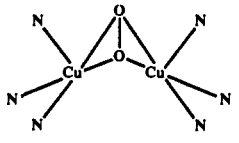
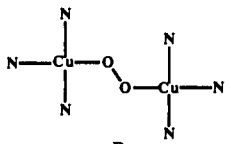
Further striking evidence for the formulation of $[\{L_3Cu\}_2(O_2)]^{2+}$ (3) and verification that it contains a bound O_2^{2-} ligand comes from its reaction with TMPA (TMPA = tris[(2-pyridyl)-methyl]amine), which is known to form a purple *trans*-μ-1,2-peroxo-bridged dicopper(II) complex (6) when $[(TMPA)Cu^I(RCN)]^+$ is reacted with O_2 at -80 °C.[5,16] We observe that addition of TMPA (-90 °C) to the brown solution of 3 instantaneously transforms it to a purple solution with spectral features identical with those of 6 (Scheme I); the yield of this conversion is 81% based on the established spectrum of 6.[16] We attribute this "peroxide transfer" reaction to the lability of unidentate L and the greater stability of the chelating TMPA ligand complex. This may prove to be an example of a dynamic "self-assembly" process[17] utilizing a "preformed" Cu_2O_2 core.

The structure of $[\{L_3Cu\}_2(O_2)]^{2+}$ (3) (CH_2Cl_2, 100 K) has been probed by X-ray absorption spectroscopy.[18] Edge comparisons with $[L_3Cu^I](PF_6)$ (2) indicate that 3 is a Cu(II) complex.[19] Simulation of the EXAFS[18] required four first-shell O/N ligands to account for the intensity of the first shell in the FT, while outer shell atom single and multiple scattering contributions from the imidazole rings alone were not sufficient to account for the intensity of the second shell in the FT. This extra intensity required either a Cu-Cu interaction or an O atom at 2.85 Å. The data could be interpreted by either of two models (A or B) shown herein.[18] Structure A contains a bent μ-η^2:η^2-peroxo ligand, a bridging mode seen in acetylene-bridged dicopper(I) complexes;[20] structure A is also closely related to that proposed for other $\{Cu_2O_2\}$ complexes previously described.[5] Kitajima and coworkers have structurally characterized a dicopper(II) complex with a planar μ-η^2:η^2-peroxo group.[4] Model B possesses a planar Cu(II) coordination and has a *trans*-μ-1,2-peroxo group as is seen in 6.[21]



A



B

	A	B
Cu-N (equatorial)	2.01 Å	2.01 Å
Cu-N(axial)	2.24 Å	
Cu-O(1)	1.92 Å	1.88 Å
Cu-O(2)	1.92 Å	2.85 Å
Cu-Cu	2.84 Å	4.30 Å

In conclusion, it is possible to generate a copper-dioxygen complex by using a simple imidazole ligand, by sufficiently lowering the temperature, thus thwarting further irreversible reduction (e.g., $Cu:O_2 = 4:1$)[22] or disproportionation. This observation is reminiscent of the behavior observed for simple iron(II) porphyrins in their 1:1 O_2 binding.[23] Interestingly, the obvious difference in properties of $[\{L_3Cu\}_2(O_2)]^{2+}$ (3) and those of oxyhemocyanin (e.g., λ_{max} = 350 nm (ϵ, 20000); Cu···Cu = 3.56 Å)[1b] suggests that 3 does not possess a $\{Cu_2O_2\}$ core structure like that observed in the protein, again illustrating the multiple structures possible for copper-dioxygen species.[5,13a,b] Further studies will be directed toward additional characterization of 3 and synthetic modifications.

Acknowledgment. We are grateful to the National Institutes of Health (GM28962, K.D.K.; NS27583, N.J.B.) for support of this research.

Supplementary Material Available: Details of the data collection and data analysis (2 pages). Ordering information is given on any current masthead page.

(15) $[\{L_3Cu\}_2(CO_3)](PF_6)_2$ (5): Anal. Calcd for $C_{31}H_{48}Cu_2F_{12}N_{12}O_3P_2$: C, 35.32; H, 4.56; N, 15.94. Found: C, 35.24; H, 4.76 N; 15.42. UV-vis (CH_2Cl_2): λ_{max} 360 (ϵ 1940), 712 (350). IR (Nujol): ν(PF) = 840 cm^{-1}, ν(CO) = 1255 cm^{-1}. EPR silent (CH_2Cl_2, 77 K). Λ_m (CH_3CN) = 250 Ω^{-1} cm^2 mol^{-1}.

(16) Jacobson, R. R.; Tyeklár, Z.; Farooq, A.; Karlin, K. D.; Liu, S.; Zubieta, J. *J. Am. Chem. Soc.* **1988**, *110*, 3690-3692.

(17) (a) Ibers, J. A.; Holm, R. H. *Science* **1980**, *209*, 223. (b) Lippard, S. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 344-361.

