What is the mechanism of phosphoryl transfer in protein kinases? A hybrid quantum mechanical/molecular mechanical study

Jason C. Hart, David W. Sheppard, Ian H. Hillier* and Neil A. Burton

Department of Chemistry, University of Manchester, Manchester, UK M13 9PL. E-mail: ian.hillier@man.ac.uk

Received (in Cambridge, UK) 17th November 1998, Accepted 20th November 1998

Hybrid QM/MM studies suggest that the conserved aspartate in protein kinases is not protonated during the phosphoryl transfer reaction, and thus does not act as a general base.

Protein kinase enzymes are critical to the regulation of cellular signalling pathways and changes in their activity has been linked to several diseases including cancer and diabetes. These enzymes phosphorylate serine (Ser), threonine (Thr) and tyrosine (Tyr) residues at target proteins *via* transfer of the γ-phosphate of adenosine triphosphate (ATP). Around 2000 different protein kinase enzymes are predicted from the human genome[1] and structural data suggest that a conserved catalytic core is present in all such enzymes.[2] A conserved phosphoryl transfer mechanism is predicted with the regions of the protein outside the catalytic core influencing substrate specificity.

A conserved aspartate (Asp) residue is present in the active site which has been widely proposed to act as a general base during the phosphorylation reaction,[3] which involves nucleophilic attack of Ser at the γ-phosphate centre. It has been suggested that the role of Asp is to deprotonate Ser, providing a more powerful nucleophile to achieve phosphate hydrolysis leading to the products shown in Fig. 1(*b*). On the other hand, recent experimental data on serine[4] and tyrosine[5] kinases have questioned whether Asp is basic enough to remove the proton from Ser (compare solution pK_a of 4 and 14 for Asp and Ser, respectively) or Tyr residues. An alternative role is proposed of aligning the OH group before reaction[4] with proton transfer to Asp occurring after the O–P bond is formed.

In an attempt to elucidate the mechanism of phosphoryl transfer in this important class of enzymes we here describe hybrid quantum mechanical (QM)/molecular mechanical (MM) calculations of the potential energy surface associated with this reaction. Such studies have been helpful in the study of the reactions in a variety of enzymes.[6–11] We employ our hybrid QM/MM code[11] which couples the QM code GAUSSIAN 94[12] with the MM code AMBER 4.0[13] to perform the calculations.

We utilised the crystal structure of cAMP dependent kinase (cAPK) complexed with ATP and a peptide inhibitor,[14] building a model of the native system by replacing the alanine residue of the inhibitor with Ser. Energy minimisation of this new residue, keeping the remainder of the active site fixed, was carried out using AMBER. This structure was used as a starting point for subsequent QM/MM calculations with the QM part of the structure consisting of the 46 atoms shown in Fig. 2. The QM region contains the Ser substrate, the conserved Asp and a lysine (Lys) residue (although our conclusions are not altered if Lys is modelled by MM), the triphosphate group and the two Mg ions which coordinate the triphosphate. The bulk of the enzyme and the non-reacting parts of the peptide and ATP substrates were kept fixed during the modelling of the reaction and were represented by around 6200 MM atoms including crystallographic waters. We chose to use the PM3[15] Hamiltonian in the QM calculations due to its success in modelling phosphoryl transfer reactions.[16]

The reaction products resulting from proton transfer from Ser to Asp along with nucleophilic attack at phosphorus leading to ADP and a phosphorylated serine (pSer) dianion [Fig. 1(*b*)] were found to be higher in energy than the reactant structure [Fig. 1(*a*)] by 36 kcal mol^{-1}. However, an alternative product [Fig. 1(*c*)] involving an ionised Asp and a pSer monoanion were found to be lower in energy than the reactants by 24 kcal mol^{-1}. Our computational scheme allows the various contributions to the large energy difference (60 kcal mol^{-1}) between structures in Fig. 1(*b*) and (*c*) to be identified. In the absence of Lys-154 and the rest of the enzyme, the two structures have essentially the same energy in spite of the bulk pK_as favouring the structure in Fig. 1(*c*). This is due to the stabilisation of the phosphate dianion by the magnesium ions. However, interaction with the bulk of the enzyme leads to the preferential stabilisation of the structure in Fig. 1(*c*), with Lys-154 contributing ~20 kcal mol^{-1}. Thus, as a number of authors[17] have emphasised, it is the

