Contribution of Transition-state Binding to the Catalytic Activity of *Bacillus subtilis* Chorismate Mutase

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The differential electrostatic interaction of chorismate, compared to the transition-state involved in its transformation to prephenate, with the active site of chorismate mutase, leads to a rate enhancement in line with experiment.

The enzyme chorismate mutase catalyses the rearrangement of chorismate **1** to prephenate **2**, a transformation at the branch point in the shikimate pathway which is responsible for the biosynthesis of aromatic amino acids in plants, fungi and bacteria.[1,2] The role of the enzyme in producing this rate acceleration, a factor of 2×10^6 compared to that in aqueous solution, is still unclear.[3–5] Indeed, it has been suggested that the chemical transformation **1** → **2** may not be the rate limiting step.[6]

Recent structural investigations of a transition-state analogue **3** bound to chorismate mutase[7,8] strongly suggest that the enzymatic rearrangement is a pericyclic process (Claisen reaction) similar to the uncatalysed reaction, which is generally agreed to proceed *via* a chair-like transition state.[9,10] In this communication we present the first quantitative modelling studies addressing the structure of the transition state **4** and the interactions that it and the reactant **1** have with the enzyme active site.

Full geometry optimisations of **1** and **4** were carried out at the Hartree–Fock *ab initio* level using a 6-31G* basis and the program GAUSSIAN92.[11] Overlapping the predicted transition state **4** with the experimental crystal structure of the analogue **3** gave an rms deviation of the heavy-atom positions of only 0.32 Å, showing the high degree of similarity between the two structures.

The intermolecular interactions between chorismate **1**, the transition state **4** and the active site of the enzyme *B. subtilis* chorismate mutase were then calculated using the program AMBER.[12] The formal atomic charges of **1** and **4** were obtained from the *ab initio* wavefunctions, following the method of Singh and Kollman.[13] Some conformational freedom of these species was permitted to maximise the overlap of the ring –OH group with its position found in the X-ray structure containing the inhibitor. Energy minimisation was then carried out to dock these two fixed structures with the enzyme site, starting with the crystallographically determined enzyme structure, but subsequently allowing for structural relaxation of those residues within 12 Å of the substrate. However, little change in the structure of the enzyme active site occurred, the rms deviations between the experimental enzyme structure and the optimised ones involving chorismate **1** and the transition state **4**, being only 0.63 and 0.55 Å respectively. All of the major interactions found experimentally[8] between the transition-state analogue and the mutase, were found in our predicted structures, shown in Fig. 1.

In Table 1 we show the intermolecular interactions contributing to the binding of **1** and **4** in the active site of the enzyme. These values were calculated for the full trimer structure of the enzyme. It is clear that the electrostatic contributions are dominant and on the basis of the calculations, a barrier lowering of ≈7 kcal mol^{-1} (1 cal = 4.184 J) is predicted, a value reasonably close to the reduction in the barrier that would arise from the observed rate enhancement (9 kcal mol^{-1})[3] considering the approximations in the calculation. This experimental value is relative to the aqueous phase and thus should be increased somewhat to directly compare with our calculated lowering which is respect to the gas phase. It is expected that relaxation of both the structure and charge distribution of the substrates in the active site will lead to a further barrier lowering. Studies to include these effects using a hybrid quantum mechanical/molecular mechanical approach[14] are underway. Our calculated structures (Fig. 1) suggest that a major source of the preferential electrostatic stabilisation of the transition state arises from enhanced hydrogen bonding between the ether oxygen and the Arg90 residue analogous to Lewis-acid catalysis of the Claisen rearrangement.

Thus, we have demonstrated the value of the transition-state analogue concept, identified the differing electrostatic interactions between the reactant and transition state in the active site and have shown that the latter gives a rate enhancement in line with that observed experimentally.

