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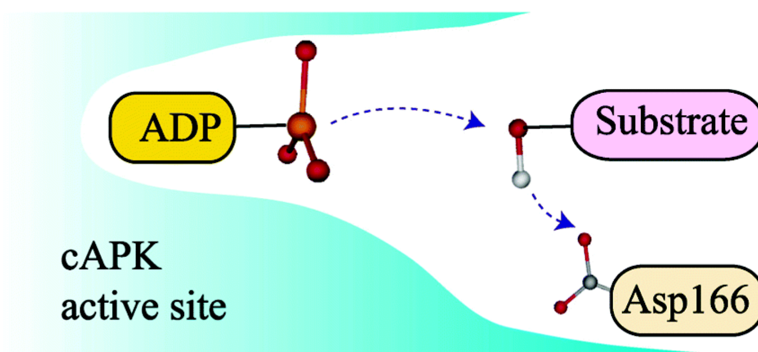

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The Role of the Putative Catalytic Base in the Phosphoryl Transfer Reaction in a Protein Kinase: First-Principles Calculations

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Protein kinases catalyze the transfer of the γ-phosphoryl group of ATP to serine, threonine, and tyrosine residues in proteins, a process essential for cell signaling. Although there may be as many as 2000 kinases in the human genome, the organization of the residues in the active sites of these enzymes is essentially the same (conserved).[1] Even after many structural and kinetic measurements, there is still considerable controversy regarding the role of these residues in the enzyme mechanism. The highly conserved aspartate (Asp166, Figure 1) has received particularly strong interest. While the essential presence of this residue has been established by mutational studies,[2] its function is still unclear. The X-ray structure[1] for cAPK kinase shows that Asp166 is located within hydrogen bonding distance of the Ser substrate OH, suggesting its role as a general base catalyst. However, the early proton transfer implied by conventional base catalysis is not supported by experimental data. The rate of phosphoryl transfer in cAPK, measured using pre-steady-state kinetics, is neither subject to a solvent deuterium isotope effect nor pH dependent.[3] Also, phosphoryl transfer in the tyrosine kinase, Csk, is insensitive to the pK_a's of fluorinated substrates,[4] indicating that the substrate nucleophile is largely neutral in the transition state (TS). The exact function of Asp166 is still debated, but the current hypothesis drawn from experimental data[3−6] is that it could play a role in either substrate orientation or late deprotonation in the reaction process.

Recent theoretical calculations have added to the controversy, yielding results apparently inconsistent with experimental findings. Semiempirical calculations (AM1, PM3)[7,8] did not assign any major role to Asp166, suggesting a mechanism with an early substrate proton transfer to the γ-phosphoryl group. While there are arguments[9] that kinetic measurements alone do not rigorously exclude such a mechanism, it seems highly unlikely in the light of recent experimental work on a Csk mutant.[6] In addition, this is inconsistent with pH data.[4,5] The fact that Asp166 does not appear to significantly alter the free energy landscape as reported in one of these calculations[8] also contradicts experimental data from mutation studies.[2]

In this Communication, we address this important divergence between theory and experiment by presenting a theoretical study of phosphoryl transfer in protein kinases using quantum mechanical calculations based on density-functional theory (DFT). This approach is significantly more accurate than prior semiempirical[7,8] approaches. Our model is based upon the X-ray structure of cAPK[1] and includes all of the essential conserved residues,[2] which have a role in the phosphoryl transfer reaction. This leads to a much larger calculation than has previously been attempted.[10] Consistent with experimental observations,[3−6,11] we find a dissociative transition state (TS) and establish that Asp166 plays an essential role in the phosphoryl transfer reaction as a "proton trap" late in the reaction process.

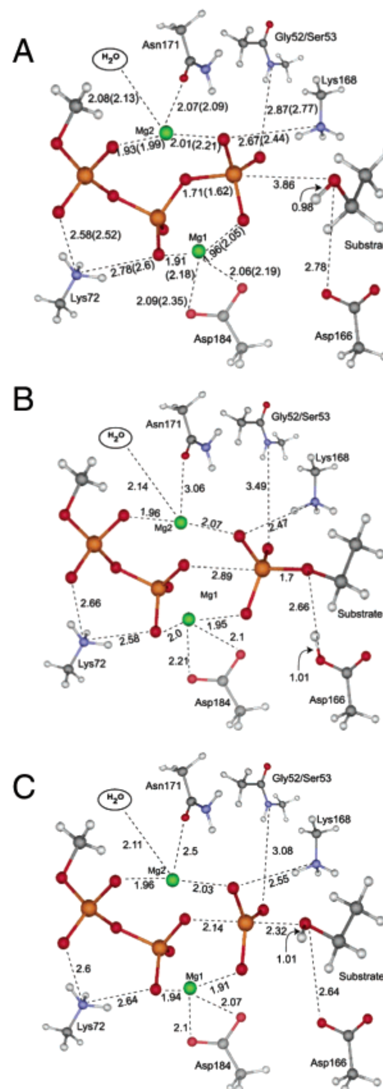

Figure 1. Optimized structures of the reactant (A), product (B), and transition (C) states. Experimental numbers are shown in brackets.

