An Antibody-Catalyzed [2,3]-Elimination Reaction

Seung Soo Yoon,† Yoko Oei, Elizabeth Sweet, and Peter G. Schultz*

Howard Hughes Medical Institute
Department of Chemistry, University of California
Berkeley, California 94720

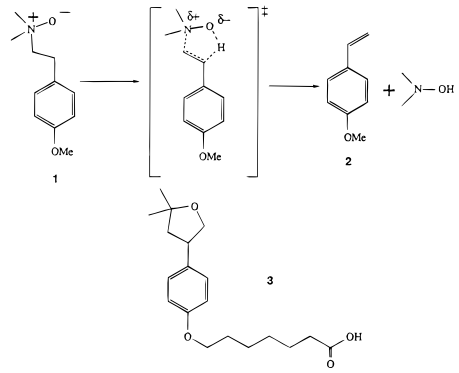
Received July 3, 1996

Although there are no obvious limitations to the types of chemical transformations that can be catalyzed by enzymes, only a few examples are known of what are formally enzyme-catalyzed, pericyclic reactions.[1] Consequently, there has been considerable interest in generating antibodies that catalyze this class of reactions, both to increase the scope of biological catalysis as well as to gain insight into the mechanisms by which such catalytic functions might evolve.[2] We now report an example of an antibody-catalyzed [2,3]-sigmatropic reaction, the Cope elimination[3] of *N*-oxide **1** to dimethylhydroxyamine and 4-methoxystyrene **2** (Scheme 1).

In order to catalyze this reaction, monoclonal antibodies were generated against the keyhole limpet hemocyanin (KLH) conjugate of substituted tetrahydrofuran **3**, which resembles the conformationally restricted transition state of the reaction.[4] Moreover, the reduced dipole monent of hapten **3** relative to the substrate is likely to induce a low-dielectric environment in the corresponding antibody combining site. Such an environment might be expected to accommodate the charge dispersion on going from substrate to transition state better than water. This effect has previously been exploited in an antibody-catalyzed decarboxylation reaction.[5] Consequently, an antibody generated to hapten **3** might be expected to catalyze the Cope elimination of substrate **1** through a combination of entropy and medium effects. The dissociative nature of the reaction should minimize product inhibition.

The *N*-hydroxysuccinyl ester of hapten **3**[6] was coupled to KLH and the resulting protein conjugate was used to immunize Balb/c mice. Twenty-three monoclonal antibodies specific for hapten **3** were obtained by standard protocols,[7] and purified by chromatography on protein A-coupled Sepharose 4B.[8] The conversion of *N*-oxide **1** to product in aqueous 5 mM NaCl, 50 mM Na_2PO_4 buffer, pH 7.5, was followed by high-performance liquid chromatography (HPLC).[9] One of the twenty-three antibodies, 21B12.1, was found to catalyze the [2,3]-elimination reaction over the background reaction, with initial rates consistent with Michaelis-Menten kinetics. The values of k_{cat} and K_m were determined by fitting the kinetic data to the Michaelis-Menten equation using a nonlinear regression program[10] and are 1.44×10^{-3} h^{-1} and 235 μM, respectively, at 37 °C. The unimolecular reaction rate constant (k_{uncat}) for the uncatalyzed reaction under the same conditions is 1.58×10^{-6} h^{-1}, corresponding to a rate enhancement of roughly 10^3 over the uncatalyzed reaction. Antibody 21B12.1 did not measurably catalyze the Cope elimination reaction of the demethylated substrate analogue, 2-(4′-hydroxyphenyl)ethyldimethylamine oxide, or the elimination reaction of the corresponding sulfoxide, 2-(4′-methoxyphenyl)ethyl methyl sulfoxide, indicating that the antibody-catalyzed reaction is highly selective. Hapten **3** inhibits the antibody-catalyzed reaction competitively with a K_i of 200 nM at 37 °C.[11] The ratio of K_i to K_m correlates roughly with the observed rate acceleration, consistent with the notion that the rate enhancement is due to the preferential binding of the transition state by antibody. The deuterated substrate, **1**-*2,2*-d_2, was prepared[12] and kinetic isotope effects were measured. The value of k_{catH}/k_{catD} is 2.78 for the antibody-

Scheme 1. Antibody-Catalyzed Cope Elimination Reaction and Transition State Analogue



* Author to whom correspondence should be addressed.

† Current address: Department of Chemistry, Sung Kyun Kwan University, Natural Science Campus, Suwon 440-746, Korea.

(1) (a) Andrews, G. D.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492. (b) Gorisch, H. *Biochemistry* **1978**, *17*, 3700. (c) Guilford, W. J.; Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 5013. (d) Laschat, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 289. (e) Oikawa, H.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Chem. Soc., Chem. Commun.* **1995**, 1321.

