Catalysis of concerted reactions by antibodies: The Claisen rearrangement

(transition state analog/monoclonal antibody/chorismate mutase model)

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Communicated by Emil Thomas Kaiser, March 28, 1988 (received for review March 15, 1988)

ABSTRACT Monoclonal antibodies were prepared against a transition state analog inhibitor of chorismate mutase (EC 5.4.99.5). One of the antibodies catalyzes the rearrangement of chorismate to prephenate with rate accelerations of more than 2 orders of magnitude compared to the uncatalyzed reaction. Saturation kinetics were observed, and at 25°C the values of k_{cat} and K_m were $1.2 \times 10^{-3} \text{ s}^{-1}$ and $5.1 \times 10^{-5} \text{ M}$ respectively. The transition state analog was shown to be a competitive inhibitor of the reaction with K_i equal to $0.6 \mu\text{M}$. These results demonstrate the feasibility of using rationally designed immunogens to generate antibodies that catalyze concerted reactions.

Recent experiments have utilized the immune system as a prolific source of specific receptor molecules for the construction of enzyme-like catalysts (1–3). Using the transition state analog approach enunciated by Jencks in 1969 (4), two groups of researchers elicited antibodies against aryl phosphonate esters that catalyze the cleavage of structurally homologous aryl esters (1, 3) and carbonates (2). In principle, catalysis results when binding interactions stabilize the transition state structure of a particular reaction relative to the bound ground state. Given the enormous specificity inherent in antibody binding interactions, and the fact that antibodies can be generated against virtually any substance, it may be possible to prepare tailored catalysts for many chemical transformations with this strategy.

Since there is presumably a low probability of generating an effective constellation of multiple catalytic groups (e.g., general acids, general bases, nucleophiles) in the binding site of an antibody during the immunization event, reactions that do not require chemical catalysis are perhaps more suitable for development. Hence, we have begun to prepare antibodies that catalyze concerted reactions, including Claisen rearrangements and Diels–Alder cyclizations. These processes are expected to be sensitive to the principal catalytic effects antibodies are likely to impart: induced strain and proximity (5). Moreover, they are important transformations available to organic chemists for the synthesis of biologically active molecules (6).

The conversion of chorismate (Scheme I, structure 1) into prephenate (structure 2) is an example of a biologically relevant Claisen rearrangement. It is the key transformation in the biosynthesis of aromatic amino acids in bacteria, fungi, and higher plants (7). The enzyme chorismate mutase (EC 5.4.99.5) accelerates this reaction 2×10^6 -fold over the uncatalyzed thermal process (8). Although the precise mechanism of rearrangement is still contested (9–11), elegant stereochemical studies have implicated a transition state with diaxial chairlike geometry (structure 3) (12).

The oxabicyclic compound **4a**, which mimics the putative transition state structure, is the best known inhibitor of chorismate mutase (13). It binds approximately 100 times more tightly to the enzyme than does chorismate (13). We have synthesized a derivative of **4a** and used it as a hapten to elicit monoclonal antibodies. Having completed a preliminary screen of 15 of the 46 resulting antibodies, we report that one of these significantly accelerates the rearrangement of chorismate to prephenate.

MATERIALS AND METHODS

Instrumentation. UV–visible spectra were measured on a Perkin–Elmer Lambda-4B spectrophotometer equipped with a thermostatically regulated cell holder. High-performance liquid chromatography (HPLC) was performed with a Hitachi model 655A-11 liquid chromatography system with a variable wavelength detector and an analytical Vydac C₁₈ 218-TP-54 reverse-phase column (The Separations Group, Hesperia, CA). NMR spectra were obtained on a Bruker NMR spectrometer (300 MHz).

