An Antibody-Catalyzed Selenoxide Elimination

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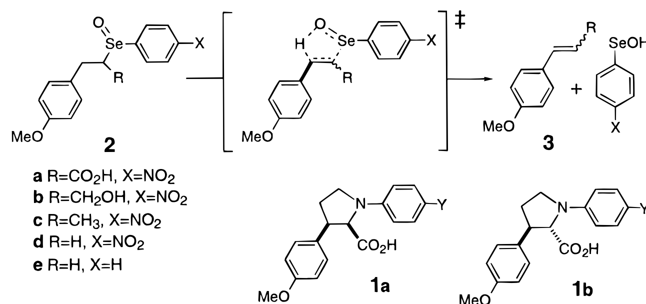
Despite their importance in chemical synthesis, pericyclic reactions are rare in cellular metabolism.[1] Engineered proteins that catalyze cycloadditions[2] and sigmatropic rearrangements[3] with high rates and selectivities are consequently of considerable interest for synthetic and mechanistic studies. Here we describe three tailored antibody catalysts for a [2,3]-sigmatropic reaction, the selenoxide elimination depicted in Scheme 1, and their distinct selectivities.

Selenoxide syn elimination affords a convenient method of introducing olefins into many molecules.[4−7] The reaction is believed to proceed via a planar, 5-membered, pericyclic transition state which is less polar than the initial state.[8,9] We reasoned that antibodies raised against proline derivatives such as **1a** and **1b** would provide a relatively low dielectric environment capable of constraining the flexible alkyl aryl selenoxides **2** in a reactive conformation, with the relative disposition of the hapten's carboxylate and 3-aryl moieties dictating the orientation of the corresponding substituents at the transition state. Although formation of *trans*-olefin products from acyclic secondary selenoxides is favored for steric reasons, antibodies prepared with the *cis*-hapten **1a** (Scheme 1) could conceivably provide sufficient binding energy to overcome the unfavorable eclipsing interactions encountered in the transition state leading to *cis*-olefins.

Racemic haptens **1a** and **1b** (Y = NHC(O)CH_2Br) were synthesized, coupled to carrier proteins, and used to produce monoclonal antibodies by standard methods.[10,11] Twenty-eight antibodies elicited by the *cis*-hapten and 20 antibodies elicited

Scheme 1



by the *trans*-hapten were purified and screened for catalytic activity using the selenoxide derivatives **2a−e**.[12,13] Three catalysts (SZ-*cis*-39C11, SZ-*cis*-42F7, and SZ-*trans*-28F8) were identified and subjected to further characterization. In each case, compounds **1a** and **1b** (Y = NO_2) are potent inhibitors of their respective antibodies,[14] indicating that catalysis is associated with the induced active site. The antibodies raised against the *cis*-hapten appear to be enantioselective, requiring 2 equiv of racemic **1a** per binding site to abolish activity, but SZ-*trans*-28F8 accommodates both enantiomers of **1b**, as judged by the 1:1 stoichiometry of inhibition.

For each of the antibodies, catalytic efficiency generally increases with decreasing size of the substituent α to the selenoxide moiety (R = CO_2H < CH_2OH < CH_3 ≲ H). Steady state kinetic parameters were determined for the best substrates (**2c−e**) from plots of initial rates versus substrate concentration and are presented in Table 1. The low K_m values suggest significant contributions to binding from the two aryl rings, while the rate enhancements over the corresponding uncatalyzed reactions ($k_{cat}/k_{uncat} \leq 10^3$) are similar in magnitude to those observed for other antibody-catalyzed sigmatropic processes.[3]

Although the selenoxide moiety itself rapidly epimerizes under the reaction conditions,[15] the additional chiral center in the secondary selenoxides influences the course of the antibody-catalyzed reactions in dramatically different ways. Thus, SZ-*trans*-28F8 evinces no chiral discrimination with substrate **2c**, converting 100% of the racemic starting material to anethole **3** (R = CH_3, >90% trans) (Figure 1). A small amount of *cis*-olefin is formed (≈8%), as in the uncatalyzed reaction, indicating more than a single binding mode for the flexible substrate. Together, these results correlate well with this antibody's ability to recognize both racemic haptens.[14] In contrast, SZ-*cis*-39C11 and SZ-*cis*-42F7 appear to be highly stereoselective as judged by catalytic conversion of only 50% of the racemic substrate (Figure 1A). *trans*-Anethole is the exclusive product of the SZ-*cis*-42F7-catalyzed reaction, but SZ-*cis*-39C11 affords a 45:55 mixture of *cis*- and *trans*-olefin (Figure 1B). The comparable energies of the SZ-*cis*-39C11-

