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# Prediction of a ligand-induced conformational change in the catalytic core of Cdc25A

Karin Kolmodin, Johan Åqvist*

*Department of Cell and Molecular Biology, Uppsala University, Biomedical Center, Box 596, SE-753 14 Uppsala, Sweden*

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**Abstract The cell cycle control phosphatases Cdc25 are dual specificity phosphatases that dephosphorylate both phosphothreonine and phosphotyrosine residues on their substrate proteins. The determination of the *apo*-protein structure of Cdc25A revealed that this enzyme has a completely different fold compared to all other phosphatases crystallised to date. The conformation of the active site residues does not seem very suitable for catalysis in this unliganded structure. We have studied some structural features of the Cdc25A *apo*-structure and a modelled Cdc25A-ligand complex by molecular dynamics simulations. The simulations predict a conformational change in the peptide backbone of the complex, which is not observed in the *apo*-structure. This ligand-induced conformational change yields a structure that is similar to other protein tyrosine phosphatase-ligand complexes that have been crystallised. The change in conformation takes place in the position between a serine and a glutamic acid residue in the phosphate binding loop. We suggest that this type of conformational change is an important molecular switch in the catalytic process.**



*Key words:* Molecular dynamics; Ramachandran plot; Protein tyrosine phosphatase; Dual specificity phosphatase

## 1. Introduction

Cdc25 is a family of dual specificity protein phosphatases (DSPs) involved in the regulation of the eukaryotic cell cycle. The Cdc25 proteins dephosphorylate the cyclin dependent kinases which are thereby activated and can ensure the cell correct timing for transition into the next phase of the cycle [1]. There are three cloned isoforms of Cdc25 in human and mammals, Cdc25A, B and C. The isoforms have high sequence identity, especially in the active site region, but are presumed to be active in different phases of the cell cycle [2]. For example, Cdc25A is found to be expressed during the G1 → S transition [3,4], whereas Cdc25B and Cdc25C are thought to regulate the entry into the mitotic phase [5].

Cdc25 proteins have an active site motif that is typical for phosphotyrosine specific protein tyrosine phosphatases (PTPs) as well as VH1 like DSPs. These enzymes all contain the characteristic H-C-$X_5$-R motif, which encompasses the nucleophilic cysteine and the arginine involved in substrate binding and transition state stabilisation. The backbone of the five residues between Cys and Arg forms the phosphate binding cradle supplying a network of hydrogen bonds to the phosphate moiety of the substrate. The common active site motif of these enzymes suggests that they all employ the same mechanism for catalysis. The catalysed reaction has been shown to proceed via a two-step pathway where the nucleophilic cysteine is phosphorylated in the first step [6]. As the leaving group is protonated by an assisting general acid, the first product, the dephosphorylated tyrosyl/seryl/threonyl residue, is released. The phosphocysteine intermediate is subsequently hydrolysed by an activated water molecule in the second step of the reaction.

The recently published crystal structure of Cdc25A revealed that this enzyme has a completely different fold than the other PTPs, and has an identical topology to that of rhodanese, a sulfur transfer protein [7]. Although different in fold, the crystal structure confirms the expected features of the characteristic active site. There are, however, a few discrepancies between Cdc25 and the PTPs that are worth mentioning. The Cdc25 proteins do not have serine or threonine immediately after the active site arginine. In the other PTPs and DSPs, the hydroxyl group of this residue is proposed to stabilise the ionised form of the nucleophilic cysteine during catalysis. Here, one of the serines (Ser-433) in the phosphate binding loop is proposed to accomplish this stabilisation instead [7]. Another difference is the fact that no auxiliary loop containing a catalytic general acid residue is observed in Cdc25. The general acid has been shown to be very important for enhancing the rate of phosphomonoester hydrolysis in the PTPs. Eckstein et al. [8] suggested from mutational experiments and sequence alignments that the conserved Asp-383 could be the general acid residue. However, this hypothesis did not hold when the crystal structure was determined. Asp-83 forms an ion pair inside the protein and is probably conserved to maintain the correct structure. Interpretations of the crystal structure suggested that Glu-431 is suitably positioned in the active site loop and may therefore function as the general acid [7]. This Glu is also totally conserved throughout the Cdc25 family.

