Antibody-Catalyzed Activation of Unfunctionalized Olefins for Highly Enantioselective Asymmetric Epoxidation

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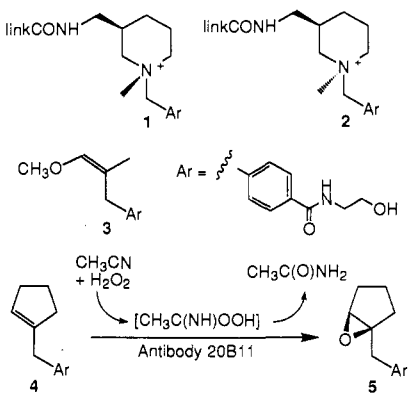
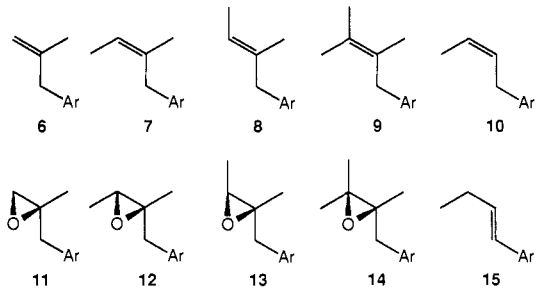
Antibodies raised against transition-state analogs have been shown to catalyze a number of chemical transformations.[1] Whereas acid–base and electrocyclic chemistry has been fairly well explored, reports of antibodies catalyzing redox reactions are scarce.[2] Herein we report the first antibody-catalyzed oxidation reaction at carbon, namely, the enantioselective epoxidation of unfunctionalized alkenes.

Epoxides are valuable chiral synthetic intermediates, and their enantioselective preparation by oxidation of achiral alkene precursors is a key reaction for many syntheses. While the Sharpless epoxidation is suitable for most allylic alcohols,[3] no such general solution exists for unfunctionalized olefins. Manganese salen complexes[4] show good selectivities for *Z* olefins (85–90% ee). Chiral metalloporphines[5] can be used for regio- and enantioselective epoxidations of styrene type olefins with 16–88% ee. The enzyme chloroperoxidase[6] has recently been shown to catalyze the epoxidation of unfunctionalized alkenes with hydrogen peroxide with 66–97% ee.

We recently reported[7] that antibodies raised against haptens **1** and **2** catalyze the hydrolysis of enol ethers to form optically pure carbonyl compounds. In the case of antibody 14D9 (anti-**1**) and enol ether **3**, it was shown that antibody catalysis originated in two effects of comparable magnitude: (1) general acid catalysis by a carboxyl group and (2) pyramidalization of the enol ether's β-carbon by hydrophobic contacts. We reasoned that the second effect should also activate alkenes analogous to **3** toward epoxidation, as the reaction involves rehybridization from sp^2 to sp^3 at the homobenzylic carbon. We therefore expected that some members of the antibody series against hapten **1** might catalyze the epoxidation of alkenes if an appropriate reagent for providing oxygen from solution could be found.



Alkenes **4** and **6–10** were prepared[8] and purified by preparative HPLC. The oxygen donor of choice had to be compatible with the antibody and its aqueous environment. This proved to be a major challenge. After a survey of possible reagents,[9] we found that the combination of hydrogen peroxide and acetonitrile[10] in aqueous buffer under neutral conditions effected clean epoxidation of our substrates without affecting the antibody itself. The species which delivers oxygen to the alkene is a peroxy carboximidic acid formed by base-catalyzed[11] addition of hydrogen peroxide to the nitrile. Although peracid formation is rate limiting, the alkene epoxidation step is kinetically observable due to the competing destruction of the peracid with H_2O_2 to form singlet oxygen and acetamide.[12] This dead cycle with H_2O_2 holds the active peracid species at a very low concentration and might explain the mildness of this reagent toward the antibody.[13] The reactivity of the alkenes under these conditions increases with carbon substitution: **6** < **7** ≈ **8** < **9**.[14]