(1) Pindur, U.; Schneider, G. H. *Chem. Soc. Rev.* **1994**, 409−415.
(2) (a) Hilvert, D.; Hill, K. W.; Nared, K. D.; Auditor, M.-T. M. *J. Am. Chem. Soc.* **1989**, *111*, 9261−9262. (b) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 7430−7431. (c) Gouverneur, V. E.; Houk, K. N.; Pascual-Teresa, B.; Beno, B.; Janda, K. D.; Lerner, R. A. *Science* **1993**, *262*, 204−208.
(3) (a) Hilvert, D.; Carpenter, S. H.; Nared, K. D.; Auditor, M. T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4953−4955. (b) Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 4841−4842. (c) Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1994**, *116*, 2211−2212.
(4) Sharpless, K. B.; Young, M. W.; Lauer, R. F. *Tetrahedron Lett.* **1973**, 1979−1982.
(5) Sharpless, K. B.; Young, M. W. *J. Org. Chem.* **1975**, *40*, 947−949.
(6) Reich, H. J.; Wollowitz, S.; Trend, J. E.; Chow, F.; Wendelborn, D. F. *J. Org. Chem.* **1978**, *43*, 1697−1705.
(7) Reich, H. J. *Acc. Chem. Res.* **1979**, *12*, 22−30.
(8) Kwart, H. *Acc. Chem. Res.* **1982**, *15*, 401−408.
(9) Kwart, L. D.; Horgan, A. G.; Kwart, H. *J. Am. Chem. Soc.* **1981**, *103*, 1232−1234.
(10) Haptens **1a** and **1b** were synthesized in racemic form from 3-(4-methoxyphenyl)proline (Chung, J. Y. L.; Wasicak, J. T.; Arnold, W. A.; May, C. S.; Nadzan, A. M.; Holladay, M. W. *J. Org. Chem.* **1990**, *55*, 270−275) by *N*-arylation with 4-fluoronitrobenzene, chromatographic separation of the cis and trans isomers, followed by reduction of the nitro group and acylation of the resulting amines with bromoacetyl bromide. All new compounds gave satisfactory spectroscopic data. Protein conjugates were prepared by alkylation of the thiols on thyroglobulin and bovine serum albumin previously modified with 2-iminothiolane. Epitope density ranged from 8 to 26 haptens per protein molecule.
(11) Immunization with the thyroglobulin conjugate of **1a** and **1b** and preparation of monoclonal antibodies were performed by standard methods (Harlow, E.; Lane, D. *Antibodies: A Laboratory Manual*; Cold Spring Harbor Lab.: New York, 1988). Hybridomas were subcloned twice and propagated in mouse ascites. Antibodies were purified by ammonium sulfate precipitation and sequential chromatography on DEAE-Sepharose, Protein G, and MonoQ ion-exchange columns.
(12) Substrates **2a−e** were prepared immediately prior to use by in situ oxidation of the corresponding selenides with excess hydrogen peroxide. The selenides were synthesized from the corresponding alcohols, activated as the mesylate, by nucleophilic displacement with a substituted arylselenolate. All new compounds gave satisfactory spectroscopic data.
(13) All kinetic measurements were performed in aqueous buffer (60 mM Tris, 100 mM NaCl, pH 8.00) at 25 °C unless otherwise indicated. Reactions were monitored spectrophotometrically (at 275 nm for **2a**, 260 nm for **2b** and **2c**, and 258 nm for **2d** and **2e**) and/or by HPLC. HPLC assays were performed on a LiChrosorb C-18 reverse-phase column (10 mm × 25 cm, eluted isocratically with mixtures of water and acetonitrile containing 0.05% trifluoroacetic acid at 1.2 mL/min). Reaction products were verified by HPLC by coinjection with authentic samples.
(14) Inhibition constants, determined as previously described (Tarasow, T. M.; Lewis, C.; Hilvert, D. *J. Am. Chem. Soc.* **1994**, *116*, 7959−7963), show that the anti-**1a** antibodies bind the *cis*-hapten more than 3 orders of magnitude more tightly than the trans. For SZ-*cis*-39C11, for example, K_i values of 47 nM and 96 μM were obtained for **1a** and **1b** (Y = NO_2), respectively. In contrast, SZ-*trans*-28F8 binds both hapten isomers with comparable affinity (K_i = 84 nM, **1a**; 82 nM, **1b**).
(15) Davis, F. A.; Reddy, R. T. *J. Org. Chem.* **1992**, *57*, 2599−2606.