We have used both local and plane wave basis implementations of DFT.[12] Local basis methods were used for structural calculations, and first-principles molecular dynamics simulations were performed using a plane wave method.[13]

Our model of the active site, Figure 1, includes the triphosphate of ATP, the two Mg^{2+} ions coordinating the triphosphate, repre-

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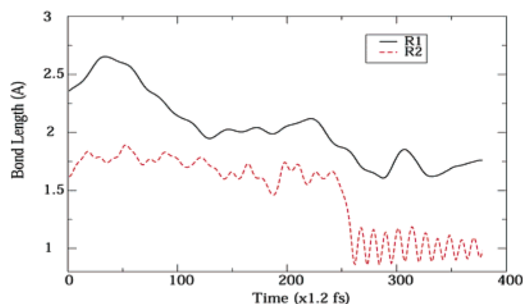

Figure 2. Time dependence of the bond distances between Ser-O and the Pγ of ATP (R_1) and between Asp166-O and Ser-H (R_2).

sentations of the Ser substrate, and Gly52/Ser53, Lys72, Asp166, Asp184, and Asn171 active site residues. The functional groups were truncated using hydrogen atoms, which together with the carbon atom were tethered according to the X-ray structure of cAPK.[1] The structural parameters of the active site after the initial optimization (see Figure 1A) are in good agreement with the inhibitor complex structure of cAPK[1]. The $O_{Asp166}-O_{Ser}$ bond length of 2.78 Å compares well to the experimental value[14] of 2.7 Å. However, the OHO bond angle of 158°, and relatively small decrease in HO_{Ser} stretching frequency (7% or 246 cm^{-1} as compared to isolated species), indicate that the $O_{Asp166}-O_{Ser}$ H-bond is relatively weak.[15] Our calculations show that the proton affinity[16] of Ser in our model protein environment has changed little (0.7% decrease) from its gas-phase value, indicating that the early removal of the substrate proton is unlikely in the protein environment.

Consistent with experimental interpretations, the lowest energy product state (PS) structure in these calculations has the proton transferred to Asp166, Figure 1B. Its energy is 9 kcal/mol below the reactant state with B3LYP (10.8 kcal/mol with GGA). The PS structure with the protonated phosphoryl group, reported as a global minimum in the PM3 calculations,[7] was found to have an energy 1 kcal/mol above the reactant state with B3LYP (1 kcal/mol below with GGA). The PS found in the AM1 calculations[8] was not stable. The protonation of the phosphoryl group is also unlikely because of interactions with Mg^{2+} ions.

Optimization of the TS corresponding to the product state with a protonated Asp166 led to a low activation barrier of 11 kcal/mol (B3LYP) (7 kcal/mol GGA) which is consistent with the observed fast reaction rate.[17] Using the average of distances from the γ-phosphoryl group to the $\beta-\gamma$ bridging oxygen (2.14 Å) and substrate hydroxyl group (2.32 Å), we predicted the TS (using Pauling's formula as suggested by Mildvan[18]) to be approximately 85% dissociative, which is in line with prior experimental predictions.[4-6,19] As predicted by pH data,[4,5] the substrate hydroxyl group is largely intact in the TS, indicating late proton transfer. A metaphosphate-like structure present in the TS also correlates with the X-ray structures of a transition state mimic.[19] Asp166 forms a stronger H-bond with Ser in the TS versus RS. The bond length and angle are 2.66 Å and 173°, while the HO_{Ser} stretching frequency is decreased by 17% (corresponding to an estimated[15] 2 kcal/mol increase in the H-bond energy as compared to the reactant state). The proton affinity of Ser decreases by 4% as compared to the RS, indicating only a slight weakening of the Ser OH bond.

The late proton transfer to Asp166 is also supported by first-principles molecular dynamics simulations. To initiate a reaction process, we started a simulation from the TS structure, Figure 1C, by assigning small initial velocity to the metaphosphate fragment toward ADP. The system evolved to a reactant-like state, returned, and crossed over the TS into the product state. The short turnaround distance in reactant configuration (2.65 Å, Figure 2) is caused by SerOH rotation early in the reaction process. The bond length variations between Ser-O and the Pγ of ATP (R_1) and between Asp166-O and Ser-H (R_2) are shown as a function of time in Figure 2. Phosphate bond formation is almost complete (R_1 shorter) before the proton transfers to Asp166 (R_2 shorter).