(18) Details of the data analysis are given in the supplementary material. The least-squares-fit indices were 1.33 and 1.50 for models A and B, respectively.

(19) The well-resolved edge feature at 8984 eV present in 2 and assigned to the 1s-4p_z transition in distorted 3-coordinate geometry is replaced in 3 by a featureless absorption edge shifted by ca. 5 eV to higher energy. (a) Blackburn, N. J.; Strange, R. W.; Reedijk, J.; Volbeda, A.; Farooq, A.; Karlin, K. D.; Zubieta, J. *Inorg. Chem.* **1989**, *28*, 1349-1357. (b) Blackburn, N. J.; Strange, R. W.; Farooq, A.; Haka, M. S.; Karlin, K. D. *J. Am. Chem. Soc.* **1988**, *110*, 4263-4272. (c) Kau, L. S.; Spira-Solomon, D. J.; Penner-Hahn, J. E.; Hodgson, K. O. *J. Am. Chem. Soc.* **1987**, *109*, 6433-6442.

(20) (a) Villacorta, G. M.; Gibson, D.; Williams, I. D.; Whang, E.; Lippard, S. J. *Organometallics* **1987**, *6*, 2426-2431. (b) Reger, D. L.; Huff, M. F.; Wolfe, T. A.; Adams, R. D. *Organometallics* **1989**, *8*, 848-850.

(21) At this time, we favor model B as the more plausible structure for $[\{L_3Cu\}_2(O_2)]^{2+}$ (3) because it possesses a reactivity pattern similar to that seen for 6.[13a,b]

(22) At room temperature, complex 2 takes up oxygen with the stoichiometry of $4Cu:1O_2$, suggesting a four-electron reduction of O_2 to give oxo-copper(II) species.

(23) Collman, J. P.; Halpert, T. R.; Suslick, K. S. In *Metal Ion Activation of Dioxygen: Metal Ions in Biology*; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1980; Vol. 2, pp 1-72, and references cited therein.

Chemoenzymatic Synthesis of Optically Active (Meth)acrylic Polymers

Alexey L. Margolin,† Paul A. Fitzpatrick,‡ Paul L. Dubin,§ and Alexander M. Klibanov*,‡

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
Merrell Dow Research Institute
9550 North Zionsville Road
Indianapolis, Indiana 46268
Department of Chemistry
Indiana University—Purdue University at Indianapolis
Indianapolis, Indiana 46205

Received January 15, 1991

Optically active polymers possess many interesting properties and have found applications in asymmetric syntheses, as chiral adsorbents for separation of racemates, and in liquid crystals.[1] A new approach to their synthesis would expand the scant arsenal of existing methods.[1] Recently, lipase-catalyzed asymmetric polycondensations have been explored for the production of optically active polyesters,[2] but the reaction rates and molecular weights obtained have been disappointing due to a plummeting reactivity of the enzymes toward higher molecular weight substrates.

Following our proposal to resolve racemic alcohols by using them as nucleophiles in asymmetric transesterifications catalyzed by lipases in neat organic solvents[3] (instead of conventional lipase-catalyzed, asymmetric hydrolysis of racemic esters in water[4]), this new strategy has become popular for the resolution of racemic alcohols,[5] as well as such other nucleophiles as amines,[6] thiols,[7]

† Merrell Dow Research Institute.
‡ Massachusetts Institute of Technology.
§ Indiana University—Purdue University at Indianapolis.

(1) Selegny, E., Ed. *Optically Active Polymers*; Reidel: Dordrecht, 1979. Ciardelli, F. In *Encyclopedia of Polymer Science and Engineering*; Mark, H. F., et al., Eds.; Wiley: New York, 1987; Vol. 10, pp 463-493.

(2) Margolin, A. L.; Crenne, J.-Y.; Klibanov, A. M. *Tetrahedron Lett.* **1987**, *28*, 1607. Wallace, J. S.; Morrow, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **1989**, *27*, 2553. Gutman, A. L.; Bravdo, T. *J. Org. Chem.* **1989**, *54*, 5645.

(3) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687. Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072.

(4) For a review, see: Jones, J. B. *Tetrahedron* **1986**, *42*, 3351.

(5) For reviews, see: Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194. Margolin, A. L. *CHEMTECH* **1991**, *21*, 160.