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Table 1. Kinetic Parameters for the Antibody-Catalyzed Syn Elimination of Selenoxide **2**[a]

antibody	substrate	k_{cat}/K_m (M^{-1} min^{-1})	k_{cat} (min^{-1})	k_{cat}/k_{uncat}
SZ-*trans*-28F8	**2c** (R = Me, X = NO_2)	120000	0.18	160
	2d (R = H, X = NO_2)	1700	0.016	210
	2e (R = H, X = H)	250	0.0012	73
SZ-*cis*-39C11	**2c** (R = Me, X = NO_2)[b]	2800	0.039	36[c]
	2d (R = H, X = NO_2)	1300	0.040	550
	2e (R = H, X = H)	2400	0.035	2200
SZ-*cis*-42F7	**2c** (R = Me, X = NO_2)	17000	0.067	62
	2d (R = H, X = NO_2)	600	0.020	270
	2e (R = H, X = H)	39	0.0021	130

[a] Assays were performed in aqueous buffer (60 mM Tris-HCl, 100 mM NaCl, pH 8.0) at 25 °C. To account for substrate depletion during assays with high antibody concentrations (1−10 μM), the data were fit to the equation $\nu = \{(k_{cat}/2)[(E + S + K_m) - [(E + S + K_m)^2 - 4ES]^{1/2}]\}$,[20] where ν is the initial rate, k_{cat} and K_m are the steady-state kinetic parameters, and E and S are the total concentrations of antibody binding site and substrate respectively. [b] Severe substrate inhibition was observed with this substrate, and the kinetic parameters were estimated from data obtained at low substrate concentration (≤30 μM). [c] As described in the text, significant yields of *cis*-anethole are obtained in the presence of antibody SZ-*cis*-39C11 but not in the uncatalyzed elimination, thus the value of k_{cat}/k_{un} substantially underestimates catalytic efficiency in this case.

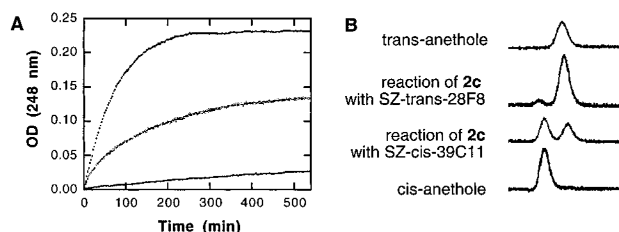

Figure 1. (A) Total time course of the antibody-catalyzed and uncatalyzed selenoxide elimination of **2c**. The reactions were performed as described[13] with 24 μM substrate at 15 °C without antibody (bottom trace), with 14 μM SZ-*cis*-39C11 (middle trace), and 2.4 μM SZ-*trans*-28F8 (top trace). They were monitored at 248 nm ($\Delta\epsilon$ = 10 000 M^{-1} cm^{-1} for both *cis*- and *trans*-anethole). (B) HPLC traces[13] of authentic *trans*-anethole (11.8 μM, 21.4 min), an aliquot of the SZ-*trans*-28F8 reaction at t = 260 min (1.6 μM cis **3**, 20.2 min; 21.3 μM trans **3**, 21.6 min), an aliquot of the SZ-*cis*-39C11 reaction at t = 376 min (5.6 μM cis **3**, 20.0 min; 7.8 μM trans **3**, 21.4 min), and authentic *cis*-anethole (11.8 μM; 19.8 min). Acetophenone (5.0 min) was used as an internal standard.

bound transition states leading to the *cis*- and *trans*-olefin products is notable and might be explained by a bifurcated binding pocket analogous to that of the crossreactive antibody DB3[16] which exhibits high affinity recognition of conformationally distinct steroids. The production of significant amounts of the *cis*-olefin must also account for the anomalously low efficiency of SZ-*cis*-39C11 with **2c** (Table 1), since the more facile pathway leading to trans product dominates the uncatalyzed reference reaction.

