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STEREOSELECTIVE OLIGOMERIZATIONS CATALYZED BY LIPASES IN ORGANIC SOLVENTS

Alexey L. Margolin, Jean-Yves Crenne, and Alexander M. Klibanov*

Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Summary: Lipases have been found to act as stereoselective catalysts in polycondensation reactions between racemic diesters and achiral diols (or vice versa) in organic solvents. Optically active trimers and pentamers have been formed as a result.

Optically active polymers possess a number of valuable and unique properties[1-3] and have found applications as catalysts and reagents for asymmetric syntheses[4], chiral adsorbents for separation of racemic mixtures[5], and liquid crystals[6]. Thus far, all synthetic optically active polymers have been prepared by chemical means, e.g., via polymerization of optically active monomers or their coupling to achiral polymers and asymmetric polymerization of racemic monomers using optically active initiators[2,3]. In this work, we have explored enzyme-catalyzed asymmetric polymerizations.

Lipases can catalyze different processes[7] when water is replaced with organic solvents as the reaction medium[8]. One of such reactions is stereoselective transesterification between a racemic ester and an alcohol (or vice versa) leading to optically active products[9]. It occurred to us that if a racemic diester and diol are used instead, then lipase may be able to catalyze stereoselective polycondensations in organic solvents[10]. The initial model system examined was comprised of bis(2-chloroethyl) (±)-2,5-dibromoadipate[11] (1) and 1,6-hexanediol (2) in toluene. Nine commercially available lipases[12] were tested as catalysts of the polycondensation

$$ClCH_2CH_2OC(O)CHBrCH_2CH_2CHBrC(O)OCH_2CH_2Cl + HO(CH_2)_6OH \longrightarrow$$

reaction[13], and nearly all were catalytically active. Lipases from Aspergillus niger and Chromobacterium sp.[14] afforded the highest activities and were employed in the subsequent, scaled-up experiments. We dissolved 3.7 mmoles of racemic 1 and 13 mmoles of 2 in 50 mL of toluene, and then 17 g of Aspergillus niger lipase[15] was added. The suspension[16] was shaken at 45°C and 250 rpm for 7 days when the degree of conversion of the ester reached approximately 50%, as judged by a gas chromatographic analysis of both

the 1 remaining and the 2-chloroethanol formed. The enzyme was then removed by filtration and the solvent and 2-chloroethanol evaporated under vacuum. The remainder was subjected to preparative HPLC (hexane/ethyl acetate gradient as the mobile phase), and the fractions obtained were analyzed by gel permeation HPLC[17]. Fractions with apparent molecular weights greater than 450 daltons were pooled and rechromatographed using preparative gel permeation HPLC. As a result, 140 mg of a trimer, 80 mg of a pentamer, and smaller quantities of higher polymers were obtained. Analysis of these products by IR revealed the presence of ester bonds and free OH groups, and ^{1}H NMR studies indicated the absence of carboxyl groups (thus excluding hydrolysis of esters) and the $ClCH_2CH_2$ moiety. Hence, the composition of the enzymatically formed trimer and pentamer is A-B-A and A-B-A-B-A, respectively, where A is the diol moiety and B is the acid moiety. Both products were found to be optically active with $[\alpha]_D^{25}$ of +4.5° and +4.3°, respectively (c 4, THF).[18,19] When Chromobacterium lipase (14 g[15]) was used as a catalyst in the same system, 140 mg of the trimer and 80 mg of the pentamer were formed[17] with $[\alpha]_D^{25}$ of +1.3° and +1.5°, respectively (c 10, THF).[18,19]

Asymmetric enzymatic oligomerization was also observed when another racemic ester, bis(2,2,2-trichloroethyl) (±)-3-methyladipate, was used where the asymmetric carbon is

$$Cl_3CCH_2OC(O)CH_2CH(CH_3)CH_2CH_2C(O)OCH_2CCl_3 + HO(CH_2)_6OH \longrightarrow$$

farther from the reaction sites than in 1. Porcine pancreatic lipase (344 mg/mL[15]) turned out to be the most active catalyst in this case, and under the same conditions as described above, 260 mg of the A-B-A trimer and 35 mg of the A-B-A-B-A pentamer[17] were obtained; $[\alpha]_D^{25}$ of their mixture was +0.3° (c 30, MeOH).[19]

In both examples described, a racemic diester and an achiral diol were involved in lipase-catalyzed production of ester oligomers. To test the generality of our methodology, we then reversed the situation and employed a chiral diol, 2,4-pentanediol, and an achiral diester, bis(2-chloroethyl) adipate[11]. Porcine pancreatic lipase, both in

$$ClCH_2CH_2OC(O)CH_2CH_2CH_2CH_2C(O)OCH_2CH_2Cl + HOCH(CH_3)CH_2CH(CH_3)OH \longrightarrow$$

toluene and in isopropyl ether, was found to be reactive only with the (2R,4R)-(-) but not with the (2S,4S)-(+) isomer of the diol in that reaction. When 10 g of the enzyme[15] was suspended[16] in 25 mL of toluene containing 10 mmoles each of racemic 2,4-pentanediol and the diester, after 14 days of shaking 350 mg of a mixture of a trimer and pentamer[17] (no 2-chloroethyl moiety by NMR; both ester bonds and free OH groups present by IR) with $[\alpha]_D^{25}$ of -7.9° (c 2, CH_2Cl_2) was formed. Hence, lipases can act as stereoselective catalysts for the production of optically active ester oligomers of different structures in organic solvents.

