a) Zaks, A.; Klibanov, A. M. *Science* **1984**, *224*, 1249. (b) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687. (c) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072. (d) Langrand, G.; Secci, M.; Buono, G.; Baratti, J.; Triantaphylides, C. *Tetrahedron Lett.* **1985**, *26*, 1857. (e) Stokes, T. M.; Oehlschlager, A. C. *Ibid.* **1987**, *28*, 2091. (f) Belan, A.; Bolte, J.; Fauve, A.; Gourcy, J. G.; Veschambre, H. *J. Org. Chem.* **1987**, *52*, 256. (g) Sonnet, P. E. *Ibid.* **1987**, *52*, 3477. (h) Theisen, P. D.; Heathcock, C. H. *Ibid.* **1988**, *53*, 2374. (i) Chem. C. S.; Wu, S. H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812. (j) Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *Ibid.* **1988**, *110*, 7200. (k) Yamamoto, K.; Nishioka, T.; Oda, J.; Yamamoto, Y. *Tetrahedron Lett.* **1988**, *29*, 1717.

(3) Margolin, A. L.; Grenne, J. Y.; Klibanov, A. M. *Tetrahedron Lett.* **1987**, *28*, 1607.

(4) Wallace, J. S.; Morrow, C. J. *J. Polym. Sci. Part A* **1989**, *27*, 2553.

(5) Gutman, A. L.; Zuobi, K.; Boltanski, A. *Tetrahedron Lett.* **1987**, *28*, 386.

(6) Gutman, A. L.; Bravdo, T. *J. Org. Chem.* **1989**, *54*, 4263.

(7) Gutman, A. L.; Oren, D.; Boltanski, A.; Bravdo, T. *Tetrahedron Lett.* **1987**, *28*, 5367.

(8) Gutman, A. L.; Knaani, D.; Kohn, D. Manuscript in preparation.

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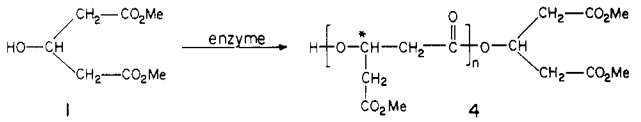
Table I. Asymmetric Polymerization of Dimethyl β-Hydroxyglutarate with Enzymes in Organic Solvents

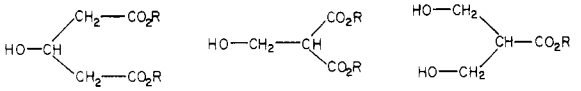


1 4

enzyme	temp, °C	chiral oligomer 4	$[\alpha]^{28}_D$, deg (c in CH_2Cl_2)	% ee[a]
LAPP	40	(+)-4 (n = 1)	+1.4 (c 1.5)	37
LAPH	40	(+)-4 (n = 1)	+2.0 (c 0.95)	35
SG	69	(+)-4 (n = 1)[b]		30
LAPP	40	(+)-4 (n = 2)[c]	+1.6 (c 0.5)	

[a] Optical purity of dimers was determined by ^{1}H NMR in the presence of the chiral shift reagent tris[3-((heptafluoropropyl)-hydroxymethylene)-(+)-camphorato]europium(III) [(+)-Eu(tfc)$_3$], as described in text. [b] Because of the small quantity of isolated material we failed to determine accurately the optical rotation of the dimer obtained with SG. However, NMR in the presence of chiral shift reagent indicated that also in this case the (+) enantiomer was obtained. [c] For trimers enantiomeric excess values could not be determined by the NMR method, due to considerable line broadening.

and may convert the achiral monomer into a single enantiomer of the polyester.



1 2 3

Following this train of thought, we selected the readily available dimethyl β-hydroxyglutarate (1, R = Me)[9] as the model prochiral monomer and examined its behavior with 13 commercially available lipases, proteases, and crude acetone powders[10] in organic solvents. Most of the enzymes tested were catalytically active, but liver acetone powder horse (LAPH), liver acetone powder pig (LAPP), and the protease from *Streptomyces griceus* (SG) afforded the highest activities and were employed in the subsequent, scaled-up polymerization experiments.

