Prediction of transition state structure in protein tyrosine phosphatase catalysis using a hybrid QM/MM potential

Jason C. Hart,[a] Neil A. Burton,[a] Ian H Hillier,*[a] Martin J. Harrison[a] and Philip Jewsbury[b]

[a] *Department of Chemistry, University of Manchester, Manchester, UK M13 9PL*
[b] *Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK SK10 4TG*

The transition state for tyrosine phosphate hydrolysis by the enzyme protein tyrosine phosphatase (PTP1B) has been determined using a hybrid QM(PM3)/MM potential, showing that the reaction is dissociative with essentially no P–S bond formation in the transition state.

The reversible phosphorylation of proteins is the major form of post-translational modification and is a key control mechanism in biochemistry for processes such as cell regulation.[1,2] Protein tyrosine phosphatases (PTPases) are a large family of enzymes that catalyse the hydrolysis of phosphotyrosine (pTyr) residues in specific proteins.[3,4] Structural[5] and kinetic[6] studies have provided a molecular understanding of PTPase activity. In brief, the enzyme active site is conserved for all PTPases and contains catalytic Cys (215 in PTP1B) and Asp (181 in PTP1B) residues. The former residue exists as the anion and acts as the nucleophile, the latter having the role of a general acid. The reaction involves the formation of a phosphoenzyme intermediate (with Cys 215 in PTP1B). Hydrolysis of this intermediate results in the regeneration of the active site.[4]

Two extreme mechanisms of phosphate ester hydrolysis are usually invoked, either dissociative or associative[3,7] which depend on the size of the negative charge on the phosphate group. In aqueous solution, kinetic data have led to the conclusion that phosphate monoesters hydrolyse *via* a transition state with little bond formation to the attacking nucleophile and extensive bond cleavage to the leaving group characteristic of a dissociative mechanism.[8] Retention of chiral properties rules out a true diffusable metaphosphate intermediate. At physiological pH monoesters such as pTyr exist as dianions and kinetic data, particularly heavy atom isotope measurements, have led to the general conclusion that PTPase catalysis proceeds *via* a dissociative transition state[6] similar to that observed in solution.[8] The characterisation of the transition state at a molecular level is naturally central to understanding the mode of action of PTPases and to the design of PTPase specific inhibitors.

The use of combined quantum mechanical/molecular mechanical (QM/MM) methods to model macromolecular reactivity is now well established,[9–13] and we have shown how such methods can be used to locate transition states for enzyme catalysed reactions[14] employing our hybrid QM/MM code[15] which couples the QM code GAUSSIAN94[16] with the MM code AMBER 4.0.[17] To further aid the understanding of the mode of action of PTPases we here describe a hybrid QM/MM study of the mechanism of phosphate hydrolysis by the enzyme PTP1B. We utilise the crystal structure of PTP1B (Ser-215) and a phosphotyrosine substrate[5] to study phosphoryl transfer between pTyr and the Cys residue (replacing Ser-215), involving proton transfer from Asp-181 to the Tyr leaving group. In our model, the pTyr substrate in the crystal structure was replaced by 4–methylphenyl phosphate to allow for substrate mobility. This enzyme–substrate structure was first energy minimised (using the AMBER 4.0 force field) and was then employed in a QM(PM3)/MM calculation, the QM part of the structure being shown in Fig. 1. The bulk of the enzyme was kept fixed during the modelling of the reaction, with the positions of the majority of the QM atoms, including the whole substrate, being allowed to vary. The QM link atoms, as well as the QM atoms of the protein backbone, were kept fixed. We chose to use the PM3[18] Hamiltonian due to its success in modelling studies of phosphate hydrolysis.[7]

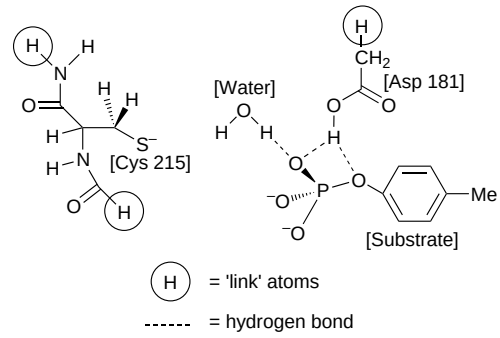

Fig. 1 QM region of active site–substrate

We have mapped out the potential energy surface for the dissociative reaction by minimising the substrate active site structure for a range of P–O (bridge) distances (while allowing the remaining QM atoms to optimise). This led to an approximate structure of the transition state, which was subsequently refined and characterised by calculation of the harmonic frequencies. The reactant and transition state structures thus obtained are shown in Fig. 2. It is clear that the transition state is largely dissociative, with a lengthened P–O bond and effectively no increase in S–P interaction compared to the reactant structure. The calculated partial atomic charges reveal a developing negative charge on the Tyr leaving group during the course of the reaction. The formal charge on this group increases from 0.80e in the reactant to 0.92e in the transition state, 0.07e of the total increase of 0.12e being associated with the bridge oxygen. In both reactant and transition state structures the proton of Asp-181 interacts with two oxygen atoms of the substrate (Fig. 2). The effect of this developing negative charge on the leaving group is to increase the interaction with the bridge oxygen compared to that