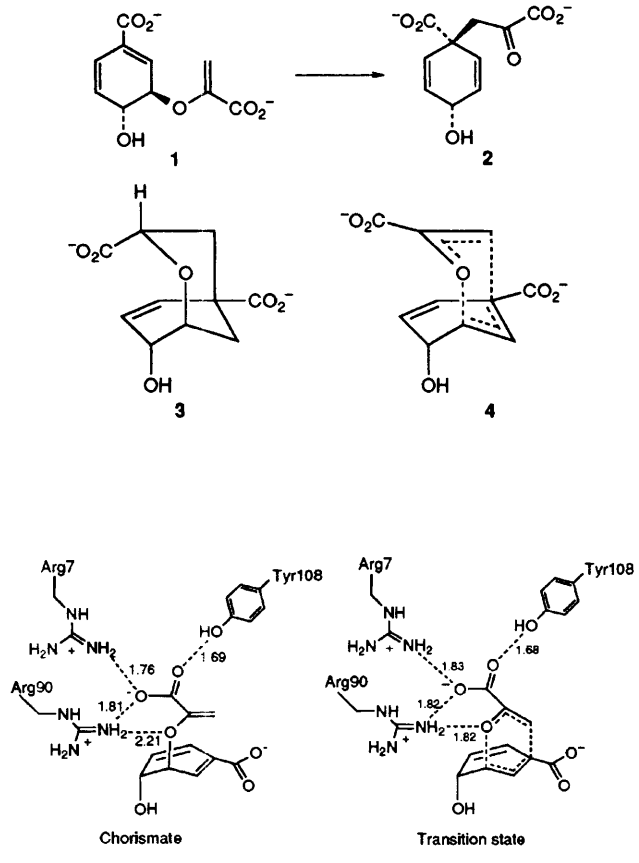

Fig. 1 Predicted interaction distances (Å) in the enzyme active site

Table 1 Substrate–enzyme interaction energies

	Interaction energy/kcal mol^{-1}	
	Electrostatic	van der Waals
Chorismate **1**	−14.6	−17.1
Transition state **4**	−20.6	−17.9

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References

1 R. Bentley, *CRC Crit. Rev. Biochem. Mol. Biol.*, 1990, **25**, 307.
2 C. Poulsen and R. Verpoorte, *Phytochemistry*, 1991, **30**, 377.
3 P. R. Andrews, G. D. Smith and I. G. Young, *Biochemistry*, 1973, **12**, 3492.
4 H. Görisch, *Biochemistry*, 1978, **17**, 3700.
5 J. V. Gray, B. Golinelli-Pimpaneau and J. R. Knowles, *Biochemistry*, 1990, **29**, 376.
6 J. V. Gray and J. R. Knowles, *Biochemistry*, 1994, **33**, 9953.
7 Y. M. Chook, H. Ke and W. N. Lipscomb, *Proc. Nat. Acad. Sci., USA*, 1993, **90**, 8600.
8 Y. M. Chook, J. V. Gray, H. Ke and W. N. Lipscomb, *J. Mol. Biol.*, 1994, **240**, 476.
9 S. D. Copley and J. R. Knowles, *J. Am. Chem. Soc.*, 1985, **107**, 5306.
10 S. G. Sogo, T. S. Widlanski, J. H. Hoare, C. E. Grimshaw, G. A. Berchtold and J. R. Knowles, *J. Am. Chem. Soc.*, 1984, **106**, 2701.
11 M. J. Frisch, G. W. Trucks, M. Head-Gordon, P. M. W. Gill, M. W. Wong, J. B. Foresman, B. G. Johnson, H. B. Schlegel, M. A. Robb, E. S. Replogle, R. Gomperts, J. L. Andrés, K. Raghavachari, J. S. Binkley, C. Gonzalez, R. L. Martin, D. J. Fox, D. J. Defrees, J. Baker, J. J. P. Stewart and J. A. Pople, GAUSSIAN92, Pittsburgh, Gaussian, Inc., 1992.
12 D. A. Pearlman, D. A. Case, J. C. Caldwell, G. L. Seibel, U. C. Singh, P. Weiner and P. A. Kollman, AMBER 4.0, University of California, San Francisco, 1991.
13 U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1984, **5**, 129.
14 B. Waszkowycz, I. H. Hillier, N. Gensmantel and D. W. Payling, *J. Chem. Soc., Perkin Trans. 2*, 1991, 225.