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Molecular dynamics simulations of human kinase protein: the influence of a conserved glycine by serine substitution in the G-loop of a CDK2 active complex

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We have performed the mutation analysis of functionally important structural elements for a cyclin dependent kinase protein *via* 2 ns molecular dynamics (MD) simulations of a crystal lattice of kinase active complex pT160-CDK2/cyclin A/ATP-Mg^{2+}/substrate. Based on the MD simulation results we discuss the influence of the structural conformation changes on the kinase activity and molecular mechanism of regulation of phosphorylation.

Studies of structures of cyclin-dependent kinase (CDK) proteins, which play a central role in regulation of a cell cycle, and of functional importance of selected structural elements of kinase cause practical interest due to high incidence of CDK genetic alteration or deregulation of CDK inhibitors in cancer cells. A catalytic subunit kinase is an evolutionarily conserved fragment, and it possesses a similar structure for human and yeast.[1] A protein molecule of human CDK2 consists of one polypeptide chain (of 298 residues in total), forming a compact globular structure that is packed into two segments: an N-terminal tail (residues 1–85), consisting of five anti-parallel β-sheets (β1–β5) and one major helix (α1), and a large C-terminal (residues 86–298), consisting mostly of α-helices (Figure 1).

At the kinase N-end, a conserved glycine-rich loop GxGxxG (G-loop) is located. The functional importance of this G-rich domain is shown for human kinase cAPC,[2,3] substitution of the first (G11) and second (G13) glycines to alanine and serine results in a crucial decrease of the cAPC activation. Substitution of the third glycine to serine *cdc28-srm* [G20S] for the saccharomyces yeast causes changes in the cell cycle, a decrease of genetic stability and an increase of radiosensitivity.[4–6] In the G-loop, the negative regulation sites are located as well, phosphorylation of the residues T14 and Y15 results in kinase inactivation. Nevertheless, it should be noted that the structural changes caused by the amino acid exchanges of the G-loop are less studied yet. Here, we analyse the substitution of serine for glycine in a conserved G-loop of human protein kinase CDK2.

For the simulation of the kinase structural changes of CDK2 due to substitution of the third glycine G16 (G20 for the yeast), we use the structure of the active complex pT160-CDK2/cyclin A/ATP-Mg^{2+} (file 1QMZ; Brookhaven Protein Data Bank http://www.pdb.org). Kinase activation occurs during the interaction of the CDK2 catalytic subunit with cyclin and consequent phosphorylation of the T160 residue in the T-loop. An active complex of protein kinase CDK2 includes a phosphorylated kinase subunit pT160-CDK2, an active molecule of ATP, which is bound with an Mg^{2+} ion, a cyclin fragment (residues 173–432), PSTAIRE (residues 45–51) and a substrate pentamer (HHASPRK), which has a phosphorylation site in the central protein region. The CDK2 complex contains 2287 water molecules.

Note that glycine G20 of yeast kinase CDC28 corresponds to glycine G16 of human kinase CDK2. Thus, performing computer MD, we changed residue S16 for residue G16, so that two sets of calculations for both complexes CDK2-G16 and CDK2-S16 under the same simulation conditions were performed.

The equilibrated atomic positions for each complex have been estimated at 2 million time steps. The integration step was 1 fs, and the total simulation time was 2 ns. Within this time, the kinase complex had to reach well-distinguished equilibrium states. Figure 1 shows the final configurations of the native (G16) and mutant (S16) proteins.

The results indicate that the final equilibrium states of the CDK2-G16 and CDK2-S16 structures differ from their corresponding initial crystal lattices. With regard to the final (2 ns) state, the structural rearrangements of the native (G16) and the mutant (S16) CDK2 complex noticeably differ from each other if we compare the details of the root-mean-square displacements (RMSD) analysis data.

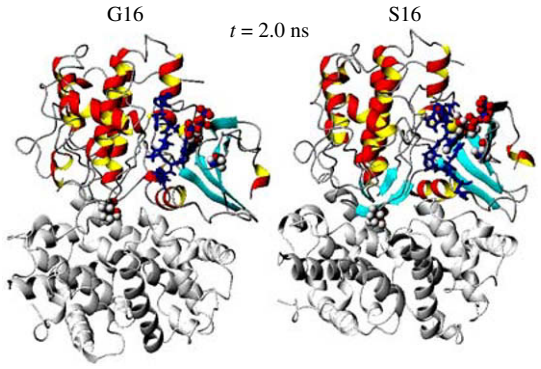

Figure 1 The final conformations for the native (G16) and the mutant (S16) CDK2 proteins. The spheres mean the atoms of the amino acid residue T160, a phosphorylation site of T-loop, and residue G16, a mutation site of glycine to serine exchange.

