An Antibody-Catalyzed Oxy-Cope Rearrangement

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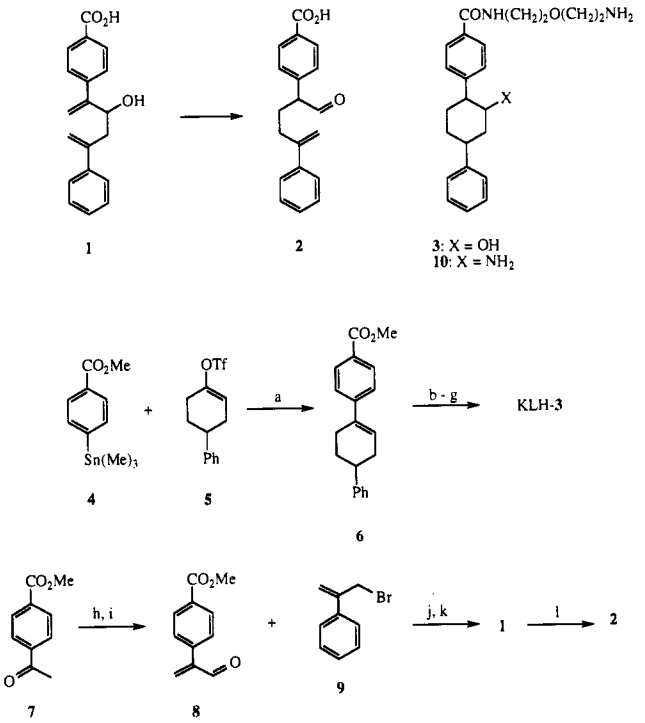
One objective in the field of antibody catalysis has been to apply what has been learned from studies of enzyme-catalyzed reactions to the design of catalysts for reactions which are rare or do not occur in nature.[1] The Cope rearrangement is an important example of one such reaction which has received considerable attention from synthetic and mechanistic chemists[2,3] but for which an enzyme has yet to be characterized. This [3,3] sigmatropic rearrangement occurs via a highly ordered chairlike transition state ($\Delta S^{\ddagger} \sim -15$ cal^{-1} mol^{-1} K^{-1}),[4,5] suggesting that an antibody which binds and orients the substrate in this chairlike conformation should catalyze the reaction by acting as an "entropy trap." Such an approach has previously been applied to the design of catalysts for two other pericyclic reactions, the Claisen rearrangement[6] and the Diels-Alder reaction.[7] We now report the generation of a number of antibodies that catalyze an oxy-Cope rearrangement and the characterization of their kinetic constants as they relate to catalytic mechanism.

Antibodies were generated against hapten **3**, which mimics the chairlike six-membered ring geometry of the transition state for the rearrangement of the disubstituted 1,5-hexadiene **1** to **2**.[8] Hapten **3** was synthesized by a Stille coupling[9] between aryltin derivative **4** and vinyl triflate **5** with subsequent hydroboration of the olefin **6** (Scheme 1). The diastereomer with all three substituents equatorial was isolated and linked to the carrier proteins keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) via a seven-atom tether. The substrate for the oxy-Cope rearrangement was synthesized by way of a Luche reaction[10] between the organozinc derivative of allyl bromide **7** and the allyl aldehyde **8**. A total of 42 monoclonal cell lines specific for **3** were generated and purified to homogeneity by protein A chromatography.[11,12] These antibodies were assayed for catalysis of the oxy-Cope rearrangement by reverse-phase high-performance liquid chromatography (HPLC) with the aldehyde product being trapped *in situ* as the oxime with hydroxylamine.[13]

Scheme 1[a]



[a] (a) LiCl, tetrakis(triphenylphosphine)palladium(0), THF (50%); (b) BH_3, THF (53%); (c) NaOH, aqueous THF (95%); (d) DCC, HOBT, $H_2N(CH_2)_2O(CH_2)_2NHBoc$ (90%); (e) trifluoroacetic acid, CH_2Cl_2 (100%); (f) thiophosgene, CH_2Cl_2; (g) KLH or BSA; (h) methyl triphenylphosphonium bromide, nBuLi, THF (67%); (i) SeO_2, tBuOOH, CH_2Cl_2 (38%); (j) Zn, aqueous NH_4Cl/THF (74%); (k) NaOH, aqueous THF (84%); (l) benzene, sealed tube, 160 °C, 4 h.

