Pergamon

Tetrahedron Letters, Vol. 37, No. 48, pp. 8675-8678, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)02007-2

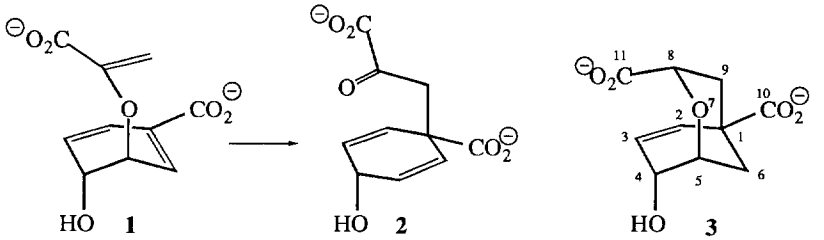
ON THE MECHANISM OF CHORISMATE MUTASES: CLUES FROM WILD-TYPE E. COLI ENZYME AND A SITE-DIRECTED MUTANT RELATED TO YEAST CHORISMATE MUTASE

Christophe C. Galopin,[a] Sheng Zhang,[b] David B. Wilson,[b] and Bruce Ganem[a]*

[a]*Department of Chemistry, Baker Laboratory*
[b]*Section of Biochemistry, Molecular and Cellular Biology*
Cornell University, Ithaca, NY 14853 USA

Abstract: Thermodynamic parameters for *E. coli* chorismate mutase are reported. In addition, site-directed mutagenesis studies provide a direct mechanistic link to yeast chorismate mutase, further indicating the importance of entropic restriction and hydrogen bonding in the enzymic catalysis of Claisen rearrangements.

The rearrangement of chorismic acid **1** to prephenic acid **2** catalyzed by chorismate mutase in the shikimic acid biosynthetic pathway represents a crucial step in the production of phenylalanine, tyrosine, and other aromatic compounds.[1] Chorismate mutases achieve millionfold rate enhancements and handily outperform most abiological catalysts, which are typically Bronsted[2] or Lewis[3] acids that achieve modest rate enhancements.[4] The best catalytic antibody engineered for mutase activity accelerates the rearrangement by 10^4.



While primary structures are known for several chorismate mutases from different organisms,[5] relatively little sequence similarity has been detected. Moreover, X-ray crystallographic studies of the N-terminal domain of the bifunctional *Escherichia coli* protein chorismate mutase/prephenate dehydratase (EcCM),[6] of *Saccharomyces cerevisiae* (ScCM),[7] and of *Bacillus subtilis* (BsCM)[8] as complexes with transition state analog **3** reveal two structurally distinct classes of enzymes.

Structures notwithstanding, none of the mutases has revealed significant mechanistic clues about catalysis. Instead, inferences about mechanism have been drawn from thermodynamic parameters measured for the *Klebsiella pneumoniae* and *Streptomyces aureofaciens* enzymes (Table, entries 4 and 5).[9] According to one longstanding hypothesis, the enzymes achieve near-zero entropy of activation (widely assumed to be characteristic of all mutases) by locking **1** in the chair conformation for rearrangement. However, the negative $\Delta S^{\ddagger}$ recently determined for BsCM (Table, entry 1) indicates that some natural chorismate mutases may be able to achieve catalysis without functioning as entropy traps.[10]

Here we report thermodynamic parameters for EcCM, one of the most widely studied enzymes in mutase research. We also present kinetic and site-directed mutagenesis studies on EcCM that provide the first direct link between the catalytic features and kinetic properties of EcCM and ScCM. Our results lend further credence to a mechanistic hypothesis in which significant conformational ordering in the E-S complex is achieved by means of hydrogen bonding and electrostatic interactions with chorismate's carboxylate groups.[11]

The 113-residue EcCM displayed a specific activity of 124 ±19 units/mg, K_M = 300 μM, and k_{cat} = 72 sec^{-1} for chorismate, as reported.[6] Activation parameters were obtained from the temperature dependence of k_{cat} between 10 °C and 37 °C. Kinetic assays were conducted in buffer containing 50 mM tris HCl (pH 7.8) by monitoring the appearance of prephenate at concentrations of chorismate ranging from 50 to 1000 μM. Each assay was acidified to convert prephenate to phenylpyruvate, then made basic with 2.5 N NaOH and monitored spectrophotometrically at 320 nm ($\epsilon_{320\ nm}$ = 17,500 $M^{-1}\ cm^{-1}$).[12] Control assays lacking enzyme were used to correct for the nonenzymic conversion of **1** to **2**. After least-squares analysis of the initial velocities, values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were calculated using the Eyring equation.