Materials. (–)-Chorismic acid (80–90%) was purchased from Sigma as the free acid and used without further purification. The concentrations of stock solutions of chorismate were determined by UV spectroscopy (ϵ_{275} , $2630 \text{ M}^{-1} \text{ cm}^{-1}$) (11). The oxabicyclic transition state analog **4a** was synthesized according to published procedures (13), together with its exo epimer (1:5 mixture, endo/exo). The desired endo isomer was purified as the bis(trimethylsilyl)ethyl ester by repeated flash chromatography and deprotected, as described (13). A glutaric acid linker was used to couple the hapten to the carrier proteins keyhole limpet hemocyanin (KLH, Pacific Bio Laboratories, Venice, CA) and bovine serum albumin (BSA, Sigma). The coupling agent **4b** was prepared in high yield from the bis(trimethylsilyl)ethyl ester of **4a** by a two-step procedure involving acylation of the secondary hydroxyl group with the mono acid chloride, mono *N*-hydroxysuccinimide ester of glutaric acid in the presence of triethylamine, followed by deprotection with tetrabutylammonium fluoride or trifluoroacetic acid immediately before use. All compounds gave satisfactory NMR spectra. Treatment of the carrier proteins with a 100-fold excess of **4b** in 10 mM borate buffer, pH 9/0.2 M NaCl for 1–2 hr at room temperature, followed by gel filtration on Sephadex G-50, gave the desired protein conjugates. Approximately 20–30 molecules of **4** could be attached to KLH and BSA by using this protocol, as estimated by amine titration (14).

Preparation and Purification of Monoclonal Antibodies. Mice (129 GIX⁺ strain) were immunized with the KLH conjugate **4c** emulsified in complete Freund's adjuvant (15). Serum titer was determined with the BSA conjugate by an enzyme-linked immunosorbent assay (ELISA) (16). A mouse

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Abbreviations: BSA, bovine serum albumin; KLH, keyhole limpet hemocyanin.

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Convincing evidence that chorismate reacts in the induced binding pocket of the antibody was obtained by examining the kinetics in the presence of the transition state analog. As shown in Fig. 1, the conversion of chorismate to prephenate is competitively inhibited by **4a**, with a K_i value of $\approx 0.6 \times 10^{-6}$ M. The ratio of K_i to K_m is similar to that seen in the case of chorismate mutase itself (13). Moreover, it correlates

Table 1. Kinetic parameters for the rearrangement of chorismate to prephenate catalyzed by monoclonal antibody 1F7

Temp., °C	$k_{\text{cat}} \times 10^3$, s ⁻¹	$K_m \times 10^5$, M	k_{cat}/K_m , M ⁻¹ ·s ⁻¹	$k_{\text{cat}}/k_{\text{un}}$
14.0	0.39 ± 0.04	4.9 ± 0.8	8.0 ± 1.4	250
25.0	1.2 ± 0.2	5.1 ± 0.9	24 ± 5	190
36.0	2.5 ± 0.2	3.8 ± 0.4	66 ± 5	110

Parameters were determined by fitting initial-rate data, obtained at 275 nm in 10 mM Tris·HCl, pH 8.0/100 mM NaCl, to the Michaelis-Menten equation. The values of k_{un} , the rate constant for the uncatalyzed thermal rearrangement, were extrapolated from the data of Andrews *et al.* (8).

roughly (within a factor of 2) with the observed rate acceleration, consistent with the notion that the enhancement is due to increased binding of the transition state by the antibody. It is interesting that the dissociation constant measured for **4a**, which contains an unacylated hydroxyl group, is 1–2 orders of magnitude larger than that for the hapten linked to BSA [estimated by ELISA titration (21)]. Since binding interactions distant from the reaction center can contribute significantly to the reaction rate (22), an acylated chorismate derivative may be an even better substrate for the antibody.

To gain some insight into the mechanism of catalysis by 1F7, the chorismate rearrangement was examined at two additional temperatures. The relevant kinetic parameters are summarized in Table 1. The entropy and enthalpy of activation ($\Delta S^\ddagger$ and $\Delta H^\ddagger$) for conversion of bound substrate to product were calculated from the temperature dependence of k_{cat} to be -22 ± 6 cal·mol⁻¹·K⁻¹ and 15 ± 2 kcal/mol (1 kcal = 4184 J) respectively. For comparison, $\Delta S^\ddagger$ is -12.85 ± 0.42 cal·mol⁻¹·K⁻¹ and $\Delta H^\ddagger$ is 20.71 ± 0.35 kcal/mol for the thermal conversion of chorismate to prephenate (8). It is apparent that the antibody stabilizes the transition state for rearrangement by significantly lowering the enthalpy of activation. This might be accomplished through the use of binding energy to induce strain into the starting material (5). The less favorable $\Delta S^\ddagger$ value for the catalyzed reaction, on the other hand, may reflect the need for a conformational change in the antibody combining site during reaction.

