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Is Chorismate Mutase a Prototypic Entropy Trap? – Activation Parameters for the *Bacillus subtilis* Enzyme

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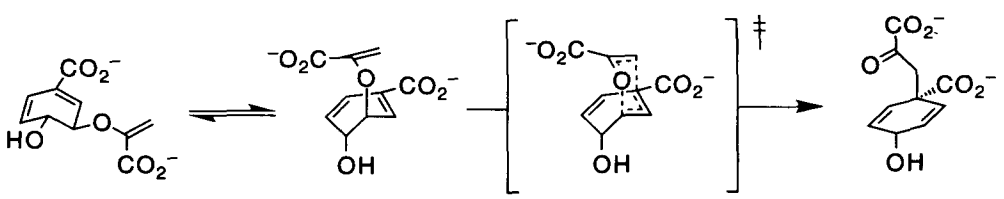
Abstract: *Chorismate mutase is thought to accelerate the chorismate-to-prephenate rearrangement in part by significantly lowering the entropy barrier for the reaction. We have determined the activation parameters for the well-characterized* Bacillus subtilis *chorismate mutase and find that* $\Delta S^{\ddagger}$ *(–9.1 ± 1.2 eu) is nearly as unfavorable as the activation entropy for the uncatalyzed process. Our results suggest that chorismate mutase catalysts show greater mechanistic versatility than commonly believed.*

The conversion of (–)-chorismate (**1**) to prephenate (**3**) by chorismate mutase constitutes a key step in the biosynthesis of the aromatic amino acids phenylalanine and tyrosine in bacteria, fungi and higher plants.[1] The reaction is formally a Claisen rearrangement and a rare example of an enzyme-catalyzed pericyclic process. The enzymatic reaction,[2,3] like the uncatalyzed thermal rearrangement,[4] proceeds through the chair-like transition state **2**, but the origins of the 10^6 to 10^7-fold rate acceleration remain poorly understood.[5] Comparison of the activation parameters for the uncatalyzed reaction and for chorismate mutases from *Klebsiella pneumoniae* and *Streptomyces aureofaciens* (Table 1) suggests that lowering the entropy barrier of the reaction by restricting the conformational degrees of freedom of the flexible chorismate molecule is a major factor in catalysis.[6] This conclusion has been generally assumed to apply to all chorismate mutases,[5] although the two enzymes on which it is based are relatively poorly characterized. Here we report activation parameters for a kinetically and structurally well-studied enzyme from *Bacillus subtilis* and show that $\Delta S^{\ddagger}$ in this case is unfavorable and comparable to that for the uncatalyzed reaction. This surprising observation contravenes conventional wisdom and suggests that chorismate mutases may exploit diverse strategies to achieve their otherwise similar catalytic effects.



1 (eq) **1** (ax) **2** **3**

The monofunctional chorismate mutase from *B. subtilis* displays simple kinetic characteristics. Its gene (*aroH*) has been cloned and expressed at high protein levels in the bacterium *Escherichia coli*.[7] Furthermore, the three-dimensional structure of the protein trimer unliganded or complexed with a transition state analog or **3** has been determined.[8,9] Using a modification[10] of published protocols,[7,11] we have purified *B. subtilis* chorismate mutase from the *E. coli* host strain KA12 transformed with plasmid pBSCM2[7] carrying *aroH*. Since our specifically engineered strain KA12[12] lacks both endogenous chorismate mutase genes, any contamination of the protein preparation with *E. coli* chorismate mutase can be excluded *a priori*. The activation parameters for the *B. subtilis* enzyme were obtained by determining the temperature dependence of k_{cat} (Figure 1). The disappearance of chorismate[13] was followed spectrophotometrically at 274 nm ($\varepsilon_{274\ nm}$ = 2.63 $mM^{-1}\ cm^{-1}$) in a buffer containing 50 mM potassium phosphate, pH 7.5, at five chorismate concentrations ranging from 20 to 500 μM. All initial velocities were corrected for the corresponding temperature-specific background reaction. At each temperature, k_{cat} was determined as described[6] assuming Michaelis-Menten kinetics.[14] Thermodynamic activation parameters were obtained from least-squares analysis of data collected from 5 °C to 45 °C (Figure 1) using the equation $k_{cat} = (kT/h)e^{-[(\Delta H^{\ddagger}/RT)-(\Delta S^{\ddagger}/R)]}$.[15]

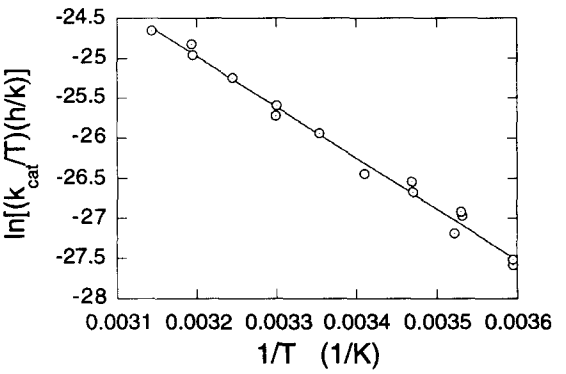


Figure 1. Temperature dependence of k_{cat}, the observed reaction constant for the rate-limiting step of *B. subtilis* chorismate mutase.