Nine monoclonals were found to be effective catalysts for the rearrangement of **1** to **2**, and the k_{cat} and K_m values for four of these antibodies were determined from Lineweaver-Burk plots (Figure 1). While three of these antibodies catalyzed the rearrangement on the order of several hundred-fold over the uncatalyzed rate, one antibody (AZ-28) was an efficient catalyst with a rate acceleration of $k_{cat}/k_{uncat} = 5300$.[14] No inhibition of the antibody-catalyzed reaction was observed after more than 40 turnovers of substrate per antibody active site (at 80 μM substrate **1** and 1 μM antibody AZ-28). Thus it appears that *in situ* generation of the oxime prevents both chemical modification of the antibody and competitive inhibition by the aldehyde product **2**.

The K_i values for all four antibodies for hapten **3** (Table 1) were determined by fitting the inhibition data to an equation for

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(1) (a) For reviews of catalytic antibodies, see: Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659-667. (b) Schultz, P. G.; Lerner, R. A. *Acc. Chem. Res.* **1993**, *26*, 391-395.

(2) For general reviews of pericyclic reactions, see: Marchand, A. P.; Lehr, R. E. *Pericyclic Reactions*; Academic Press: New York, 1977. Woodward, R. B.; Hoffmann, R. *The Conservation of Orbital Symmetry*; Cambridge University Press: London, 1972.

(3) For reviews of the Cope rearrangement, see: Rhoads, S. J.; Raulins, N. R. *Org. React.* **1975**, *22*, 1-252. Doering, W.v.E.; Roth, W. R. *Angew. Chem., Int. Ed.* **1963**, *2*, 115.

(4) Doering, W.v.E.; Roth, W. R. *Tetrahedron* **1962**, *18*, 67.

(5) Foster, G. E.; Cope, A. C.; Daniels, F. *J. Am. Chem. Soc.* **1947**, *69*, 1893.

(6) (a) Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A.; Schulz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 4841. (b) Hilvert, D.; Carpenter, S. H.; Nared, K. D.; Auditor, M. T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4953. (c) Jackson, D. Y.; Liang, M. N.; Bartlett, P. A.; Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 182. (d) Campbell, P. A.; Tarasow, T.; Massefski, W.; Wright, P. E.; Hilvert, D. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8663.

(7) (a) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 7430-7431. (b) Hilvert, D.; Hill, K. W.; Nared, K.; Auditor, M. T. M. *J. Am. Chem. Soc.* **1989**, *111*, 9261. (c) Gouverneur, V. E.; Houk, K. N.; Pascual-Theresa, B.; Beno, B.; Janda, K. D.; Lerner, R. A. *Science* **1993**, *262*, 204.

(8) Dewar, M. J. S.; Wade, L. E. *J. Am. Chem. Soc.* **1977**, *99*, 4417-4424.

(9) Scott, W. J.; Crisp, G. T.; Stille, J. K. *J. Am. Chem. Soc.* **1984**, *106*, 4630.

(10) Wilson, S. R.; Guazzaroni, M. E. *J. Org. Chem.* **1989**, *54*, 3087.

(11) Shokat, K. M. Ph.D. Thesis, University of California, Berkeley, CA, 1991.

(12) Kronvall, G.; Grey, H.; Williams, R. *J. Immunol.* **1972**, *105*, 1116.

(13) All reactions were carried out in aqueous buffer composed of 20 mM MES, 100 mM NaCl, 5 mM hydroxylamine, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 2% acetonitrile at pH 6.0 and 25 °C. The aldehyde product was trapped *in situ* as the oxime with hydroxylamine. Reactions were assayed by HPLC using a Microsorb 5-μm C_{18} column with a 25-cm bed and a 4.6-mm i.d. using a gradient beginning with 43% acetonitrile in 0.1% aqueous trifluoroacetic acid, increasing to 53% acetonitrile over 11.5 min, and then 100% acetonitrile at 14 min with a flow rate of 1.0 mL per minute. Compounds were detected by UV at 240 nm, and the retention times of **8** and **9** were 11.0 and 12.5 min, respectively. Product formation was quantitated against the internal standard, 3-nitrobenzoic acid (retention time = 5.7 min). All kinetic runs were performed in duplicate at five different substrate concentrations with six data points per concentration (80, 100, 125, 200, and 500 μM) at less than 3% reaction completion. Antibody concentrations were 4 μM in binding sites except for AZ-28, which was assayed at a concentration of 1 μM binding sites.