FIG. 1. Lineweaver-Burk plot for Claisen rearrangement of chorismate by monoclonal antibody 1F7. Velocities were determined as described in Table 1. x, No inhibitor present; ♦, inhibited by 6.4 μM **4a**; □, inhibited by 15 μM **4a**.

The Claisen rearrangement of chorismate is a prototype of a broad class of concerted chemical reactions of enormous practical and theoretical interest. Using a rationally designed immunogen, we have successfully elicited an antibody that significantly accelerates the rate of this process. The design principles outlined above can now be extended to the development of other antibodies that are shape-selective rather than chemoselective. The ability to generate tailored catalysts for these important transformations has far-reaching implications for medicine and industry.

Note Added in Proof. Recent experiments with (±)-chorismate have established that the antibody-catalyzed rearrangement not only is accelerated over background but also occurs in a highly stereoselective fashion. (+)-Chorismate, unlike the (−) isomer, does not appear to be a substrate for 1F7, demonstrating further the enzyme-like nature of this tailored antibody binding site.

We are grateful to Diane Schloeder for expert technical advice and to Drs. Darryl Rideout and Alan Schwabacher for a critical reading of the manuscript. This work was supported in part by a Junior Faculty Research Award to D.H. from the American Cancer Society and by Grant GM38273 from the National Institutes of Health to D.H.

- Tramontano, A., Janda, K. D. & Lerner, R. A. (1986) *Science* **234**, 1566–1570.
- Jacobs, J. W., Schultz, P. G., Sugawara, R. & Powell, M. (1987) *J. Am. Chem. Soc.* **109**, 2174–2176.
- Napper, A. D., Benkovic, S. J., Tramontano, A. & Lerner, R. A. (1987) *Science* **237**, 1041–1043.
- Jencks, W. P. (1969) *Catalysis in Chemistry and Enzymology* (McGraw Hill, New York), p. 288.
- Jencks, W. P. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol.* **43**, 219–410.
- March, J. (1985) *Advanced Organic Chemistry* (Wiley, New York), pp. 745–758, 1028–1032.
- Weiss, U. & Edwards, J. M. (1980) *The Biosynthesis of Aromatic Amino Compounds* (Wiley, New York), pp. 134–184.
- Andrews, P. R., Smith, G. D. & Young, I. G. (1973) *Biochemistry* **18**, 3492–3498.
- Copley, S. D. & Knowles, J. R. (1987) *J. Am. Chem. Soc.* **109**, 5008–5013.
- Gajewski, J. J., Jurayj, J., Kimbrough, D. R., Gande, M. E., Ganem, B. & Carpenter, B. K. (1987) *J. Am. Chem. Soc.* **109**, 1170–1186.
- Addadi, L., Jaffe, E. K. & Knowles, J. R. (1984) *Biochemistry* **23**, 4494–4501.
- Sogo, S. G., Widlanski, T. S., Hoare, J. H., Grimshaw, C. E., Berchthold, G. A. & Knowles, J. R. (1984) *J. Am. Chem. Soc.* **106**, 2701–2703.
- Bartlett, P. A. & Johnson, C. R. (1985) *J. Am. Chem. Soc.* **107**, 7792–7793.
- Habeeb, A. F. S. A. (1966) *Anal. Biochem.* **14**, 328–336.
- Niman, H. L. & Elder, J. H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4524–4528.
- Goding, J. W. (1983) *Monoclonal Antibodies: Principles and Practice* (Academic, New York), pp. 234–255.
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. & Klenk, D. C. (1985) *Anal. Biochem.* **150**, 76–85.
- Kabat, E. A. (1976) *Structural Concepts in Immunology and Immunochemistry* (Holt, Reinhart and Winston, New York), p. 227.
- Yamaoka, K., Tanigawara, Y., Nakagawa, T. & Uno, T. (1981) *J. Pharmacobio. Dyn.* **4**, 879–885.
- Görisch, H. & Lingens, F. (1974) *Biochemistry* **13**, 3790–3794.
- Butler, J. E. (1980) In *Enzyme-Immunoassay*, ed. Maggio, E. T. (CRC, Boca Raton, FL), pp. 41–42.
- Moore, S. A. & Jencks, W. P. (1982) *J. Biol. Chem.* **257**, 10893–10907.