To probe the origins of catalysis, the reaction of primary selenoxide **2e** with SZ-*cis*-39C11 was examined in greater detail. Comparison of the activation parameters for the reaction in the antibody pocket ($\Delta H^{\ddagger}$ = 19.7 ± 1.2 kcal/mol and $\Delta S^{\ddagger}$ = −7.8 ± 4.1 eu) and in aqueous buffer ($\Delta H^{\ddagger}$ = 26.3 ± 0.15 kcal/mol and $\Delta S^{\ddagger}$ = +0.014 ± 0.47 eu) shows that transition state stabilization is achieved through enthalpic rather than entropic means. The more favorable activation entropy for the uncatalyzed reaction may reflect the release of ordered solvent molecules as the transition state is approached. The selenoxide substrate is a good hydrogen bond acceptor and will be solvated to a much greater extent in aqueous buffer than the comparatively less-polar transition state. Differences in solvation of the antibody-bound ground and transition states are likely to be much less pronounced. Because selenoxide syn eliminations are not subject to acid and base catalysis,[6] partitioning the substrate into the less-polar medium of the antibody binding site alone could account for much of the observed rate enhancement,[17,18] while the conformational constraints of the active site would dictate the observed selectivity. Indeed, the elimination of **2e** is dramatically accelerated by aprotic solvents: log(k_{obsd}) correlates with the solvatochromic polarity measure $E_T(30)$ over a reactivity range of 10^4 (r = 0.973) (Figure 2). The rate achieved by SZ-*cis*-39C11 (k_{cat} = 0.036 min^{-1}) is similar to that found with the aprotic solvent 1,2-dichloroethane ($E_T(30)$ = 41.3; k_{obsd} = 0.0440 min^{-1}). Notably, reaction in the latter solvent is also characterized by an unfavorable activation entropy relative to aqueous buffer ($\Delta H^{\ddagger}$ = 20.3 ± 0.5 kcal/mol; $\Delta S^{\ddagger}$ = −4.8 ± 1.7 eu).

(16) Arevalo, J. H.; Taussig, M. J.; Wilson, I. A. *Nature* **1993**, *365*, 859−863.

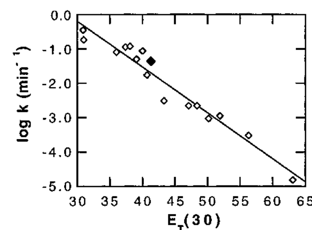

Figure 2. Rate constants for the reaction of compound **2e** (25 °C) plotted against the solvent solvatochromic polarity parameter $E_T(30)$ in cyclohexane, *n*-hexane, 1,4-dioxane, tetrahydrofuran, ethyl acetate, chloroform, methyl acetate, dichloromethane, 1,2-dichloroethane (filled diamond), *tert*-butyl alcohol, 2-butanol, isopropyl alcohol, *n*-butanol, ethanol, ethylene glycol, and aqueous buffer (Tris-HCl 60 mM, NaCl 100 mM, pH 8.0) in the order of increasing $E_T(30)$ value.

In summary, these experiments expand the scope of antibody catalysis to a new class of pericyclic reactions for which natural enzymes are unknown.[19] More importantly, they illustrate how conformational constraints imposed through hapten structure can be exploited together with medium effects to control chemical selectivity and reactivity. Improvements in hapten design, coupled with more extensive screening of the immune response, may yield synthetically useful catalysts for a wide range of solvent-sensitive processes.

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(17) Lewis, C.; Kramer, T.; Robinson, S.; Hilvert, D. *Science* **1991**, *253*, 1019−1022.

(18) By analogy, other proteins with generic hydrophobic binding pockets should also accelerate these transformations. In fact, BSA, which promotes other medium-sensitive reactions (Kikuchi, K.; Thorn, S. N.; Hilvert, D. *J. Am. Chem. Soc.* **1996**, *118*, 8184−8185; Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *Nature* **1996**, *383*, 60−63), catalyzes the selenoxide elimination of **1e** (k_{cat} = 0.0022 min^{-1}, K_m = 170 μM), but depending on the substrate concentration, its efficiency is 20 to 200-fold lower than that of SZ-*cis*-39C11. In addition, BSA does not catalyze the formation of *cis*-olefins from secondary selenoxides.

(19) Antibodies that catalyze a related [2,3]-sigmatropic elimination reaction of an *N*-oxide were recently reported (Yoon, S. S.; Oei, Y.; Sweet, E.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 11686−11687). As in our study, medium effects were identified as a major contributor to catalytic efficiency.

(20) Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*; John Wiley & Sons: New York, 1975; pp 72−77.