Using **6–9**, **4**, and **15** as test olefins, an extensive survey for possible catalysts was conducted. For catalysis of epoxide formation, 22 antibodies against **1** and 20 antibodies against **2** were assayed.[15] Nine anti-**1** antibodies and six anti-**2** antibodies showed rate enhancement of epoxide formation over background for either **6** (six anti-**1** and two anti-**2**), **7** (four anti-**1** and four anti-**2**), **8** (four anti-**1** and one anti-**2**), or **4** (two anti-**1**, no anti-

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(9) Magnesium monoperphthalate (resulting in rapid cleavage of the antibody), manganese(III) tetrakis(4-carboxyphenyl)porphine with hydrogen peroxide (destruction of the cofactor itself), and iron(III) tetrakis(pentafluorophenyl)porphine (insoluble in water). General references: Brougham, P.; Cooper, M. S.; Cummerson, D.A.; Heaney, H.; Thompson, N. *Synthesis* **1987**, 1015. Harriman, A.; Porter, G. *J. Chem. Soc., Faraday Trans. 2* **1979**, *75*, 1532. Also see ref. 5.

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(13) The uncatalyzed reaction is directly proportional to both the alkene (20–500 μM) and the acetonitrile concentration (5–383 mM). Measurements in 50 mM phosphate buffer, 50 mM sulfate, at pH 6.56 and 725 mM H_2O_2. There was no decrease in catalysis when the antibody-catalyzed epoxidation was followed over a period of 48 h using the conditions described below.

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Table 1. Kinetic Data Obtained for Different Alkenes with Antibody 20B11

alkene	K_M (μM)	k_{cat} (s^{-1})	k_{uncat} (s^{-1})	k_{cat}/k_{uncat}	ee (%)[a]
6	260	1.4×10^{-5}	2.4×10^{-7}	60	
7	120	6.4×10^{-5}	5.2×10^{-7}	125	67
8	140	3.0×10^{-5}	5.9×10^{-7}	50	>98
4	85	5.0×10^{-5}	1.1×10^{-6}	40	>98
9	60	3.6×10^{-5}	2.9×10^{-6}	15	

[a] Analysis on chiral phase HPLC (Chiralcel OD). The numbers correspond to product formed by catalytic antibody. The analyzed samples contain approximately 30% of racemic product formed by the uncatalyzed reaction. The optical purity for the bulk product was 47, 64, and 71% ee for **7**, **8**, and **4**, respectively. Also see text and footnotes 16 and 17. The absolute configuration of the epoxides was not determined.

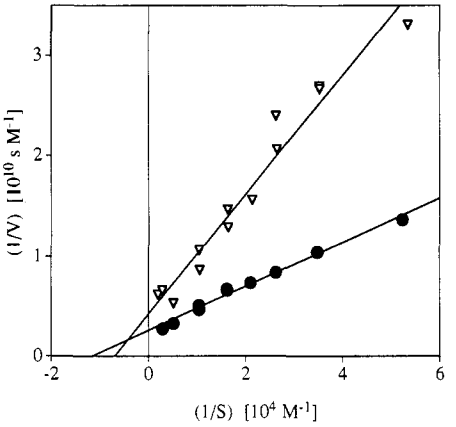

Figure 1. Lineweaver–Burk plot of reaction rates for the antibody-catalyzed epoxidation of substrates 8 (▽) and 4 (●), measured in 50 mM phosphate buffer, 50 mM in sulfate at pH 6.56 and 8 μM antibody 20B11.