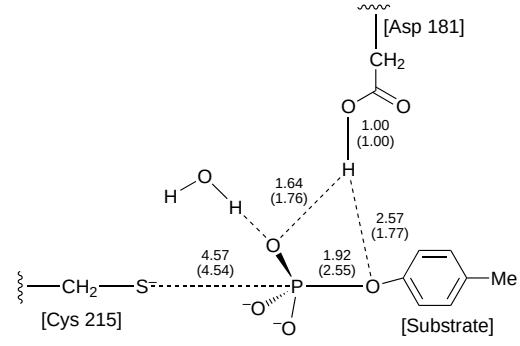

Fig. 2 Active site–substrate geometry. Reactant structure (Å) and, in parenthesis, transition state structure.

involving the terminal oxygen. However, we do not find proton transfer from the asparate group to have occurred at the transition state. The energy barrier between the reactant and transition state structures is 27.2 kJ mol^{-1}. Further along the reaction pathway to the product, there is a second barrier corresponding to proton transfer to the leaving OPh group along with S–P bond formation. However, this second barrier is at lower energy than the dissociative transition state that we have identified so that the P–O bond cleavage is rate determining in line with experiment.[6]

Thus, the first theoretical prediction of the nature of the transition state for a PTP–catalysed reaction, which we have presented here where the transition state corresponds to considerable P–O bond lengthening, with little P–S bond formation, is in general agreement with the conclusions from kinetic measurements. Of particular interest is the quite flat potential energy profile in the region of the transition state with a change of only 7 kJ mol^{-1} when the P–O length changes from 2.10 Å to the optimal value of 2.55 Å in the transition state (Fig. 2). This flat energy surface is also reflected in the low value of the imaginary frequency of the transition state (114*i* cm^{-1}). Such a flat surface suggests that there will be considerable geometric freedom in the design of suitable inhibitors of this enzymic reaction.

We thank the EPSRC for support of this research.

Footnote

* E-mail: ian.hillier@man.ac.uk

References

1 E. G. Krebs, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1122.
2 E. H. Fischer, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1130.
3 J. B. Vincent and M. W. Crowder, *Phosphatases in Cell Metabolism and Signal Transduction: Structure, Function and Mechanism*, Springer, New York, 1995.
4 D. Barford, Z. Jia and N. K. Tonks, *Nat. Struct. Biol.*, 1995, **2**, 1043.
5 Z. Jia, D. Barford, A. J. Flint and N. K. Tonks, *Science*, 1995, **268**, 1754.
6 A. C. Hengge, G. A. Sowa, L. Wu and Z. Y. Zhang, *Biochemistry.*, 1995, **34**, 13982.
7 J. Wilkie and D. Gani, *J. Chem. Soc., Perkin Trans. 2*, 1996, 783.
8 A. C. Hengge, W. A. Edens and H. Elsing, *J. Am. Chem. Soc.*, 1994, **116**, 5045.
9 A. Warshel and M. Levitt, *J. Mol. Biol.*, 1976, **103**, 227.
10 M. J. Field, P. A. Bash and M. Karplus, *J. Comput. Chem.*, 1990, **11**, 700.
11 J. Aqvist and A. Warshel, *Chem. Rev.*, 1993, **93**, 2523.
12 U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1986, **7**, 718.
13 B. Waszkowycz, I. H. Hillier, N. Gensmantel and D. W. Payling, *J. Chem. Soc., Perkin Trans. 2*, 1991, 225.
14 M. J. Harrison, N. A. Burton, I. H. Hillier and I. R. Gould, *Chem. Commun.*, 1996, 2769.
15 M. J. Harrison, PhD Thesis, The University of Manchester, 1997.
16 M. J. Frisch, G. W. Trucks, H. B. Schlegel, P. M. W. Gill, B. G. Johnson, M. A. Robb, J . R. Cheeseman, T. A. Keith, G. A. Petersson, J. A. Montgomery, K. Raghavachari, M. A. Al-Laham, V. G. Zakrzewski, J. V. Ortiz, J. B. Foresman, J. Cioslowski, B. B. Stefanov, A. Nanayakkara, M. Challacombe, C. Y. Peng, P. Y. Ayala, W. Chen, M. W. Wong, J. L. Andres, E. S. Replogle, R. Gomberts, R. L. Martin, D. J. Fox, J. S. Binkley, D. J. Defrees, J. Baker, J. P. Stewart, M. Head-Gordon, C. Gonzalez and J. A. Pople, GAUSSIAN94 , Gaussian Inc., Pittsburgh PA, 1995.
17 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, 1994.
18 J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 209

Received in Cambridge, UK, 4th April 1997; 7/02304I