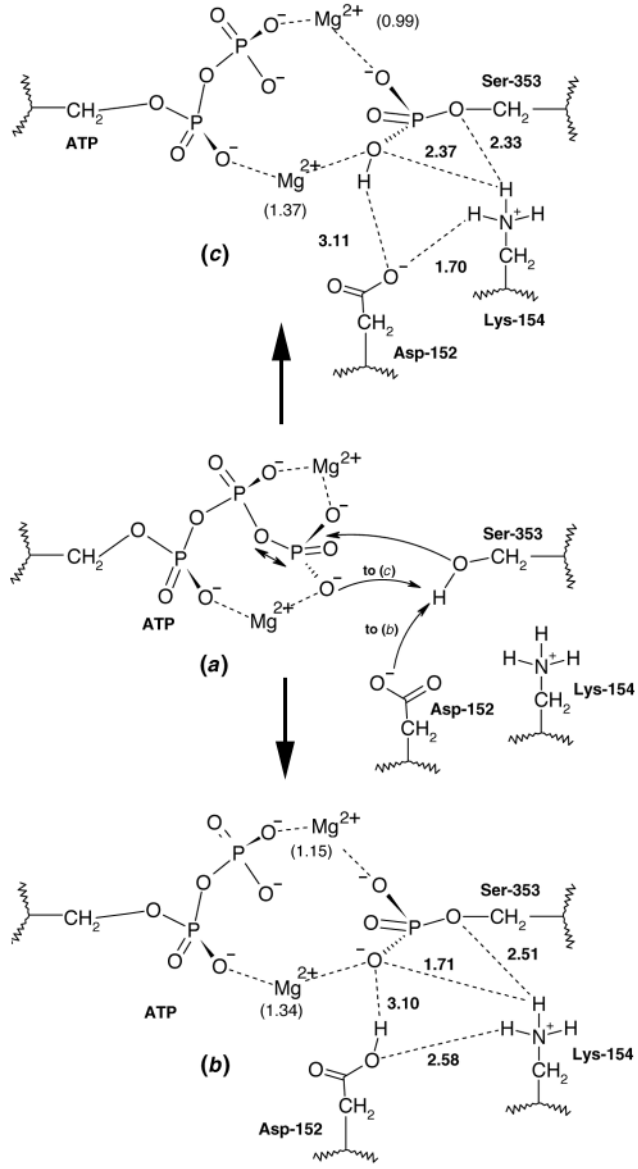

Fig. 1 Alternative mechanisms of phosphoryl transfer in protein kinase, with optimised structures (Å) and, in parenthesis, atomic charges.

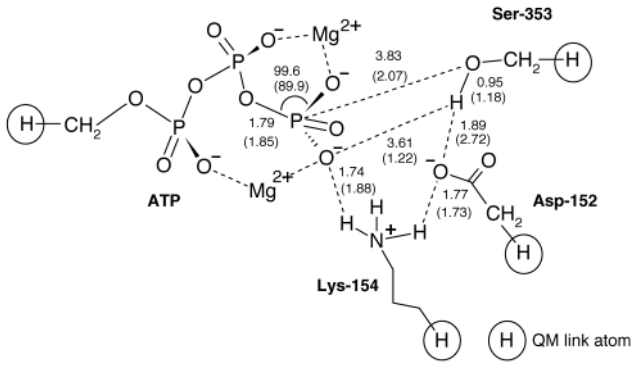

Fig. 2 Optimised reactant, and in parenthesis, transition state geometry (Å) in cAMP dependent kinase.

local electrostatic microenvironment that is important in determining the effective pK_as of the residues, rather than the bulk values (6.3[18] and 4.0[19] for pSer and Asp respectively).