Preparative enzymatic experiments were carried out in 25 mL of dry hexane, between 1 g of substrate and 1 g of enzyme,[11] by shaking the suspension[12] at 40 °C and 200 rpm with LAPH and LAPP, or under reflux (69 °C) with SG. Reaction progress was monitored by NMR: the monomer (1, R = Me) has a characteristic singlet at 3.69 ppm due to the two equivalent Me esters and a multiplet at 4.45 ppm due to the CH adjacent to OH; in the oligomer 4 these signals correspond to the end units, while the singlet at 3.67 ppm and the multiplet at 5.50 ppm are due to the Me ester and the CH adjacent to -O- ester of the newly formed internal units. Comparison of the relative intensities of the appropriate signals enabled us to estimate the degree of polymerization.

Reactions were terminated by filtering off the enzyme, when the NMR analysis indicated the presence of a trimer on average. The product mixture was fractionated by solubility: unreacted monomer and low oligomers were obtained from the decanted hexane solution and from washing the enzyme with cold chloroform, while the higher oligomers were recovered by extracting the enzyme powder with boiling chloroform. Although the reactions were very slow (10 days were required to obtain a trimer), they were stereospecific. In all cases (Table I), the dimers and trimers, which were carefully purified by preparative TLC on silica gel,[13] were optically active,[14] due to the newly created asymmetric center in the oligomer 4 (marked with an asterisk). The relatively low optical rotations are not surprising, because two of the substituents on the asymmetric center are very similar (two ester groups). The optical purity of the dimers was accurately determined by ^{1}H NMR in the presence of the chiral shift reagent, [(+)-Eu(tfc)$_3$]. Its complexation with the hydroxyl group of 4 causes unequal downfield shifts for the CH_2 protons adjacent to the chiral center.

This novel approach enabled us to carry out asymmetric synthesis of a chiral polymer from an achiral monomer. An important feature of chiral polymers such as 4 is that they possess functional groups, which may be used for cross-linking and as anchors for attaching achiral reagents. The seemingly modest 30–37% ee values are in fact considerably higher than the best values reported for *chemical* polymerization of prochiral monomers with asymmetric catalysts.[15] Furthermore, we believe that polymerization rates and ee values may be higher for monomers with the more reactive primary hydroxyl groups and/or activated esters (such as 2 or 3, R = CF_3CH_2). Studies in this direction are currently underway in our laboratory.

Acknowledgment. We thank the Israel Academy of Sciences and Humanities for financial support.

(9) The synthesis of the dimethyl β-hydroxyglutarate (1) involved esterification of acetonedicarboxylic acid via the acid chloride to give the dimethyl 3-oxoglutarate as in the following: Kumar, B.; Verma R. K. *Synth. Commun.* **1984**, 1359. This keto ester was then reduced with $NaBH_4$ as in the following: Nozaki, H.; Kikondo, K.; Nakanisi, O.; Sisiso, K. *Tetrahedron* **1963**, *19*, 1617. The product was purified by vacuum distillation (110 °C, 0.1 mmHg) and its purity was confirmed by ^{1}H NMR.

(10) Enzymes were obtained as follows: lipases from porcine pancreas, *Candida cylindracea*, and wheat germ, from Sigma Chemical Co.; lipases from *Pseudomonas* sp., *Aspergillus niger*, *Mucor* sp. MAP, *Rhizopus niveus* N, and *Rhizopus oryzae* FAP, from Amano Pharmaceutical Co.; Proteases from *Bacillus subtilis* (subtillisin Carlsberg), *Steptomyces griseus* and chimotrypsin from Sigma; and the acetone powders from horse liver and pig liver were obtained from Sigma. When necessary, enzymes were "pH-adjusted", by lyophilizing from an aqueous solution at pH = 7.5, the pH optimal for the enzymatic activity as described in the following: Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192.

(11) The seemingly large amounts of the enzymes used in this work are misleading, as the commercial preparations employed were very crude. No reaction took place in the absence of enzymes under the conditions used.

(12) All enzymes are totally insoluble in hexane.

(13) Preparative TLC separations were performed on plates coated with a 1-mm thick layer of silica gel 60 PF 254, eluting with 95:5 ether–hexane. Under these conditions quantitative separation was obtained between monomer (R_f = 0.36), dimer (R_f = 0.22), and trimer (R_f = 0.14).

(14) Since oligomers 4 have not been obtained in chiral form before, we do not know what the absolute configuration is.

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