Activation parameters determined for EcCM (Table, entry 2) are in good agreement with values for the enzymes from *K. pneumoniae* and *S. aureofaciens*. In particular, the value of $\Delta S^{\ddagger}$ (-3.0 ± 1.6 eu) indicates that conformational control of enzyme-bound chorismate plays a substantial role in catalysis. This finding complements recent site-directed mutagenesis studies on EcCM, which suggest that Lys39, Arg11', and Arg28 rotationally constrain chorismate via electrostatics and hydrogen bonding (Figure).[13,14] It seems clear that BsCM notwithstanding,[10] entropic restriction figures prominently in most enzyme-catalyzed rearrangements of **1**.

Like other mutases, both EcCM and BsCM reduce $\Delta H^{\ddagger}$ by ca. 5 kcal/mol, apparently by stabilizing C5-O7 bond-breaking in the rearrangement transition state. In EcCM, the enthalpic reduction is thought to arise from hydrogen bonding between the carboxamide of Gln88 and O7 of **1** (Figure),[11] whereas in BsCM, a nearby carboxylate anion at Glu78 is proposed to stabilize positive charge developing on the cyclohexadienyl ring as O7 dissociates.[15] To assess the relative catalytic efficiencies of glutamine and glutamate against a common active site framework, we now report additional kinetic studies on a Gln88Glu mutant of EcCM recently produced in our lab[13] and by Cload *et al.*[14] using site-specific mutagenesis.

At the pH optimum of EcCM (7.8), the Gln88Glu mutant displays only 1% of wild-type enzyme activity.[13,14] Interestingly, the Gln88Glu mutant closely resembles ScCM, which also has a glutamate (Glu246) at the position corresponding to Gln88 in EcCM. Unlike EcCM, however, ScCM shows a pH optimum at 5.5.[16] Moreover, ScCM displays virtually no activity at pH 7.8, suggesting that active catalysis requires Glu246 in its protonated (carboxylic acid) form. In agreement with this hypothesis, the activity of the Gln88Glu mutant EcCM was highly pH-dependent, rising dramatically at its optimal pH (4.5, acetate buffer) to 140% of wild-type EcCM activity. The added rate enhancement accrues from enhanced turnover (k_{cat} = 9700 min^{-1} for the mutant; k_{cat} = 3700 min^{-1} for wild-type, both at pH 4.5).

Further insight into the nature of catalysis by the Gln88Glu mutant comes from its activation parameters, which were determined at pH 4.5 (Table, entry 3).[17] Both $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are, within experimental error, the same as for wild-type EcCM. We conclude that catalysis by the Gln88Glu mutant requires an undissociated carboxylic acid to function as a hydrogen bond donor in much the same fashion as Glu246 in ScCM and Gln88 in wild-type

TABLE
Thermodynamic Parameters for the Catalyzed and Uncatalyzed Reaction of Chorismate to Prephenate*

Catalyst	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (e.u.)
BsCM	12.7 ± 0.4	-9.1 ± 1.2
EcCM wild type, pH 7.8	16.3 ± 0.5	-3.0 ± 1.6
EcCM (Q88E), pH 4.5	16.6 ± 0.4	0.2 ± 1.7
K. pneumoniae	15.9 ± 0.4	-1.1 ± 1.2
S. aureofaciens	14.5 ± 0.4	-1.6 ± 1.1
Catalytic Antibody IF7	15 ± 2	-22 ± 6
Catalytic Antibody 11F1-2E11	18.3	-1.2
uncatalyzed	20.5 ± 0.4	-12.9 ± 0.4

*Entries 1 and 4-8 as cited in ref. 10

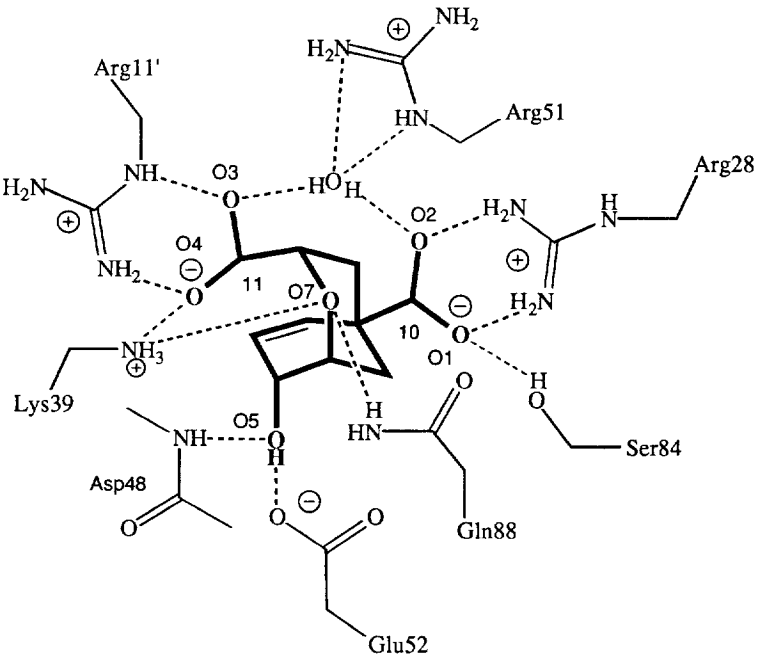


FIGURE
Hydrogen Bonding and Electrostatic Interactions of Inhibitor 3 with Relevant Side Chains of EcCM