(2) (a) Hilvert, D.; Hill, K. W.; Nared, K. D.; Auditor, M. *J. Am. Chem. Soc.* **1989**, *111*, 9261. (b) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 7430. (c) Hilvert, D.; Carpenter, S. H.; Nared, K. D.; Auditor, M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4953. (d) Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 4841. (e) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1994**, *116*, 2211. (f) Gouverneur, V. E.; Houk, K. N.; Pascul-Teresa, B. D.; Beno, B.; Janda, K. D.; Lerner, R. A. *Science* **1993**, *262*, 204. (g) Yli-Kauhaluoma, J. T.; Ashley, J. A.; Lo, C.-H.; Tucker, L.; Wolfe, M. M.; Janda, K. D. *J. Am. Chem. Soc.* **1995**, *117*, 7041. (h) Suckling, C. J.; Tedford, M. C.; Bence, L. M.; Irvine, J. I.; Stimson, W. H. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1925.

(3) Cope, A. C.; Foster, T. T.; Towel, P. H. *J. Am. Chem. Soc.* **1949**, *71*, 3939.

(4) Kwart, H. *Acc. Chem. Res.* **1982**, *15*, 401.

(5) (a) Lewis, C.; Kramer, T.; Robinson, S.; Hilvert, D. *Nature* **1991**, *253*, 1019. (b) Lewis, C.; Paneth, P.; O'Leahy, M. H.; Hilvert, D. *J. Am. Chem. Soc.* **1993**, *115*, 1410.

(6) Hapten **3** was synthesized from the commercially available starting material, 2(5*H*)-furanone. A palladium-catalyzed coupling reaction of starting material with 4-iodoanisole followed by hydrogenation, Grignard reaction with methyl magnesium bromide, and acid-catalyzed cyclization in the presence of *p*-toluenesulfonic acid afforded 2-dimethyl-4-(4′-methoxyphenyl)tetrahydrofuran. Removal of the methyl protecting group of phenol with sodium hydride and ethanethiol, followed by Mitsunobu alkylation with ethyl 6-hydroxyhexanoate and subsequent hydrolysis of the ethyl ester, provided hapten **3**. Substrate **1** was synthesized by an ethyl-(3-dimethylaminopropyl)dicarbodiimide coupling reaction of 4-methoxyphenylacetic acid with dimethylamine, followed by reduction with borane−THF complex and oxidation with *m*-chloroperbenzoic acid.

(7) Jacobs, J. W. Ph.D. Thesis, University of California, Berkeley, CA 94720.

(8) (a) Kronvall, G.; Grey, H.; Williams, R. *J. Immunol.* **1972**, *105*, 1116. (b) Harlow, E.; Lane, D. *Antibodies. A Laboratory Manual*; Cold Spring Harbor Laboratory: New York, 1988.

(9) HPLC assays were carried out with a Microsorb C18 reverse-phase column with a gradient starting a 10% acetonitrile in water and increasing to 100% acetonitrile over 25 min. Product formation was monitored at 270 nm and quantitated against the internal standard, 4-(*N*-ethylamido)-benzoic acid methyl ester. The retention time of the product formed in the catalyzed and uncatalyzed reaction is identical with that of commercially available 4-methoxystyrene.

(10) Initial rates were measured at five different substrate concentrations (100, 175, 250, 500, and 1000 μM) and the initial rate data were fitted to the Michaelis-Menten equation $v = V_{max}[\mathbf{1}]/(K_m + [\mathbf{1}])$ using the Levenverg−Marquart algorithm of the Kaleida Graph computer program (Abelbeck software). Antibody concentrations were 4.3 μM in binding sites.

(11) Inhibition data were fitted to following equation:

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

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: Williams, J. W.; Morrison, J. F. *Methods Enzymol.* **1979**, *63*, 437.

(12) Reduction of *p*-anisoyl chloride with sodium borodeuteride followed by mesylation, replacement with cyanide, reduction with $LiAlH_4$, reductive dimethylation, and oxidation with *m*-chloroperbenzoic acid provided the deuterated substrate.

S0002-7863(96)02257-3 CCC: $12.00

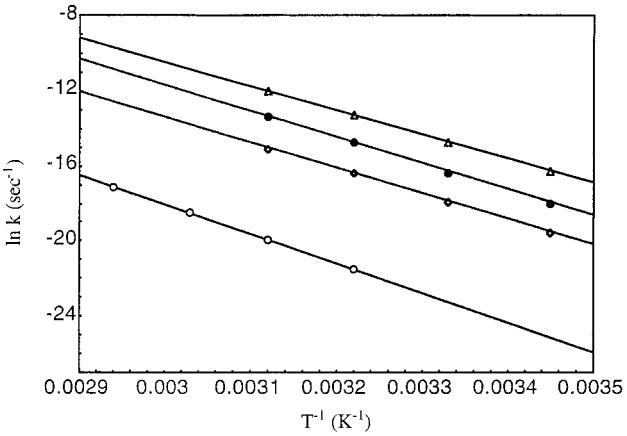


Figure 1. Arrhenius plot for the antibody 21B12.1 catalyzed reaction (●) and the uncatalyzed reactions in 1,4-dioxane (◊), DMF (△), and aqueous buffer (○).