Figure 2 shows the structural rearrangement of the atomic positions of the CDK2 complex through the estimation of the RMSD values. As is seen, the RMSD between the equilibrium structures of CDK2-G16 and CDK2-S16 differ by an average of 2 Å. We also compared the RMSD behaviour separately in selected fragments of the CDK2. The analysis of the RMSD values shows that the structure deviations in kinase and cyclin regions are about 1.8–1.6 Å. The changes in G-loop positions are on the average 2 Å, in T-loop 1.4 Å, and substrate and PSTAIRE fragments are strongly deformed. Thus, the introduction of a single G16S substitution causes a larger atomic displacement in the structural elements, resulting in greater fluctuation of the whole protein structure.

Note that the differences in the structural behaviour of these protein complexes are mostly distinguishable in fragments that

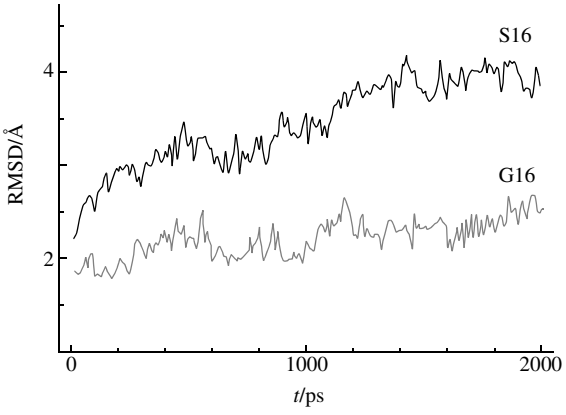

Figure 2 The behaviour of the RMSD (root-mean-square displacements) for the native (G16) and mutant (S16) CDK2 proteins.

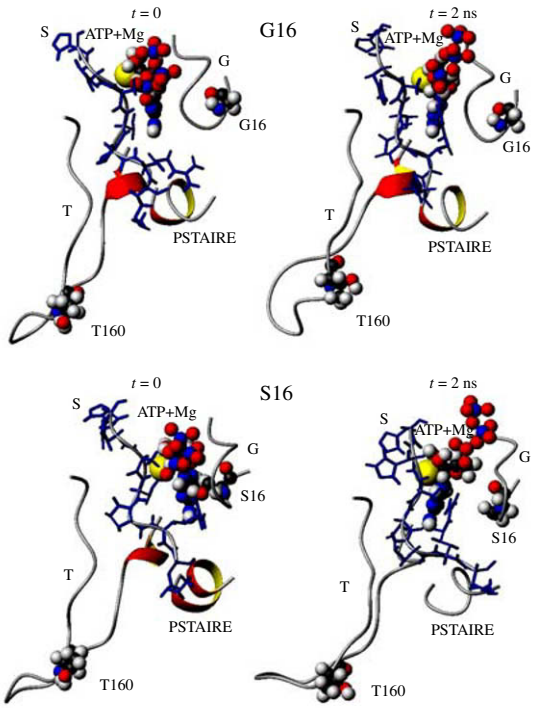

Figure 3 Comparison of the initial ($t = 0$) and final ($t = 2$ ns) conformations of the 'key structural elements' of the native (G16) and mutant (S16) CDK2 complexes. The mutation (G/S16) and phosphorylation (T160) sites, as well as the complexes ATP-Mg^{2+}, fragment PSTAIRE, G-, T-loops and substrate (S) are shown.

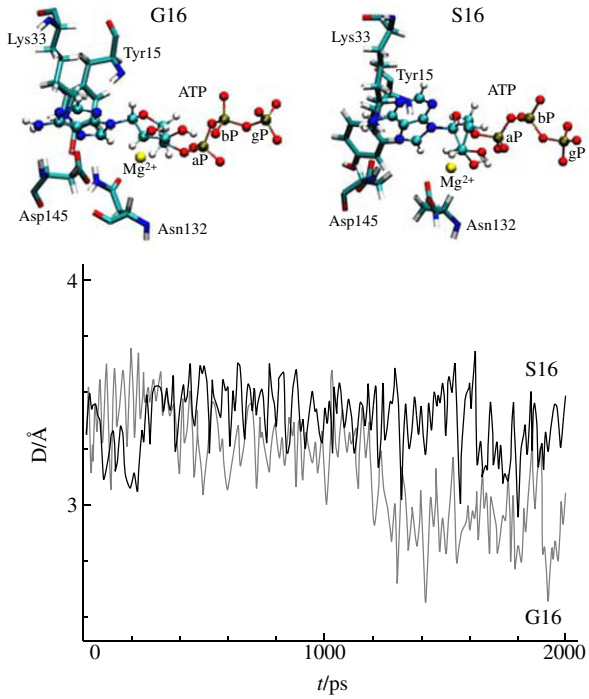

Figure 4 The localization of the ATP binding site (top snapshots) and the distance diagrams between the ATP, Mg ion and mutation site G/S16 (bottom).

play a central role in the CDK2 activation mechanism, in functionality of the kinase protein. These structural elements are, first of all, the regions of the conformation of the ATP-Mg^{2+} complex, the G- and T-loops and substrate S (see Figure 3).