EcCM. While electrostatic effects by charged residues may affect other aspects of mutase catalysis, our data lend additional credence to the importance of H-bonding in reducing the activation enthalpy of Claisen rearrangements,[18] as suggested by experimentally observed solvent effects on nonenzymic rearrangements,[19,20] Monte Carlo simulations in aqueous solvents,[21] *ab initio* studies on enzyme and antibody catalysis,[22] as well as by recent studies on synthetic, hydrogen-bonding ureas that catalyze the rearrangement of allyl vinyl ethers.[23]

ACKNOWLEDGMENT: Financial support by grants from the National Institutes of Health (NIH GM 24054) is gratefully acknowledged.

REFERENCES

1. Haslam, E. *Shikimic Acid Metabolism and Metabolites;* John Wiley & Sons: New York, **1993**.
2. (a) Claisen, L. *Chem. Ber.* **1912,** *45,* 3157; (b) Murray, R. D. H.; Sutcliffe, M.; Hasegawa, M. *Tetrahedron* **1975,** *31,* 2966.
3. Nonoshita, K.; Banno, H.; Maruoka, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1990,** *112,* 316.
4. Maruoka, K.; Saito, S.; Yamamoto, H. *J. Am. Chem. Soc.* **1995,** *117,* 1165.
5. Romero, R. M.; Roberts, M. F.; Phillipson, J. D. *Phytochemistry* **1995,** *40,* 1015.
6. Lee, A. Y.; Karplus, P. A.; Ganem, B.; Clardy, J. *J. Am. Chem. Soc.* **1995,** *117,* 3627.
7. Xue, Y.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1995,** *92,* 10595.
8. Haynes, M. R.; Stura, E. A.; Hilvert, D.; Wilson, I. A. *Science* **1994,** *263,* 646.
9. Gorisch, H. *Biochemistry* **1978,** *17,* 3700.
10. Kast, P.; Asif-Ullah, M.; Hilvert, D. *Tetrahedron Lett.* **1996,** *37,* 2691.
11. Lee, A. Y.; Clardy, J.; Stewart, J. D.; Ganem, B. *Chemistry and Biology* **1995,** *2,* 195.
12. Nishioka, L.; Woodin, T. *Anal. Biochem.* **1972,** *45,* 617.
13. Zhang, S.; Kongsaeree, P.; Clardy, J.; Wilson, D. B.; Ganem, B. *Bioorg. Med. Chem.* **1996,** *4,* 1015.
14. Cload, S. T.; Liu, D. R.; Pastor, R. M.; Schultz, P. G. *J. Am. Chem. Soc.* **1996,** *118,* 1787.
15. Kast, P.; Hartgerink, J. D.; Asif-Ullah, M.; Hilvert, D. *J. Am. Chem. Soc.* **1996,** *118,* 3069.
16. Schmidheini, T.; Mösch, H.-U.; Evans, J. N. S.; Braus, G. *Biochemistry* **1990,** *29,* 3660.
17. Activation parameters were not obtained at pH 7.8, since the mutant enzyme could not be saturated with substrate (K_m >10,000 μM).
18. Ganem, B. *Angew. Chem. Int. Ed. Engl.* **1996,** *35,* 936.
19. Coates, R. M.; Rogers, B. D.; Hobbs, S. J.; Peck, D. R.; Curran, D. P. *J. Am. Chem. Soc.* **1987,** *109,* 1160.
20. Gajewski, J. J.; Jurayj, J.; Kimbrough, D. R.; Gande, M. E.; Ganem, B.; Carpenter, B. K. *J. Am. Chem. Soc.* **1987,** *109,* 1170.
21. Severance, D. L.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1992,** *114,* 10966.
22. Wiest, O.; Houk, K. N. *J. Am. Chem. Soc.* **1995,** *117,* 11628.
23. Curran, D. P.; Kuo, L. H. *Tetrahedron Lett.* **1995,** *36,* 6647.

(*Received in USA* 23 *September* 1996; *accepted* 7 *October* 1996)