The crystal structure of Cdc25A was determined in absence of an inhibitor or substrate analogue in the active site. This resulted in an open conformation with the arginine side-chain in a position that would not be optimal for transition state stabilisation. Furthermore, the backbone of the phosphate binding loop is found in an unexpected conformation. In order to study some structural aspects of Cdc25, we performed molecular dynamics (MD) simulations of the Cdc25A *apo*-structure and a modelled Cdc25A-sulfate ion complex.

## 2. Materials and methods

All MD simulations were run using the software package Q [9]

*Corresponding author. Fax: (46)-18-536971.
E-mail: aqvist@xray.bmc.uu.se


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together with the GROMOS87 force field [10]. The initial protein coordinates were taken from the crystal structure of Cdc25A, PDB accession code 1C25 [7]. The active site of the enzyme was solvated by a 16 Å sphere of SPC water molecules. The solvent surface was subjected to radial and polarisational restraints as described in [9]. Protein atoms outside the simulation sphere were restrained to their crystallographic coordinates and interacted only via bonds, angles and torsions across the boundary. The system was heated to a temperature of 300 K by a stepwise equilibration protocol where the protein atoms were restrained to their initial coordinates. After the heating procedure, the restraints were turned off and MD was run at constant temperature 300 K with a time step of 1 fs. The trajectories of the *apo*-structure were run straightforwardly, whereas the initial structure of the Cdc25-ligand complex was obtained by a few minor structural modifications. The crystal structure of the low molecular weight PTP in complex with sulfate [11] (PDB entry 1PHR) was used as a template for the modelling. The backbone atoms of the loop residues 430–436 in Cdc25 and 12–18 in the low molecular weight PTP were superimposed (Fig. 1A). The resulting position of the sulfate ion was then transferred to the corresponding coordinates in the Cdc25 structure. The torsional angles of the Arg-436 side-chain were modified so that the guanidinium group interacted with two of the sulfate oxygens, similar to the binding mode found in all other crystallised PTP-ligand complexes. To avoid clashes, the side-chains of Glu-431 and Phe-432 were also slightly moved.

## 3. Results

The MD trajectories of the *apo*-structure in water were run for 65 ps at 300 K and showed relatively stable energies and well retained structures. Simulation of the modelled complex of Cdc25A and $SO_4^{2-}$ yielded stable trajectories after 2.5 ps simulation at 300 K. However, already at 2 ps, a conformational change in the backbone peptide bond between residue Ser-434 and Glu-435 occurred. In the starting (crystal) structure, the dipoles of this peptide bond are 'inverted' compared to the pattern seen in the other H-C-$X_5$-R containing structures. 'Inverted' here refers to the fact that the carbonyl oxygen is pointing into the phosphate binding site and the amide nitrogen is pointing outwards. This conformation would be electrostatically unfavourable when there is a negatively charged substrate bound in the phosphate binding loop and thus the dipoles flip over to the preferred conformation when the sulfate ion is present (Fig. 1B). The conformational change was clearly monitored by measuring the Ramachandran angles of residues Ser-434 and Glu-435 during the MD trajectory (Fig. 2). The diagram shows that residue 435 has a strained conformation in the starting structure with the Ramachandran angles $\varphi$ and $\psi$ being unstable and located outside the allowed regions. As the trajectory proceeds, the torsional angle $\varphi$ changes and the Ramachandran trajectory ends up in the allowed region of the diagram. For Ser-434, mainly the $\psi$ angle changes its value and the Ramachandran plot trajectory is displaced from the allowed region defined by β-structures into the region typical for α-helical structures.

The sulfate ion in the active site was replaced by a phenyl phosphate molecule in a new set of MD simulations. The average structures showed that the phenyl ring preferably interacts with the side-chain of F432, which has been proposed to be important for phosphotyrosine recognition [7]. In this binding mode, where the two phenyl rings stack with each other, Glu-431 does not appear to be in a proper position for proton donation to the bridging oxygen of the substrate. The side-chain of Glu-431 is also surrounded by basic residues which would not stabilise the protonated form of the glutamic acid required for catalysis in other PTPs. As far as stabilisation of the nucleophilic cysteine is concerned, the simulations showed that Ser-433 is able to interact with the ionised sulfur atom, although they are quite distant in the crystal structure (4.6 Å). However, the interaction is not very stable and the hydroxyl group of the serine residue rather interacts with the carbonyl oxygen of Leu-465 (2.8 Å apart in the crystal structure) during most parts of the trajectory.