By comparing the initial and final conformations in the 'key fragments' between the native and mutant CDK2 complex, it is easy to observe that the shape of the G-loop in the mutant (CDK2-S16) structure is more deformed than that in the native (CDK2-G16) one. The conformation of the T-loop in CDK2-S16 is more distinguishable than that in CDK2-G16. In the CDK2-S16 variant, the T-loop is reoriented from the cyclin 'cleft' to the kinase direction.

A binding site of the ATP molecule in CDK2 is located in a cleft between the N and C terminals.[7] The Mg^{2+} ion and several amino acid residues of a catalytic subunit, including Lys33 and Asp145, determine a proper rearrangement of the phosphate groups, thereby facilitating the pass of γ-phosphate from ATP to the protein substrate. Figure 4 indicates that the O_2 oxygen of the ribose and the O_{1A} oxygen of the Pα-ATP participate in the octahedral orientation of Mg^{2+}. Other two ligands, the residues Asp145 and Asn132, coordinate a proper location of Mg^{2+}. Thus, this catalytic unit (*viz.*, Lys33, Asn132, Asp145) takes an active part in the orientation of ATP phosphates and the coordination of Mg^{2+}. This segment, as is well established, is crucial for the CDK2 catalysis mechanism.[7]

The distance deviations between the ATP, Mg^{2+} ion and mutation site (G/S16) in the above catalytic region are presented separately in Figure 4 (bottom diagrams). Note that, in G16 and S16 variants, starting from the same configurations, both the ATP position and its orientation with regard to the Mg^{2+} are considerably different from each other. Namely, the distance between the ATP and Mg^{2+} in the native (G16) structure decreases in comparison to that for the mutant (S16) one. (In native protein, the ATP molecule seems more closely located to the Mg^{2+} ion.) This results in that the C5 group of the ribose (this site is located between the ribose and Pα-ATP) and adenine have to be reoriented in the Mg^{2+} direction. Such a picture, nevertheless, is not observed for the mutant (S16) structure. Thus, G/S16 mutation acts in such a way that the ATP molecule 'loses or weakens' its ability to exchange actively with the Mg^{2+} ion.

In conclusion, the replacement of glycine by serine (G16S) in a conserved G-loop may essentially modify the relative orientation of the ATP molecule with regard to Mg^{2+} and also rearrange the other fragments of the kinase protein. As a result, one may expect that G16S mutation could cause greater structural changes in all kinase subunit and cyclin regions. The distance between the phosphorylated Ser of the substrate and the ATP γ-phosphate is increased to about 3 Å. These structural changes will inevitably involve kinase activity, decrease or modification of substrate phosphorylation, *etc*. This is due to the fact that the kinase functional activity is strongly sensitive to the relative location of substrate, γ-ATP, G- and T-loops, to the conformation of an active centre. Apparently, the above observations based on the MD results for the kinase structural changes may correlate with the molecular mechanism of the kinase binding with cyclin, substrates, kinase activity and regulation of phosphorylation.

For the details of computational methods, see Supplementary Materials which are available free *via* http://www.turpion.org/suppl/mc/2337/suppl2337.pdf.

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References

1 H. L. De Bondt, J. Rosenblah, J. Jancarik, H. D. Jones, D. O. Morgan and S.-H. Kim, *Nature*, 1993, **363**, 595.
2 R. T. Aimes, W. Hemmer and S. S. Taylor, *Biochemistry*, 2000, **39**, 8325.
3 D. A. Johnson, P. Akamine, E. Radzio-Andzelm, N. Madhusudan and S. S. Taylor, *Chem. Rev.*, 2001, **101**, 2243.
4 A. B. Devin, T. Prosvirova, V. T. Peshekhonov, O. V. Chepurnaya, M. E. Smirnova, N. A. Koltovaya, E. N. Troitskaya and I. P. Arman, *Yeast*, 1990, **6**, 231.
5 N. A. Koltovaya, I. P. Arman and A. B. Devin, *Yeast*, 1998, **14**, 133.
6 N. A. Koltovaya, K. Yu. Kadyshevskaya and A. B. Devin, *Yeast*, 1995, **11**, 73.
7 P. D. Jeffrey, A. A. Russo, K. Polyak, E. Gibbs, J. Hurwitz, J. Massague and N. P. Paoletich, *Nature*, 1995, **373**, 313.

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