Antibody-Catalyzed Enantioselective Epoxide Hydrolysis

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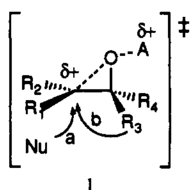
Received November 30, 1992

Acid-catalyzed opening of epoxides is not a trivial process, as the reaction may lead to a number of products, depending on the substrate and reaction conditions.[1] Interaction of an acid (A^+) with an epoxide generates an electron-deficient species (I) that



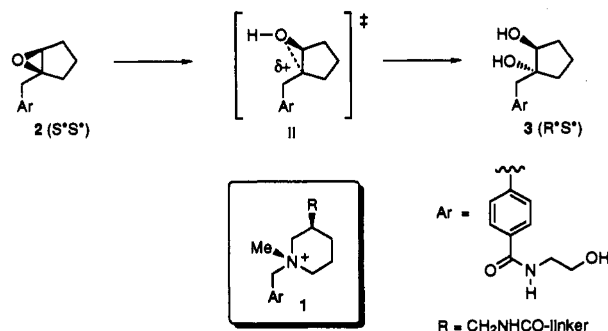
I

may undergo either external nucleophilic attack (a) to produce alcohol (e.g., diol when the nucleophile is water) or a group migration (b) to give a carbonyl compound. Additional aspects of regioselectivity and stereochemistry at the two vicinal carbon centers add to the complexity of this reaction. Nature meets the challenge of selective epoxide opening with a number of enzymes. For example, microsomal epoxide hydrolase[2] and leukotriene A_4 hydrolase[3] form 1,2- and 1,8-diol, respectively, whereas squalene oxide cyclase induces formation of new C–C bonds with skeletal rearrangement.[4]

Aiming at catalysis of enantioselective epoxide hydrolysis with monoclonal antibodies (Mabs),[5] we considered charged analogs of I to mimic the developing positive charge on one of the carbon atoms. For example, acid-catalyzed hydrolysis of epoxide 2 to produce the trans diol 3 (Scheme I) is likely to occur via transition state II, with ring opening at the tertiary carbon center.[6] We reasoned that II, which involves a considerable amount of positive charge at this center, could be mimicked by the quaternary ammoniun cation 1, which features a positively charged nitrogen atom at the appropriate position. Moreover, we expected that the asymmetric environment of the protein would induce kinetic resolution of the racemic substrate. Mabs which have been elicited against hapten 1 have already been shown to catalyze acid-promoted hydrolytic reactions, such as cleavage of cyclic acetals and ketals and enantioselective protonolysis of enol ethers.[7] Here we report on the first example of an antibody-catalyzed epoxide hydrolysis.[8]

Hydrolysis of 2[9] to produce diol 3 is catalyzed by 14D9 in aqueous acidic solutions (Table I). Similarly, epoxide 4[10] is

Scheme I



catalytically hydrolyzed by the same antibody to produce diol 5 (Chart I). This enzyme-like catalysis is evident from the observed Michaelis–Menten kinetics and from the fact that catalysis is totally inhibited in the presence of stoichiometric quantities (with respect to 14D9) of the inhibitor 6.[7,11] The aromatic moiety (Ar) of both substrates is designed to provide most of the binding energy to the antibody.[6a] Their epoxide function is positioned according to II, assuming that the hydrolysis is acid-catalyzed with the expected ring-opening at the more substituted carbon.[6] Indeed, the pH–rate profiles of hydrolysis of 2 (Figure 1)[12] and 4 (data not shown) suggest that these reactions are acid-catalyzed. This is consistent with the observation that the competing reactions with chloride ions to produce chlorohydrin 7 and 8 are not catalyzed by this antibody.[13] Accordingly, addition of other nucleophiles, including sodium azide, aminoethanol, and mercaptoethanol (all found to attack preferentially at the less substituted carbon atom), is not catalyzed by 14D9.