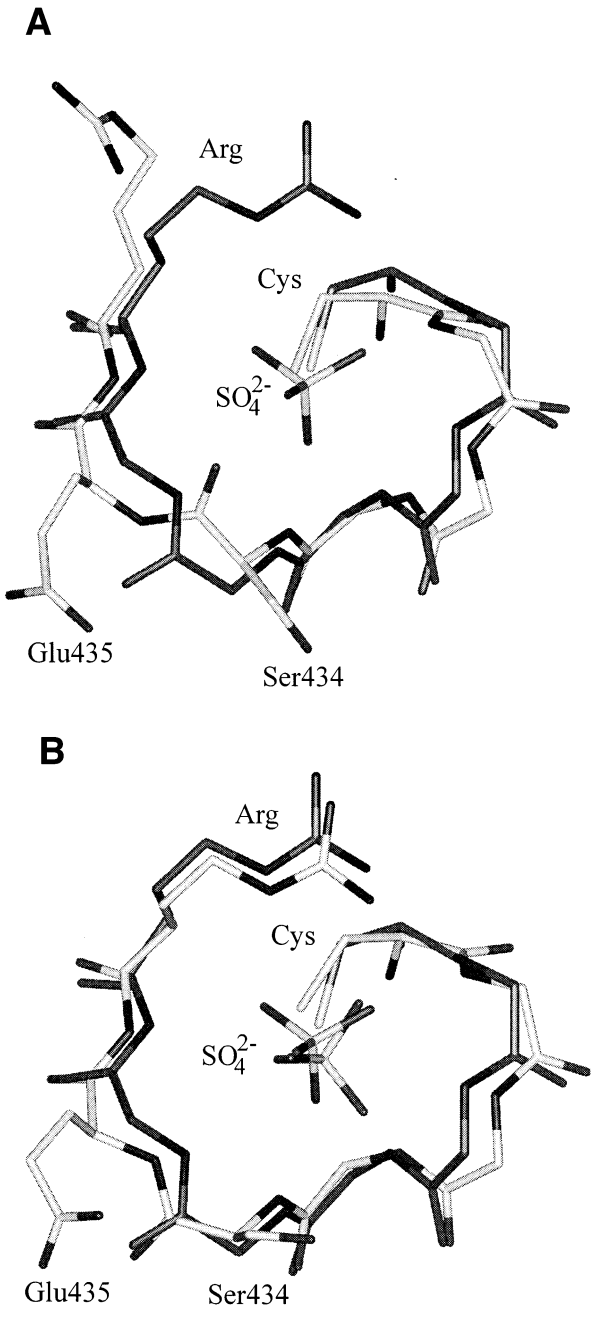


Fig. 1. A: Superposition of the backbone atoms of residues 430–436 in Cdc25A (white, PDB entry 1C25) and 12–18 in low molecular weight PTP (grey, PDB entry 1PHR). The sulfate ion is found in the crystal structure 1PHR. In addition to Ser-434 and Glu-435, only the totally conserved side-chains Cys and Arg are shown for clarity. B: Average MD structure of the ligand complex with Cdc25A after the observed conformational change, superimposed on low molecular weight PTP as in A.

