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Density functional study of the enzymatic reaction catalyzed by a cyclin-dependent kinase

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Density functional theory (DFT) calculations were carried out to study the molecular mechanism of the phosphoryl transfer reaction catalyzed by cyclin-dependent kinases (CDKs). The DFT study presented here shows that CDKs catalyze the phosphoryl transfer reaction from ATP to the serine substrate through a single step mechanism with a S_N2-like transition state.

The regulation of the eukaryotic cell cycle depends on the action of several related Ser/Thr protein kinases called cyclin-dependent kinases (CDKs), which are transiently activated at specific steps of the cycle.[1] Increasing evidence is arising about the connection between the CDKs regulation and some human diseases.[2,3] Several crystallographic studies have recently disclosed the structural determinants of representative members of this protein family, also providing details at the atomic level about the CDKs molecular mechanism.[4] However, the enzymatic reaction has not yet been investigated in depth. In this context, first principle quantum chemical calculations would improve the understanding of the phosphoryl transfer reaction from ATP to substrate serine catalyzed by CDKs:

$$\text{Ser-OH} + \text{ATP} \rightarrow \text{Ser-OPO}_3^{2-} + \text{ADP}$$

Here, density functional calculations[5] (DFT/B3LYP)[6] were carried out on a model system based on the crystal structure of the Michaelis complex of CDK2 with cyclin A, ATP, and an optimal peptide substrate (HHA**S**PRK) (resolution 2.2 Å; PDB code 1QMZ).[4b] This enzymatic complex provided a good starting point for the present study. The presence of both cyclin A and phosphorylated Thr160 guaranteed that the conformation of the CDK2 catalytic site residues was the active one.

In order to cope with the high computational demand of quantum chemical calculations, both a simplified structure of the Michaelis complex, and the locally dense basis set (LDBS) were used. LDBS has been demonstrated to provide very similar results compared to a full basis set,[7] and it has already been applied to study the phosphate hydrolysis in a biological system.[8] The structural model used here was composed by the truncated forms of Asp145, Asn132, the serine substrate, and ATP replaced by an acetate, an acetamide, an ethyl alcohol molecule, and a methyl triphosphate moiety, respectively. In addition, the catalytic cation Mg^{2+} and a water molecule were also included (Fig. 1).[9] At the reagents, all the model system atoms were in their crystallographic positions. Furthermore, to preserve the geometry of the catalytic site, the external methyl groups of Asp145 and Asn132 were kept fixed to their crystallographic positions. It should be noted that Asp127 was not included in the final model system only after the role of its carboxylate as general base was investigated in depth. Actually, all computations carried out to detect the involvement of Asp127 in deprotonating the serine failed. This result is in line with previous studies about phosphoryl transfer reaction in similar biological systems.[10]

The phosphoryl transfer reaction is believed to occur through two possible molecular mechanisms, either a dissociative or an associative one.[11] The present study supports that the reaction

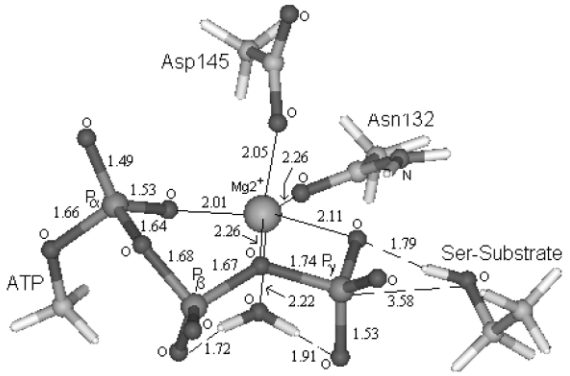

Fig. 1 DFT optimized structure of the reagent model system (distances in Å). The truncated form of Asp145, Asn132, ATP, and the serine substrate, along with the Mg^{2+} ion and a water molecule are shown.

catalyzed by CDKs occurs through a metaphosphate transition state (TS). Moreover, all attempts performed to identify an energetically stable intermediate failed. Actually, the geometry of the TS shown in Fig. 2 – identified by analytical computation of the Hessian matrix and by the characterization of the imaginary frequency – resembles the typical structure of the metaphosphate anion. This suggests a rather associative mechanism consistent with previous studies concerning phosphoryl transfer reactions catalyzed by other protein kinases.[12] The reaction mechanism turned out to be a concerted single step with a S_N2-like transition state (Fig. 2). As a matter of fact, by analyzing the TS features it was possible to detect that, when the system was in the saddle point, the substrate serine was almost deprotonated. This clearly arose by an inspection of the potential energy surface (PES), which was obtained by intrinsic reaction coordinate (IRC) calculations, starting from the TS geometry (Fig. 3). Actually, along the reaction coordinate – from the reagents to the products – the first observed chemical event was the formation of the highly nucleophilic agent $SerO^-$ through a proton transfer (PT) process. This occurred in a flat

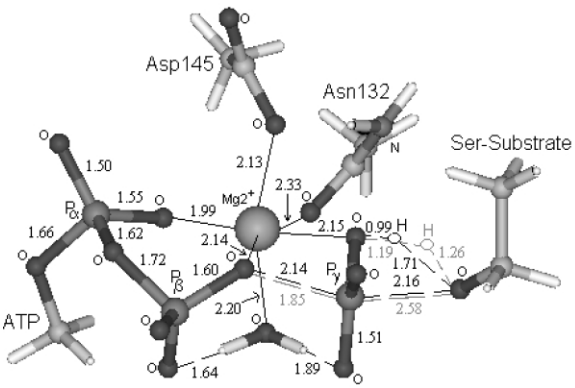

Fig. 2 DFT optimized structure of the transition state (distances in Å). The shaded hydrogen atom and bonds show the proton transfer optimized geometry (point □ in Fig. 3) along the reaction path.