Table 1. Comparison of Thermodynamic Parameters for Catalyzed and Uncatalyzed Chorismate-to-Prephenate Rearrangements

Source of chorismate mutase activity	$\Delta G^{\ddagger a}$ [kcal mol^{-1}]	$\Delta H^{\ddagger}$ [kcal mol^{-1}]	$\Delta S^{\ddagger}$ [cal $mol^{-1}\ K^{-1}$ (eu)]
Uncatalyzed reaction[16]	24.5	20.7 ± 0.4	−12.9 ± 0.4
Bacillus subtilis[b]	15.4	12.7 ± 0.4	−9.1 ± 1.2
Klebsiella pneumoniae[c]	16.2	15.9 ± 0.4	−1.1 ± 1.2[d]
Streptomyces aureofaciens[6]	15.0	14.5 ± 0.4	−1.6 ± 1.1[d]
Catalytic antibody 11F1-2E11[17]	18.7	18.3	−1.2
Catalytic antibody 1F7[18]	21.6	15 ± 2	−22 ± 6

[a]Calculated for 25 °C. [b]This work. [c]Formerly known as *Aerobacter aerogenes*.[6] [d]Assuming two (*K. pneumoniae*) and three (*S. aureofaciens*) active sites per enzyme molecule.[6]

At 30 °C, we found a k_{cat} of 46 (± 3) s^{-1} per active site and a Michaelis constant K_m of 67 (± 5) μM, which are similar to values obtained under comparable conditions by Knowles and coworkers.[7] As shown in Table 1, the entropy of activation for the *B. subtilis* chorismate mutase ($\Delta S^{\ddagger}$ = –9.1 eu) is nearly as unfavorable as $\Delta S^{\ddagger}$ for the uncatalyzed reaction.[16] Consequently, the rate acceleration achieved by the enzyme results in large part from decreasing the activation enthalpy by 8.0 kcal/mol; the $-T\Delta\Delta S^{\ddagger}$ term contributes only –1.1 kcal/mol, or 12%, to the gain in free energy of activation at 25 °C. This finding contrasts with that obtained for the enzymes from *K. pneumoniae* and *S. aureofaciens* and for the catalytic antibody 11F1-2E11[17] where the entropic contribution to $\Delta\Delta G^{\ddagger}$ ranges from 35% to 60%. It has generally been assumed that the activation parameters in the latter three cases actually reflect the chemical transformation and are typical for the enzyme-catalyzed Claisen rearrangement.[5] In contrast, product dissociation may be rate-limiting at substrate saturation for the *B. subtilis* enzyme,[19] although contradictory evidence has been presented.[11,19] Meaningful interpretation of the unfavorable $\Delta S^{\ddagger}$ for this enzyme must therefore await further experimental data, for instance from kinetic isotope effect studies. Nevertheless, given the similarity in k_{cat} values of the natural enzymes,[6,7] the differences in the activation parameters are striking and imply different detailed catalytic mechanisms.

Despite an unfavorable $\Delta S^{\ddagger}$, *B. subtilis* chorismate mutase is likely to exert considerable conformational control over the flexible chorismate molecule. The active site is highly complementary to the reactive pseudodiaxial conformation of the substrate, with multiple hydrogen-bonding and electrostatic interactions poised to lock the enolpyruvyl side chain in place over the cyclohexadienyl ring.[8,9] Similarly, the catalytic antibody 1F7,[18] which has the most unfavorable $\Delta S^{\ddagger}$ of all the chorismate mutase catalysts examined (Table 1), appears to bind this high energy species preferentially.[20,21] Nevertheless, the energetic contribution of such conformational control in the sense of a classical entropy trap[22] is experimentally inaccessible if steps other than the chemical rearrangement are rate-limiting. Stabilization of the putative dipolar transition state through electrostatic and hydrogen-bonding interactions,[23-27] also inferable from the crystal structures[9,21,28,29] and experimentally examined for the *B. subtilis* enzyme,[30,31] could well provide the major catalytic effect.[5] In this respect, 1F7 is clearly less optimized than its natural counterparts.[21] While much emphasis has been put on the favorable $\Delta S^{\ddagger}$ of several chorismate mutases in the past,[5,6,17] our results with the *B. subtilis* enzyme caution against overgeneralization of these activation parameters and suggest that natural chorismate mutases may show greater mechanistic versatility than commonly believed.

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