catalyzed reaction, suggesting that the rate-determining transition state for the reaction involves some degree of C−H bond breaking. For the uncatalyzed reaction in aqueous buffer, the value of k_{uncatH}/k_{uncatD} is 2.34.[14]

To gain additional insight into the mechanism of catalysis by antibody, 21B12.1, the kinetics of the catalyzed (k_{cat}) and uncatalyzed (k_{uncat}) elimination reactions were examined as a function of temperature (Figure 1). The activation parameters for the uncatalyzed reaction are $\Delta H^{\ddagger} = 30.6$ kcal/mol and $\Delta S^{\ddagger} = -2.84$ eu and the values for the antibody-catalyzed reaction are $\Delta H^{\ddagger} = 27.2$ kcal/mol and $\Delta S^{\ddagger} = -0.29$ eu, in aqueous 5 mM NaCl, 50 mM Na_2PO_4 buffer, pH 7.5.[15] The difference in $\Delta G^{\ddagger}$ between the antibody-catalyzed reaction and the uncatalyzed reaction is 4.2 kcal/mol at 37 °C, consistent with the roughly 1000-fold rate acceleration. The fact that the entropy of activation for the antibody-catalyzed reaction is about zero suggests that the antibody constrains the substrate in a favorable conformation for the elimination reaction. However, the antibody functions primarily by lowering the enthalpy of activation for reaction, consistent with solvation effects on the reaction.

To provide additional evidence for this idea, we measured the elimination reaction of substrate **1** in dimethylformamide (DMF) and 1,4-dioxane. The values of k_{uncat} are 2.76×10^{-4} h^{-1} in 1,4-dioxane ($E_T = 36$)[16] and 5.79×10^{-3} h^{-1} in DMF ($E_T = 43$)[16] at 37 °C. The activation parameters for the uncatalyzed reaction in 1,4-dioxane are $\Delta H^{\ddagger} = 26.6$ kcal/mol and $\Delta S^{\ddagger} = -5.71$ eu. The values for the uncatalyzed reaction in DMF are $\Delta H^{\ddagger} = 25.3$ kcal/mol and $\Delta S^{\ddagger} = -3.55$ eu.[16] The change of reaction medium from water ($E_T = 63.1$)[16] to organic solvent was found to significantly accelerate the *N*-oxide elimination reaction, largely by lowering the enthalpy of activation. These results are consistent with the notion that the medium effects play a significant role in this antibody-catalyzed reaction. The lack of a quantitative correlation of k_{uncat} with the solvent parameter E_T[16] likely reflects the differing abilities of DMF and dioxane to stabilize both substrate **1** and a transition state in which partial proton transfer is occurring. Given the substrate specificity of the antibody and the small fraction of active antibodies relative to those that bind hapten **3**, other factors are clearly influencing catalysis. Additional mechanistic and structural studies on antibody 21B12.1, the germline precursor, and noncatalytic antibodies specific for **3** may clarify this issue.

In summary, we have successfully elicited an antibody that significantly accelerates the rate of [2,3]-sigmatropic elimination reaction. Mechanistic studies underscore the importance of medium effects in this antibody-catalyzed reaction and suggest that such effects,[17] when combined with entropic effects as well as general acid−base and nucleophilic catalysis, could lead to large additive reductions in the $\Delta G^{\ddagger}$ of catalytic antibodies.

Acknowledgment. We are grateful for financial support for this work from the National Institutes of Health (Grant No. R01AI24695). P.G.S. is a Howard Hughes Medical Institute Investigator. S.S.Y. is supported by a fellowship from the Helen Hay Whitney Foundation.

JA962257W

(13) Equimolar concentrations of **1** and **1**-*2,2*-d_2 were combined and added to the antibody solution. Reaction products (<1% conversion) were purified by HPLC and analyzed by mass spectrometry. The signal resulting from **2** and the deuterated product were separately integrated and the ratio of the integral (P_H/P_D) was taken as the mole ratio of product. The value of k_{catH}/k_{catD} is uncorrected for secondary isotope effects.

(14) For the uncatalyzed reaction, the values of k_H/k_D are 2.52 and 2.54 in dioxane and DMF, respectively.

(15) The reaction rates (k_{cat} and k_{uncat}) were measured at four different temperatures (47, 37, 27, and 17 °C) for the antibody-catalyzed reaction in water and the uncatalyzed reaction in 1,4-dioxane and DMF, and (67, 57, 47, and 37 °C) for the uncatalyzed reaction in aqueous buffer. The activation parameters were determined from an Arrhenius plot.[18]

(16) Dimroth, K.; Reichardt, C.; Siepmann, T.; Bohlmann, F. *Ann. Chem.* **1963**, *661*, 1.

(17) (a) Chiao, W.-B.; Saunders, W. H., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 2802. (b) Cram, D. J.; Sahyun, M. R.; Knox, G. R. *J. Am. Chem. Soc.* **1962**, *84*, 1734.

(18) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper & Row: New York, 1987; p 209.