In view of this result we have modelled the potential energy surface for the reaction which involves proton transfer from Ser to a terminal oxygen of ATP and nucleophilic attack at the phosphorus atom of ATP leading to the structure shown in Fig. 1(*c*) without the formation of a high energy serine anion intermediate. The properly characterised transition state (Fig. 2, imaginary frequency 2064*i* cm^{-1}) involves a concerted cleavage of the Ser O–H and P–O(bridge) bonds and formation of H–O(P) and P–O(Ser) bonds, and has a barrier of 39 kcal mol^{-1}. The alternative mechanism involving transfer of the proton from Ser to Asp either before or after O–P bond formation leads to a neutral Asp residue [Fig. 1(*b*)] and has higher barriers in the region of 45–50 kcal mol^{-1}, as well as leading to the higher energy product.

Thus although our calculations at the PM3 level are only semi-quantitative they do suggest that the role of the Asp residue may not be as a general base, but rather to stabilise protonated pSer in the active site [Fig. 1(*c*)]. Such stabilisation by protonation is often observed when two charged groups are in close proximity[20] and the position of a proton between Asp and Glu residues in a protein has been established by high resolution crystallography.[21] The relative pK_a values of pSer and Asp in model systems would suggest that protonation of pSer will be favoured in line with our computational results. A similar mechanism may also be involved in other enzyme catalysed phosphoryl transfer reactions, such as those involving protein tyrosine phosphatases, where a conserved Asp residue is found and is considered to be a general acid.[22] Here protonation of the phosphate terminal oxygen rather than Asp may occur in the reactants. Thus possible enzyme mechanisms other than those in which the Asp is a general acid or base should be considered in these important and widely occurring classes of enzymes.

We thank the EPSRC for support of this work.

Notes and references

1 T. Hunter, *Biochem. Soc. Trans.*, 1996, **24**, 307.
2 D. Bossemeyer, *FEBS Lett.*, 1995, **369**, 57.
3 A. S. Mildvan, *Proteins: Struct., Funct., Genet.*, 1997, **29**, 401.
4 J. Zhou and J. A. Adams, *Biochemistry*, 1997, **36**, 2977.
5 K. Kim and P. A. Cole, *J. Am. Chem. Soc.*, 1997, **119**, 11 096.
6 A. Warshel and M. Levitt, *J. Mol. Biol.*, 1976, **103**, 227.
7 M. J. Field, P. A. Bash and M. Karplus, *J. Comput. Chem.*, 1990, **11**, 700.
8 J. Aqvist and A. Washel, *Chem. Rev.*, 1993, **93**, 2523.
9 U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1986, **7**, 718.
10 B. Waszkowycz, I. H. Hillier, N. Gensmantel and D. W. Payling, *J. Chem. Soc., Perkin Trans. 2*, 1991, 225.
11 M. J. Harrison, N. A. Burton and I. H. Hillier, *J. Am. Chem. Soc.*, 1997, **119**, 12 285.
12 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. Gomperts, 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.
13 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.
14 D. R. Knighton, J. Zheng, L. F. Ten Eyck, N.-H. Xuong, S. S. Taylor and J. M. Sowadski, *Science*, 1991, **253**, 414.
15 J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 209.
16 J. Wilkie and D. Gani, *J. Chem. Soc., Perkin Trans. 2*, 1996, 783.
17 A. Warshel and J. Aqvist, *Annu. Rev. Biophys. Biophys. Chem.*, 1991, **20**, 267; P. A. Frey, *Science*, 1995, **269**, 104.
18 D. J. Graves and S. Luo, *Biochem. Biophys. Res. Commun.*, 1994, **205**, 618.
19 A. Fersht, *Enzyme Structure and Mechanism*, W. H. Freeman, New York, 1985.
20 T. Bugg, *An Introduction to Enzyme and Coenzyme Chemistry*, Blackwell Science, Oxford, 1997.
21 J. R. Helliwell, *Nat. Struct. Biol.*, 1997, **4**, 874.
22 Z.-Y. Zhang, *Crit. Rev. Biochem. Mol. Biol.*, 1998, **33**, 1.

Communication 8/08955H