Enantioselectivity is one of the greatest advantages of antibody catalysis. Expecting to see kinetic resolution of epoxides 2 and 4 in the antibody-catalyzed reactions, we carried out a preparative-scale hydrolysis of racemic 2 and 4 to give 3 and 5, respectively.[14] Indeed, HPLC analysis of the diacetate 9 showed that this product

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(8) An antibody-catalyzed formation of a tetrahydropyran ring from an epoxy alcohol has been recently reported: Janda, K. D.; Shevlin, C. G.; Lerner, R. A. *Science* **1993**, *259*, 490.

(9) Epoxide 2 was prepared in a three-step sequence: (a) cross-coupling of 1-chlorocyclopentene with (4-(methoxycarbonyl)benzyl)zinc bromide in the presence of $Pd(PPh_3)_4$ (Negishi, E.-I.; Matsushita, H.; Nobuhisa, O. *Tetrahedron Lett.* **1981**, *22*, 2715) to give (4-(methoxycarbonyl)phenyl)-1-cyclopentenylmethane; (b) amidation of the latter with 2-aminoethanol at 100 °C for 4 h; and (c) epoxidation with *m*-chloroperbenzoic acid in dichloromethane.

(10) Epoxide 4 was prepared similarly to the above described synthesis of 2, starting with 2-bromopropene instead of 1-chlorocyclopentene and using $Pd(PPh_3)_2Cl_2$ instead of $Pd(PPh_3)_4$ in the cross-coupling reaction.

(11) Assay conditions: antibody (2–10 μM), substrate (20–500 μM), either phosphate-buffered saline (PBS) or acetate-buffered saline (ABS) (50 mM, pH 5.57, 100 mM NaCl). All reactions were carried out at 24 °C and monitored at 254 nm by RP-HPLC (Hitachi L-6200A equipped with an AS-2000 autosampler and a Supelcosil LC-18 column (25 cm × 4.6 mm, 5 μm) using acetonitrile/water gradient at 1.5 mL/min.

(12) Double reciprocal plot of the experimental values $1/k_{cat}$ vs $1/[H_3O^+]$ is linear (11 points, $r^2 = 0.996$, data not shown). This is consistent with the participation of an ionizable antibody side chain in catalysis and gives the values $pK = 5.8$ for the dissociation constant of that side chain (probably either an aspartate or glutamate residue) and $(k_{cat})_{max} = 3.4 \times 10^{-5}$ s^{-1} for the maximum turnover number of the antibody at low pH. The calculated line was obtained by using these values in the following equation: $1/k_{cat} = 1/(k_{cat})_{max} + (1/[H_3O^+])K/(k_{cat})_{max}$.[7d] The background reaction is the sum of a pH-independent reaction, $k_0 = 1.08 \times 10^{-8}$ s^{-1}, and a hydronium ion-catalyzed reaction, $k_{H^+} = 0.026$ M^{-1} s^{-1}. The calculated line was obtained using these values in the following equation: $k_{uncat} = k_0 + [H_3O^+]k_{H^+}$.

(13) Chlorohydrin 8 is probably formed via an acid-catalyzed S_N2-type attack of a chloride ion at the epoxide primary carbon (Addy, J. K.; Parker, R. E. *J. Chem. Soc.* **1965**, 644). Its rate of formation increases linearly with NaCl concentration in both buffer solutions. Typically, at pH range of 5–6.5, 24 °C and 100 mM NaCl, both 5 and 8 are formed in comparable amounts.

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Table I. Kinetic Data for 14D9-Catalyzed Hydrolysis of Epoxides to Vicinal Diols[a]

substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/k_{uncat}	ee (%)[b]
2	25	2.5×10^{-5}	440	87[15]
4	85	3.4×10^{-5}	100	0
4S	95	3.5×10^{-5}	105	
4R	75	3.3×10^{-5}	100	

[a] All reactions were carried out at pH 5.6, 24 °C, in phosphate buffer (50 mM) and NaCl (100 mM).[11] [b] Enantiomeric excess of diol **3** was measured by HPLC using a chiral column,[14] while that of diol **5** was determined by ^{1}H NMR of the corresponding Mosher's esters.[17,18]

is obtained in 87% ee.[15] However, in the case of antibody-catalyzed hydrolysis of **4**, no measurable optical enrichment of diol **5** could be detected by ^{1}H NMR analysis of its Mosher ester derivative.[16,17] Accordingly, hydrolysis of either of the enantiomerically enriched epoxides **4S** and **4R**[18] is catalyzed to the same extent by 14D9 (Table I). This is an interesting observation, considering the seemingly minor structural difference between **2** and **4**. The extra two methylene groups in **2** are apparently responsible for the stereodifferentiating interactions that allow for kinetic resolution. The five-membered ring of **2** (as compared with **4**) probably provides a tighter fit to the antibody hydrophobic pocket that complements the piperidinium portion of **1**.