(14) The background rate (k_{uncat}) was determined under conditions identical to those for the antibody-catalyzed reaction and found to be 4.9×10^{-6} min^{-1}. The uncatalyzed reaction showed no significant pH dependence between pH 6.0 and 8.0.

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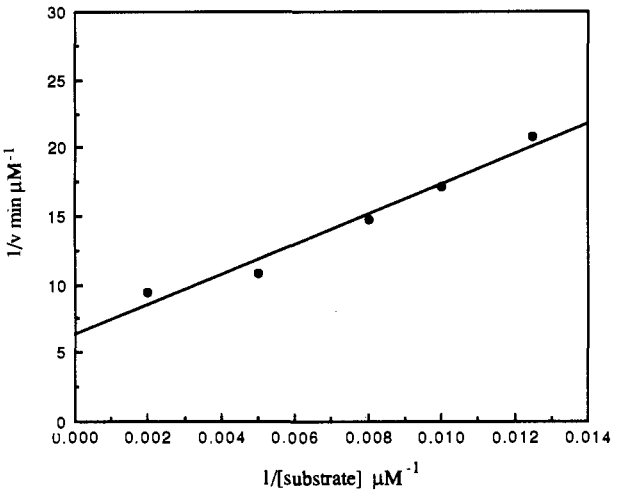

Figure 1. Lineweaver–Burk plot for antibody AZ-28.

tight-binding competitive inhibitors[15–18] and ranged from 30 to 160 nM. A log plot of the K_i vs K_m/k_{cat} values of these four antibodies shows a linear trend over 2 orders of magnitude, whereas the corresponding log plot of K_i vs K_m shows no linearity. These results suggest that hapten **3** is in fact acting as a transition-state analogue in the generation of these catalytic antibodies.[19,20] Analysis of the reaction stereochemistry and activation parameters will provide additional insight into this novel biological catalyst. In addition, antibodies are currently being generated against hapten **10**, in which the positively-charged ammonium ion is expected to elicit a general base in the antibody combining site which might accelerate the rearrangement via an oxyanion substituent effect.[21]

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Supplementary Material Available: Full experimental details for the synthesis of the compounds described in Scheme 1 (13 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) K_i values were determined by measuring the reaction rate over a range of six hapten concentrations (starting with 0.2 μM and increasing to 1.0 μM in the case of AZ-28 and 2.0 μM for the others) at a substrate concentration of 80 μM and using the same assay conditions described above. Curve fitting[16] was accomplished using the KaleidaGraph data analysis program (Synergy Software) on a Macintosh Quadra 700. These K_i values represent the apparent K_i at the substrate concentration of 80 μM and should be considered relative to each other.

(16) Inhibition data were fitted to the following equation:

$$v_i/v_0 = ([E_t] - [I] - K_i' + \{([E_t] - [I] + K_i')^2 + 4K_i'[I]\}^{0.5})/[E_t]$$

Where $[E_t]$ = total concentration of antibody binding sites, [I] = concentration of inhibitor, $K_i' = K_i(1 + [S]/K_m)$, [S] = substrate concentration, v_i = initial velocity measured in the presence of inhibitor, and v_0 = initial velocity in the absence of inhibitor.

(17) Cha, S. *Biochem. Pharmacol.* **1975**, *24*, 2177.

(18) Angeles, T. S.; Smith, R. G.; Darsley, M. J.; Sugasawara, R.; Sanchez, R. I.; Kenten, J.; Schultz, P. G.; Martin, M. T. *Biochemistry* **1993**, *32*, 12128–12135.

(19) (a) Wolfenden, R. *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 271–306. (b) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618–4624.

(20) Phillips, M. A.; Kaplan, A. P.; Rutter, W. J.; Bartlett, P. A. *Biochemistry* **1992**, *31*, 959–963.

(21) Evans, D. A.; Golub, A. M. *J. Am. Chem. Soc.* **1975**, *97*, 4765.