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10. Lipase-catalyzed polycondensation between achiral dicarboxylic acids and primary glycols in aqueous-organic mixtures was recently reported (Okumura, S.; Iwai, M.; Tominaga, Y. Agric. Biol. Chem. 1984, 48, 2805-2808). Also, Ajima et al. (Ajima, A.; Yoshimoto, T.; Takahashi, K.; Tamaura, Y.; Saito, Y.; Inada, Y. Biotechnol. Lett. 1985, 7, 303-306) claimed a polycondensation of achiral 10-hydroxydecanoic acid catalyzed by lipase in benzene but no convincing evidence was presented.
11. All diesters were synthesized from the corresponding acyl chlorides and chlorinated EtOH according to the general procedure: Sonntag, N.O.V. Chem. Rev. 1953, 52, 237-416.
12. Lipases were obtained as follows: porcine pancreatic, Candida cylindracea, and wheat germ from Sigma Chemical Co.; Aspergillus niger K, Mucor sp. MAP, Rhizopus niveus N, Rhizopus oryzae FAP, and lipoprotein lipase from Pseudomonas sp. from Amano International Enzyme Co.; Chromobacterium sp. CV from Finnsugar Biochemicals. Specific activities of lipases were 11, 500, 8.6, 10, 10, 45, 750, 2190, and 120 units/mg solid, respectively.
13. Lipase concentrations in toluene were 100 mg/mL, except for lipoprotein lipase (5 mg/mL). The reaction mixtures were shaken at 45°C; periodically, aliquots were withdrawn and assayed by gas chromatography[9a].
14. All lipases were tested both "straight from the bottle" and "pH-adjusted"[7], i.e., lyophilized from an aqueous solution of the pH optimal for the enzymatic activity. Only for the Chromobacterium lipase such treatment resulted in a significant (15-fold) increase in the specific activity, and therefore this enzyme was always lyophilized from pH 7.0 prior to use.

15. The seemingly high amounts of lipases used in these experiments are somewhat misleading, as the commercial preparations employed were very crude. No appreciable reaction was observed in the absence of the enzyme. Furthermore, when porcine pancreatic lipase was irreversibly inactivated with the active center-specific reagent diethyl p-nitrophenyl phosphate (Maylie, M.F.; Charles, M.; Sarda, L.; Desnuelle, P. Biochim Biophys. Acta 1969, 178, 196-198), the enzyme lost its ability to catalyze transesterification reactions in organic solvents.
16. All lipases are insoluble in the organic solvents used.
17. An Ultrastyragel column (1000 A pore size, Waters Associates), packed in THF, was used for all gel permeation HPLC experiments. The column was calibrated (here and hereafter THF was employed as the mobile phase) using 8 different polyethylene glycols with molecular weights from 200 to 8,000 daltons. The molecular weights of the enzymatic products were within 20% from those calculated for the trimers and pentamers. This difference is presumably due to the approximation of the enzymatically formed oligoesters with polyethylene glycols. The composition of the trimers and pentamers was always confirmed by IR and NMR analyses (see text).
18. We did not determine the enantiomeric excess of these esters, because the commercial starting material, 2,5-dibromoadipic acid, consisted of a mixture of (±) and meso forms (Buchman, E.R.; Reims, A.O.; Skei, T.; Schlatter, M.J. J. Am. Chem. Soc. 1942, 64, 2696-2700) and because of serious difficulties encountered in hydrolysis of the oligoesters not leading to racemization.
19. Although specific optical rotations for esters of 2,5-dibromoadipic and 3-methyladipic acids are not known, comparison of our data with much higher values for the free acids (Dictionary of Organic Compounds; Oxford University Press: New York, 1965; vol. 2, p. 908 and vol. 4, p. 2117, respectively) suggests rather modest (although significant) ee's for the enzymatically produced oligomers. It is worth noting that while we are not aware of studies on asymmetric polycondensations of racemic monomers with optically active initiators, polymerization processes of that type usually afford low optical purities, e.g., less than 10% in the case of α-olefin polymerizations (Pino, P.; Oschwald, A.; Ciardelli, F.; Carlini, C.; Chielini, E. In Coordination Polymerization; Chien, J.C.W, Ed.; Academic Press: New York, 1975; pp. 25-72).
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