Antibody 14D9 catalyzes the hydrolysis of **2** and **4** with multiple turnovers, as demonstrated by the fact that no measurable loss of activity was detected even after five consecutive reaction cycles under substrate-saturating conditions.[16] This stands in remarkable contrast to the recently reported case of an enone isomerizing Mab, where stoichiometric reaction of an epoxide substrate with a side chain in the active site resulted in loss of activity.[19] That antibody was elicited against a tertiary amine quite similar to our hapten **1**. The use of a quaternary ammonium function in the hapten probably generates a combining site with electrostatic interactions strong enough for catalysis, while keeping potentially reactive residues at a safe distance from the reaction center. This concept might be applicable in the design of catalytic antibodies for other reactions involving reactive substrates.

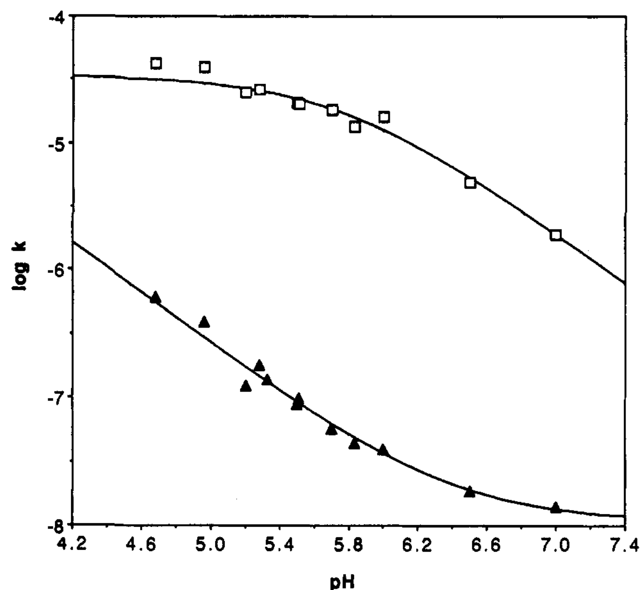
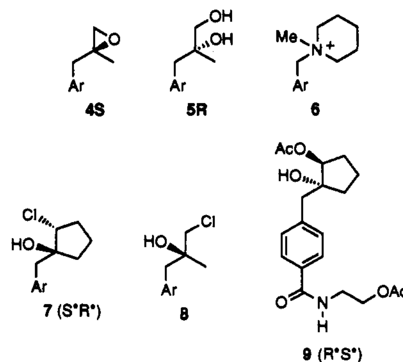


Figure 1. pH-rate profile for the hydrolysis of epoxide **2**.[11,12] The upper line and data points (□) represent the calculated and experimental log k (s^{-1}) of the antibody 14D9-catalyzed reaction, respectively. Similarly, the lower line and points (▲) represent the background (uncatalyzed) reaction. Assays were carried out in 50 mM acetate (pH 4.6–5.8) or phosphate (pH 5.2–7.0) with 100 mM NaCl at 24 °C.

Chart I



In conclusion, the first antibody-catalyzed epoxide hydrolysis reaction has been achieved. The enantioselectivity of this reaction appears to depend on the structure of the substrate. Complete kinetic resolution has been achieved with the cyclic substrate **2**. By contrast, both enantiomers of the smaller substrate **4** are equally reactive with this catalytic antibody. These observations highlight the importance of secondary interactions between substrate and antibody, and suggests that such interactions are a necessary feature to be built in to obtain efficient, enantioselective antibody catalysts.