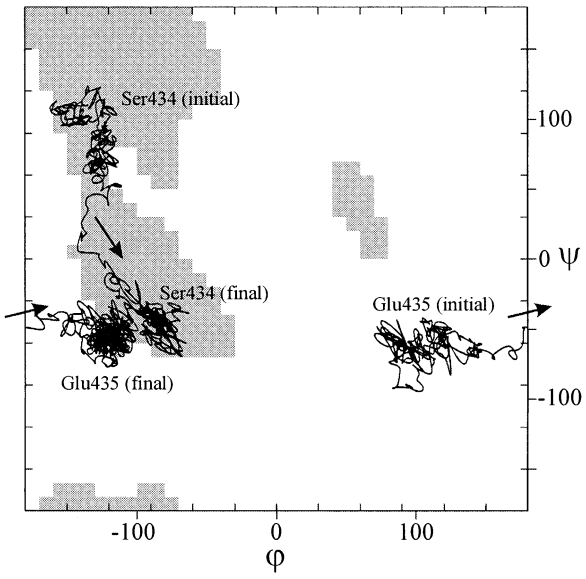


Fig. 2. Ramachandran plot trajectory of Ser-434 and Glu-435 during the first 5 ps of MD simulation at 300 K. The arrows indicate the direction of the trajectories. Shaded areas correspond to the generally allow regions for $\varphi$ and $\psi$.

## 4. Discussion

All crystal structures of PTPs and DSPs in complex with various ligands have a common structure of the active site with the backbone amides of the loop residues pointing into the centre of the crevice in order to stabilise the equatorial oxygens of the phosphomimetic group. The conformation of the corresponding region in the structure of Cdc25A, where one of the peptide bonds is pointing its amide group in the opposite direction, has not been observed before. This conformation would be very disfavoured in the enzyme-substrate complex with the negatively charged phosphate group positioned in the binding loop. It would also have a destabilising effect on the transition state which requires maximal stabilisation of the equatorial oxygens for efficient catalysis.

In this work, we have studied the effect of docking an oxyanion in the active site of Cdc25A. By running MD simulations of the modelled complex, we found that the whole peptide bond spontaneously flips so as to stabilise the substrate/product analogue. The result is not totally unexpected, but may have interesting implications. It may indicate that also other conformational changes occur upon substrate binding, for instance, the side-chain conformations of Ser-434 and Glu-435. It is thus possible that the observed conformational change is a mechanism to orient these residues in favourable positions for catalysis or substrate recognition. This would then be similar to the ligand-induced loop closure that occurs in, for example, PTP1B. It is tempting to propose that Glu-435 could act as a general acid as it comes closer to the active site upon this conformational change. However, mutation of the corresponding residue in Cdc25B yielded an enzyme with similar activity as the wild-type [12].

Cdc25A is similar to the low molecular weight PTP in that there is an additional cysteine residue that appears able to form a disulfide bridge with the catalytic cysteine under certain conditions [7,13]. In the latter case, this residue (reduced Cys-17) has been proposed to play a role in the hydrolysis of the phosphoenzyme intermediate by interaction with the water nucleophile [14]. However, in Cdc25A, the non-catalytic cysteine (Cys-384) is located in a position where such a function could not be accomplished.

The general acid found in all tyrosine specific phosphatases and VH1 like DSPs has not yet been unambiguously identified in Cdc25. The absolutely conserved Glu-431 is a good candidate. However, its position and local environment in our enzyme-substrate model do not seem optimal for this function. The side-chain would have to adopt a strained conformation in order to reach the bridging oxygen of the substrate, since the Arg-436 side-chain displaces Glu-431 from its crystallographic position. Site-directed mutagenesis is required to answer the question regarding the importance of this residue. It has been reported that replacement of the five amino acids between the active site cysteine and arginine (Glu-Phe-Ser-Ser-Glu) with (Ser-Ala-Gly-Val-Gly) completely abolishes activity [15]. However, this could be due to other effects than the lack of a general acid residue. In the case of Cdc25, it cannot be excluded that the enzyme actually functions without such a general acid group. The turnover rate of Cdc25 with pNPP as substrate is generally so low that it is comparable to the rate of a PTP with the general acid mutated [16–18]. The unusually shallow binding site in Cdc25 makes the leaving group more solvent accessible compared to the case in, for example, the low molecular weight PTP which is narrow and hydrophobic in this region [11,19]. Efficient solvation of the leaving group by water molecules instead of using a general acid for protonating the leaving group might be sufficient for catalysis in this case. Further biochemical investigations and structural studies of Cdc25 complexes should hopefully be able to clarify the detailed action of this important enzyme family.

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