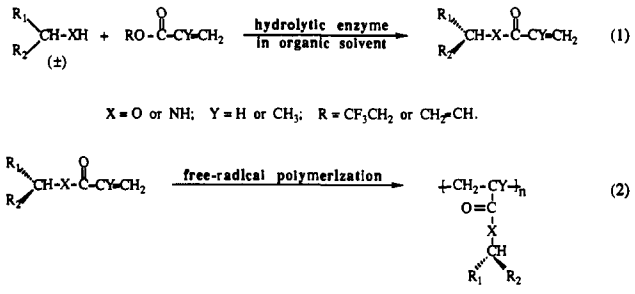
0002-7863/91/1513-4693$02.50/0

Table I. Chemoenzymatic Preparation of Optically Active Poly(meth)acrylates and Polymethacrylamides (Scheme I)[a]

substrates				molecular weight of polymer,[d] Da	ee of polymer,[e] %
nucleophile	acylating agent	enzyme[b]	chiral monomer formed[c] (ee, %)		
(±)-1-indanol	trifluoroethyl acrylate	lipase[11]	(*R*)-acrylate (98)	4×10^6	98
(±)-2-benzylpropanol	trifluoroethyl methacrylate	lipase[11]	(*S*)-methacrylate (90)	2×10^6	90
(±)-1,2,3,4-tetrahydro-1-naphthol	vinyl acrylate	lipase[11]	(*R*)-acrylate (99)	2×10^5	98
(±)-1-(1-naphthyl)ethylamine	trifluoroethyl methacrylate	subtilisin[13]	(*S*)-methacrylamide (>98[f])	1×10^5	96
(±)-phenylalaninamide	trifluoroethyl methacrylate	subtilisin[13]	(*S*)-methacrylamide (77)	3×10^5	76

[a] In the *enzymatic* step of Scheme I, reaction mixtures were vigorously shaken under the following conditions (from top to bottom): nucleophile concentrations 0.15, 0.15, 0.15, 0.50, and 0.12 M; (meth)acrylate ester concentrations 0.45, 0.45, 0.15, 0.50, and 0.12 M; enzyme concentrations 50, 50, 40, 20, and 10 mg/mL; solvent volumes 100, 56, 45, 15, and 60 mL; temperatures 25, 25, 25, 30, and 45 °C; reaction times 3.5, 3.5, 4, 36, and 48 h. Lipase- and subtilisin-catalyzed acylations were carried out in *tert*-butyl methyl ether and 3-methyl-3-pentanol, respectively, dehydrated prior to use by shaking with 3-Å molecular sieves (Linde). No appreciable reaction was observed without enzyme. In the *chemical* step of Scheme I, monomers were subjected to free-radical polymerization initiated by 2,2-azobisisobutyronitrile[12] (70 °C, O_2-free, 3 h) in the bulk (for (meth)acrylates) or in solution (for methacrylamides, 0.5 M in benzene and DMF, respectively). [b] Lipase was used as supplied by the manufacturer. Subtilisin was dissolved (5 mg/mL) in 20 mM aqueous potassium phosphate buffer (pH 7.8), followed by lyophilization and incubation in a controlled-humidity chamber at 11% relative humidity for 48 h. All acylations were initiated by addition of lipase or subtilisin to the reaction mixtures; the suspensions were homogenized by a 10-s sonication. [c] The products' characteristics were as follows (from top to bottom): isolated yields per reactive enantiomer 90, 52, 82, 36, and 46%; GC purities 98, 99, 100, 99, and 100%; $[\alpha]^{25}_D$ +96.2° (*c* 0.58, $CHCl_3$), +14.4° (*c* 1.0, $CHCl_3$), +99.1° (*c* 1.4, $CHCl_3$), +25.0° (*c* 1.0, MeOH), and +2.1° (*c* 1.0, MeOH). The products were purified by silica gel column chromatography, and their identities were confirmed by high-resolution MS, ^1H NMR, and IR spectroscopies. The ee values for the (meth)acrylates were determined by HPLC on a Chiralcel OD (Daicel) column and for the methacrylamides by capillary GC on a Chirasil-Val (Alltech) column. The absolute configurations were assigned by comparison with authentic enantiomers. [d] The polymers' molecular weights and dimensions were measured by static light scattering (a Photal instrument from Polymer Labs). Weight average molecular weights (M_w) and radii of gyration (R_g, from top to bottom, 57, 54, 43, 23, and 23 nm) were determined from Zimm plots obtained in good solvents: THF (entry 1), DMF (entries 2, 4, and 5), and toluene (entry 3). M_w values were calculated from the refractive index increments (dn/dc) reported for similar systems.[14] Poly[(*R*)-(+)-1-indolyl acrylate] was also characterized by dynamic light scattering,[15] giving an apparent Stokes radius (R_s) of 35 nm. The R_g/R_s ratio for this polymer in THF, 1.6, is in agreement with that predicted for flexible chains in good solvents.[16] [e] The polymers' ee values were determined as those of the chiral alcohols and amines prepared from the polymers by alkaline hydrolysis (25 °C, 3 days) in dioxane for entries 1 and 3, acid hydrolysis (purging with HBr, 25 °C, 3 days) in benzene for entry 2, and acid hydrolysis (6 N HCl, 110 °C, 20 h) in water for entries 4 and 5. The $[\alpha]^{25}_D$ values for the polymers were as follows (from top to bottom): +59.5° (*c* 0.52, $CHCl_3$), +6.6° (*c* 0.86, $CHCl_3$), +61.7° (*c* 1.2, $CHCl_3$), +24.2° (*c* 1.0, DMF), and −3.3° (*c* 1.0, DMF). [f] The sensitivity limit of our method.