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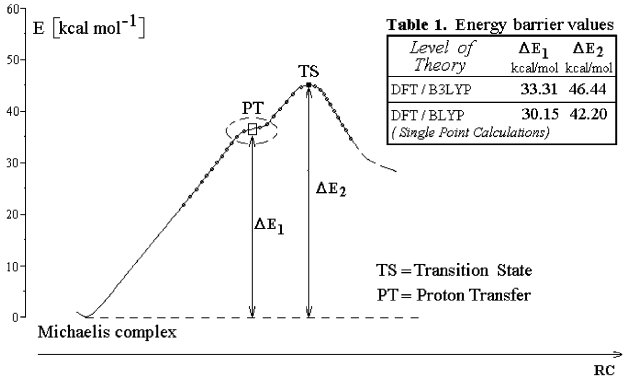

Table 1. Energy barrier values

Level of Theory	ΔE_1 kcal/mol	ΔE_2 kcal/mol
DFT / B3LYP	33.31	46.44
DFT / BLYP *(Single Point Calculations)*	30.15	42.20

Fig. 3 DFT energy profile along the optimized reaction coordinate (**RC**). The flat region in which the proton transfer (PT) occurs is shown. The optimized geometry along the IRC used to calculate ΔE_1(indicative value) for the PT event is shown (□). ΔE_1 and ΔE_2 energy values are reported in **Table 1**. DFT/BLYP single point energy values are also reported.

region of the PES: after the PT the reaction energy kept on increasing, leading directly to the TS. This might be due to the fact that $SerO^-$ is a very strong nucleophile and, thus, it cannot lead to a stable reaction intermediate bearing an alcoholate moiety. Therefore, after PT, the serine kept on approaching the γ-phosphorus to complete the substrate nucleophilic attack to ATP, leading to the reaction products (Fig. 4).

The energy barrier determined here ($\Delta E_2 \approx 46$ kcal mol^{-1}, Table 1) is indeed lower than that calculated at the DFT/B3LYP level *in vacuo* for the methylphosphate hydrolysis (*ca.* 70 kcal mol^{-1}).[8] The electrostatic effects of the active site, where the Mg^{2+} cation holds a prominent position, are responsible for the energy barrier lowering, showing the fundamental role of the active site residues in increasing the reaction rate. In this respect, it was also noticed that along the reaction coordinate the octahedral Mg^{2+} coordination shell was well maintained, stabilizing both the geometry and the overall negative charge of the metaphosphate anion (*i.e.*, TS).[13] However, the energy barrier for this enzymatic reaction is expected to be much lower than 46 kcal mol^{-1}, due to the contribution of the overall protein electric field, which was neglected in our calculations. Calculations in progress using a mixed code (QM/MM)[14] will hopefully unravel this point. In this regard, DFT/BLYP (exchange and correlation functional supported by the QM/MM code[14]) single

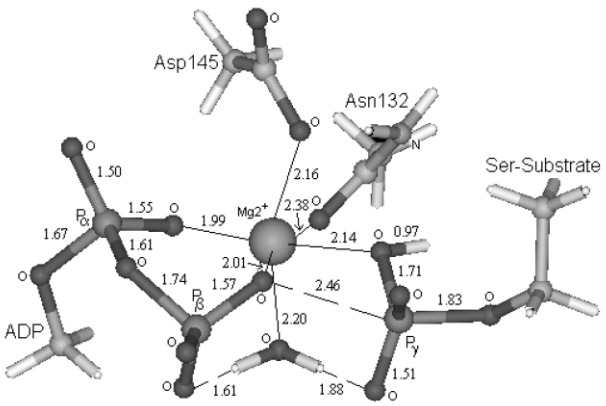

Fig. 4 DFT optimized structure of the latest point along the reaction pathway (IRC) from the TS to the products (distances in Å). The γ-phosphate is completely transferred from ATP to the substrate, leading to ADP and the phosphorylated serine.

point calculations were also carried out (the energy values are reported in Table 1).

In summary, the phosphoryl transfer reaction catalyzed by CDK2 was investigated for the first time by means of DFT calculations. Considering the high amino acid conservation of the ATP binding site within the whole family of the CDKs,[2] it is reasonable to assume that results obtained for CDK2 can have a general use in understanding the enzymatic molecular mechanism of other pharmacologically relevant CDKs, like CDK1[2] and CDK5.[3] The reaction occurred through a concerted single step with a S_N2-like transition state. The geometry of the identified TS is in good agreement with some recent crystallographic structures of transition state analogues.[12b,15] Furthermore, it was also observed that during the catalysis, a proton transfer process between the serine substrate and ATP took place just before the $SerO^-$ nucleophilic attack. This key event might be considered a sort of peculiarity of CDKs enzymatic reaction. For instance, in the GTP hydrolysis catalyzed by the G protein Cdc42, the direct proton transfer between the catalytic water and the γ-phosphate was both geometrically and energetically ruled out.[16] Finally, the results presented here, along with some recent studies concerning the CDKs active sites flexibility,[17] might be of paramount importance, when designing CDK inhibitors of therapeutic relevance.

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