2). The antibody catalysis was quantitatively inhibited by addition of hapten, thereby showing that the reaction is taking place in the antibody combining site. In these samples the reaction rate (k_{uncat}, Table 1) was in accordance with the measured background rate with antibody-free samples. One antibody (20B11, anti-**1**) was characterized in detail. This antibody catalyzed the epoxidation of **4** and **6–9** (Figure 1, Table 1), following Michaelis–Menten kinetics. No catalytic activity was detected with **10** and **15**, suggesting that proper placement of the double bond and double substitution at the homobenzylic carbon are essential for catalysis. Kinetic data for acetonitrile were collected using substrate **7** (200 μM), giving an apparent K_M of 180 mM and k_{cat} = 1.7×10^{-4} s^{-1} for 725 mM H_2O_2 at pH 6.56.

The enantioselectivity of the antibody-catalyzed epoxidation was investigated for three substrates as follows: samples of 380 μL were prepared, containing the highly purified antibody (23 μM), the alkene **7** (respectively **8** or **4**) (250 μM), acetonitrile (287 mM), and H_2O_2 (725 mM) in 50 mM phosphate buffer, 50 mM sulfate, pH 6.56. After incubation overnight,[16] the epoxide products **12**, **13**, and **5** were isolated by reversed-phase HPLC and analyzed on a chiral HPLC column.[17] As shown in Table 1, asymmetric induction by the antibody is complete for **8** and **4**. The incomplete induction observed with **7** suggests that this substrate might adopt two reactive conformations within the antibody binding site.

In summary, we have shown that the acetonitrile/hydrogen peroxide system is compatible with proteins in physiological buffers. It can be used to achieve antibody-catalyzed, enantioselective epoxidation. Future research will address improvement of catalysis using a hapten with a better match to the geometry of the epoxidation reaction.[18] In any event, the system differs from conventional methods in that a chiral environment with exquisite specificity can be induced at will.

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Supplementary Material Available: Synthetic procedures and analytical data for compounds **4–15**, HPLC traces of the separation on a chiral phase column for **12**, **13**, and **5**, and details of the x-ray structural analysis of **12** (21 pages); listing of observed and calculated structure factors for **12** (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(15) Assay conditions: 50 mM phosphate, pH 6.56, 50 mM Na_2SO_4. Sulfate replaces chloride to avoid chlorohydrine formation. The ionic strength is required to preserve the antibody structure. Alkene concentration = 250 μM, antibody concentration = 1.5–1.8 mg/mL, [H_2O_2] = 725 mM, [CH_3CN] = 96–287 mM. In each case, epoxide formation was followed by reversed-phase HPLC (Vydac 218TP54, 0.46 cm i.d. × 25 cm, flow 1.5 mL/min, eluent 20–25% CH_3CN in H_2O, 0.1% trifluoroacetic acid; UV detection (λ = 240 nm)) using an internal standard (*N*-ethyl benzamide for **6–8**, and **15**, *N*-propyl-*o*-toluamide for **4** and **9**). Another 55 antibodies against three haptens related to **1** and **2** featuring six-membered rings with one ammonium center were tested, none of them showing catalytic activity.

(16) Two aliquots of 15 μL were taken out and one of them mixed with 1 μL of an 8.3 mM solution of inhibitor (final concentration 250 μM). After incubation overnight, epoxide formation had reached 31.8% (respectively 30.7%, 32.5%) in the catalyzed sample and 9.4% (respectively 10.9%, 9.0%) in the inhibited sample for **7**, **8**, and **4**. At that stage, the antibody had carried out, on average, 3.5 turnovers/active site and still showed the full catalytic activity.

(17) Daicel Chiralcel OD HPLC column (0.46 cm i.d. × 25 cm, 70% hexane, 30% 2-propanol, flow 1.0 mL/min; UV detection (λ = 240 nm)). t_R (**12**) = 13.3 min, 17.2 min; ratio 2.8:1. t_R(**13**) = 15.2 min, 22.3 min; ratio 4.5:1. t_R(**5**) = 10.6 min, 18.8 min; ratio 5.9:1. For **11** we were unable to resolve the two enantiomers on this and three other chiral HPLC columns.

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