Scheme I



and hydroperoxides.[8] In this study, we have incorporated it into a chemoenzymatic methodology for the facile production of optically active polymers of high molecular weight, as depicted in Scheme I.[9] First, a lipase or a protease is used as an asymmetric catalyst for stereoselective acylation of a racemic alcohol or amine, respectively, with a (meth)acrylate ester (eq 1). Second, the resultant monomer is chemically polymerized to form an optically active polymer (eq 2).

We chose commercially available monomers, trifluoroethyl (meth)acrylate and vinyl acrylate, as acylating agents.[10] Lipase P[11] was employed as an asymmetric transesterification catalyst in anhydrous *tert*-butyl methyl ether. Three racemic alcohols, both secondary (1-indanol and 1,2,3,4-tetrahydro-1-naphthol) and primary (2-benzyl-1-propanol), were readily enzymatically acylated to afford optically active (meth)acrylates with enantiomeric excesses in the range from 90 to 99% (Table I). After chromatographic purification, the optically active monomers were subjected to free-radical bulk polymerization.[12] The latter proceeded with no racemization, as evidenced by the essentially unchanged ee's of the alcohols obtained by cleaving the ester bonds in the polymers, compared to those of the monomeric (meth)acrylates. The molecular weights of the optically active poly(meth)acrylates produced were from 200 000 to 4 000 000 Da (daltons) (i.e., 2–4 orders of magnitude higher than those obtained by fully enzymatic polycondensations[2]).

A similar methodology (Scheme I) was employed for the synthesis of optically active polymethacrylamides, except that subtilisin Carlsberg[13] was used as an asymmetric catalyst in anhydrous 3-methyl-3-pentanol. Racemic 1-(1-naphthyl)ethylamine and phenylalaninamide were enzymatically acylated to give the corresponding *S* amides with ee's of >98% and 77% (Table I). These optically active methacrylamides were then purified and polymerized (again, without appreciable racemization) to yield polymers with molecular weights of 100 000 and 300 000 Da. (Note that in the latter case an unusual poly(amino acid) was formed.)

The chemoenzymatic strategy described herein combines the keen enantioselectivity of enzymatic resolutions with high molecular weights and efficiency of free-radical polymerizations of (meth)acrylic monomers. Thus it can be generally used for the synthesis of new optically active materials. We are currently extending this approach to regio- and chemoselective (meth)acryloylations catalyzed by hydrolases in organic solvents, followed by free-radical polymerizations, to prepare homopolymers from multifunctional monomeric precursors.

(6) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 3094. Gotor, V.; Brieva, R.; Rebolledo, F. *J. Chem. Soc., Chem. Commun.* **1988**, 957.
(7) Bianchi, D.; Cesti, P. *J. Org. Chem.* **1990**, *55*, 5657.
(8) Baba, N.; Mimura, M.; Hiratake, J.; Uchida, K.; Oda, J. *Agric. Biol. Chem.* **1988**, *52*, 2685.
(9) When this project was in progress, a report appeared (Ghogare, A.; Sudesh Kumar, G. *J. Chem. Soc., Chem. Commun.* **1990**, 134) on asymmetric acylation of (±)-2-ethylhexan-1-ol with *O*-acryloyl oximes catalyzed by porcine pancreatic lipase in tetrahydrofuran, followed by benzoyl peroxide initiated polymerization. The optical purities and absolute configurations for the monomers or the polymers were not determined.
(10) Activated esters were chosen to accelerate enzymatic acylations and to shift the thermodynamic equilibrium in reaction 1 to the right.
(11) *Pseudomonas* sp. (presumably *Pseudomonas cepacia*) lipase from Amano International Enzyme Co.
(12) Sandler, S. R.; Karo, W. *Polymer Synthesis*; Academic Press: New York, 1974; Vol. 1, pp 266–308.
(13) *Bacillus licheniformis* serine protease from Sigma Chemical Co.
(14) Huglin, M. B., Ed. *Light Scattering from Polymer Solutions*; Academic Press: New York, 1972; Chapter 6.
(15) Dubin, P. L.; Vea, M. E. Y.; Fallon, M. A.; Thé, S. S.; Rigsbee, D. R.; Gan, L. M. *Langmuir* **1990**, *6*, 1422.
(16) Benmouna, M.; Akcasu, A. Z. *Macromolecules* **1978**, *11*, 1187.
(17) This work was supported by NIH Grant GM 39794 to A.M.K.