In summary, both structural and dynamical calculations provide compelling evidence for a largely dissociative reaction mechanism in which Asp166 accepts the substrate proton during the reaction process. However, this occurs at a point in the reaction coordinate where bond cleavage at the PO bridging position is already well advanced. Therefore, Asp166 is crucial to the reaction process serving as a "proton trap" that locks the transferred phosphoryl group to the substrate. These findings are supported by the existing experimental data[4-6,11,19,20] and resolve prior inconsistencies between theory[7,8] and experiment.

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References

(1) Taylor, S. S.; Knighton, D. R.; Zheng, J. H.; Sowadski, J. M.; Gibbs, C. S.; Zoller, M. J. *Trends Biochem. Sci.* **1993**, *18*, 84−89. Johnson, D. A.; Akamine, P.; Radzio-Andzelm, E.; Madhusudan; Taylor, S. *Chem. Rev.* **2001**, *101*, 2243−2270.
(2) Cole, P. A.; Grace, M. R.; Phillips, R. S.; Burn, P.; Walsh, C. T. *J. Biol. Chem.* **1995**, *270*, 22105. Gibbs, C. S.; Zoller, M. J. *J. Biol. Chem.* **1991**, *266*, 8923−8931.
(3) Zhou, J.; Adams, J. A. *Biochemistry* **1997**, *36*, 2977−2984.
(4) Kim, K.; Cole, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 11096−11097.
(5) Kim, K.; Cole, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 6851−6858.
(6) Williams, D. M.; Cole, P. A. *J. Am. Chem. Soc.* **2002**, *124*, 5956.
(7) Hart, J. C.; Hillier, I. H.; Burton, N. A.; Sheppard, D. W. *Chem. Commun.* **1999**, 79.
(8) Hutter, M. C.; Helms, V. *Protein Sci.* **1999**, *8*, 2728−2733.
(9) Florian, J.; Warshel, A. *J. Am. Chem. Soc.* **1997**, *119*, 5473. Aqvist, J.; Kolmogodin, K.; Florian, J.; Warshel, A. *Chem. Biol.* **1999**, *6*, R71.
(10) Hirano, Y.; Hata, M.; Hoshino, T.; Tsuda, M. *J. Phys. Chem.* **2002**, *106*, 5788.
(11) Parang, K.; Till, J. H.; Ablooglu, A. J.; Kohanski, R. A.; Hubbard, S. R.; Cole, P. *Nat. Struct. Biol.* **2001**, *8*, 37. Granot, J.; Mildvan, A. S.; Bramson, H. N.; Kaiser, E. T. *Biochemistry* **1980**, *19*, 3537.
(12) NWChem version 4.5, local basis calculations were based on Ahlrichs pVDZ basis; plane wave calculations were based on Hamann pseudo-potentials with a 15.87 Å cubic cell and an energy cutoff of 80 Ry.
(13) Valiev, M.; Bylaska, E. J.; Gramada, A.; Weare., J. H. In *Reviews in Modern Quantum Chemistry*; Sen, K. D., Ed.; World Scientific: Singapore, 2002; p 1684.
(14) Madhusudan; Trafny, E. A.; Xuong, N. H.; Adams, J. A.; Teneyck, L. F.; Taylor, S. S.; Sowadski, J. M. *Protein Sci.* **1994**, *3*, 176−187.
(15) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford Press: New York, 1997; p 12. Joesten, M. D.; Schaad, L. J. *Hydrogen Bonding*; Dekker: New York, 1974; p 221.
(16) The proton affinity of Ser was defined as the energy required to remove the proton from the Ser-OH group. Its gas-phase value was 407 kcal/mol.
(17) Grant, B. D.; Adams, J. A. *Biochemistry* **1996**, *35*, 2022.
(18) Mildvan, A. S. *Proteins: Struct., Funct., Genet.* **1997**, *29*, 401−416.
(19) Madhusudan; Akamine, P.; Xuong, N.-H.; Taylor, S. S. *Nat. Struct. Biol.* **2002**, *9*, 273. Cook, A.; Lowe, E. D.; Chrysina, E. D.; Skamnaki, V. T.; Oikonomakos, N. G.; Johnson, L. N. *Biochemistry* **2002**, *41*, 7301.
(20) Ablooglu, A. J.; Framkel, M.; Rusinova, E.; Ross, J. B.; Kohanski, R. A. *J. Biol. Chem.* **2001**, *276*, 46933−46940.

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