Acknowledgment. E.K. thanks the U.S.-Israel Binational Science Foundation and the U.S. National Institutes of Health, and J.L.R. thanks the Swiss National Science Foundation for financial support.

(14) A 1.8-mL PBS (50 mM, pH 5.57) solution containing racemic epoxide **2** (510 μM) and 14D9 (40 μM) was kept at 24 °C. A control reaction mixture contained the same ingredients except 14D9. After 24 h the mixture was diluted with PBS up to 5 mL and filtered–dialyzed to 1 mL; more PBS (4 mL) was added, and the mixture was filtered–dialyzed again to 1 mL. The crude product was dissolved in a mixture of triethylamine and acetic anhydride, stirred at room temperature for 16 h, and then worked up with water and ethyl acetate (control experiments showed that under these conditions the epoxide function in **2** is absolutely stable and triol **3** is quantitatively converted to diacetate **9**). The mixture was analyzed by HPLC equipped with Chiralcel OD-H column (Daicel Chemical Industries) using hexane:2-propanol (9:1) at 1 mL/min. The two enantiomeric products **9** (their absolute configuration is yet unknown) appeared at 31.3 and 35.5 min. The chemical yield of **7** in this reaction was 20.5% (by HPLC). In a control reaction, carried out without antibody under identical conditions, the chemical yield was 2.8% with a 1:1 ratio between the two enantiomers of **9**.

(15) Considering the fact that 13.5% of **3** originates from a spontaneous, uncatalyzed hydrolysis of **2** (as determined by a control experiment), the actual enantioselectivity is greater than 98%.

(16) A preparative-scale reaction with racemic **4** was carried out under the same conditions described in ref 14 for substrate **2**. After 24 h the mixture was diluted with PBS up to 5 mL and filtered–dialyzed to 1 mL; more PBS (4 mL) was added, and the mixture was filtered–dialyzed again to 1 mL. The aqueous filtrate was stored at −80 °C. More substrate was added (to reach 510 μM), and four more cycles were carried out in the same manner. After five cycles the combined filtrate was saturated with NaCl, extracted with 2-propanol, and immediately separated by column chromatography (silica gel, EtOAc). Both samples of triol **5** from the catalyzed as well as the uncatalyzed reactions exhibited the same 1:1 ratio of enantiomeric products, as was evident from ^{1}H NMR analysis of their Mosher's esters.[17]

(17) Mosher's esters (Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543) of **5** were prepared as described earlier (Keinan, E.; Sinha, S. C.; Sinha-Bagchi, A. *J. Org. Chem.* **1992**, *57*, 3631) using acetonitrile as a solvent. In the case of the triols **5S** and **5R**, the two primary alcohols were esterified. Enantiomeric enrichment was determined by integration of the ^{1}H NMR signals related to the methyl carbinol group in the corresponding Mosher's esters: δS, 1.179; R, 1.166.

(18) Using the asymmetric dihydroxylation reaction ((a) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768. (b) Keinan, E.; Sinha, S. C.; Sinha-Bagchi, A.; Wang, Z.-M.; Zhang, X.-L.; Sharpless, K. B. *Tetrahedron Lett.* **1992**, *33*, 6411), 3-(4-(methoxycarbonyl)phenyl)-2-methylpropene was reacted with either AD-mix-α or with AD-mix-β to produce the corresponding (S)- and (R)-diols in 39% and 40% ee, respectively. This was determined by integration of the ^{1}H NMR signals related to the methyl carbinol group in the corresponding Mosher's monoester: δ S, 1.168; R, 1.181. This level of enantiomeric purity may be increased by using benzyl instead of methyl esters and by recrystallizing the corresponding diols from cold diethyl ether. The diols were converted to the corresponding substrates **4*S*** and **4*R*** via a four-step sequence: (a) monotosylation of the primary alcohol with 1 equiv of p-toluenesulfonyl chloride in pyridine; (b) ring closure with K_2CO_3 in methanol; (c) ester hydrolysis with KOH in aqueous methanol; and (d) amidation with 2-aminoethanol using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (EDC) and N-hydroxysuccinimide in DMF.

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