Nanoseconds long molecular dynamics (MD) trajectories of the CDK2/ATP complex were analyzed. The MD simulations of substitution CDK2-G16S (CDC28-G20S) shows a conformational change of CDK2 structure resulting in the moving of the G-loop away from ATP and a new rearrangement of amino acids in the T-loop.

Introduction

The cyclin dependent kinases (CDKs) are a subfamily of serine/threonine-specific protein kinases. The enzymes catalyze a transfer of the gamma-phosphate of adenosine triphosphate (ATP) to a protein substrate. CDKs are crucial regulators of the timing and co-ordination of eukaryotic cell cycle events. Transient activation of these kinases at specific cell stages is thought to trigger the principal transitions of the cell cycle, including DNA replication and entry into mitosis. In yeast, both transitions are controlled by single CDK (CDC28 in *Saccharomyces cerevisiae* [3]). In human cells, cell cycle events are governed by several CDKs. CDK4-cyclin D is necessary for passage through G1, CDK2-cyclin E is necessary for the transition from G1 to S phase, CDK2-cyclin A is necessary for progression through S, and CDC2-cyclin B is necessary for the transition from G2 to M phase. Two of these proteins CDK2 and CDC2/CDK1 have long been studied extensively [1-7]

Results

We simulated the CDK2-G16/ATP of the wild-type structure and compared the conformational changes with CDK2-S16/ATP structure. For CDK2-G16 and CDK2-S16 structures we have also generated a number of the animation movies to display the real-time dynamical motions. We concentrate on the positional changes between the ATP, residue 16 and T-loop.

If to make the comparison of the pictures of the initial and final states of wild-type CDK2-G16/ATP structure, it is easy to see that there is not a visually large difference between
these two structures. Moreover, the position of the amino acid residue 16 does not change within 1 million time step MD steps in comparison to that at the initial configuration. So, for the wild-type protein we found that it keeps its conformation stable relatively to the original state.

Regarding the CDK2-S16 variant, a completely different picture of the dynamics and conformational changes has seen. First of all, the amino acid residue 16 moves dramatically far from the ATP location site. In comparison to the wild structure, the distance between the position S16 and ATP, as it seen from the snapshots, increases of about 2-2.5 times at average. At the same time, such movement results on a relative shift of the T160 residue and the whole T- and G-loops positions. On the one hand, it is obvious that the increase of the ATP-S16 distance influences on the picture of the hydrogen bonds formation involving the ATP and G-loop. On the other hand, the mutation induced CDK2' conformational change results in an interhelical protein movement, covering a phosphorylation point (viz. T160).

**Discussion**

The G-loop enables protein kinase to adopt a wide range of backbone conformations. The significance of this domain is demonstrated by the fact that substitution of the glycine residues in the G-loop, particularly the first and the second glycine (GxGxxG) with either alanine or serine results in a drastic decrease in cAPK activity. The functional importance of the G-loop has been described in detail for cAPK [8-11], but its importance for CDK regulation has not been yet discussed. It is believed that the G-loop catalytic function – that is, correct ATP binding and alignment – is the same as its function in cAPK, but it exhibits a new inhibitory function for CDK [12]. We have obtained the mutation cdc28-srm that possesses a pleotropic manifestation in yeast cells. Analyses have shown that this mutation is localized in the glycine-rich loop (G-loop) and is a substitution of the third glycine with serine.

The crystal structure of inactive monomeric CDK2 showed that the T-loop (residues 147-153) would block access of substrates to the active site and that ATP would bind with the wrong geometry for efficient catalysis. Binding to cyclin A simultaneously moves the T-loop of CDK2 away from the substrate binding cleft and repositions of the G-loop (residues 11-18), so that they can interact properly with the ATP phosphates. This complex has a low but detectable activity. Less dramatic changes occur in the structure of the CDK2/cyclin A complex following activation by phosphorylation of T160 [13]. The phosphorylation of T160 might relieve a proposed steric interference between the substrate and the unphosphorylated T-loop and help to organize an acidic patch containing E162, E208 and D235 by repositioning E162, a T-loop residue. The
serine of the peptide substrate is hydrogen bonded to the ATP gamma-phosphate oxygen, to the catalytic aspartate D127, and to the conserved lysine K129 [14].

MD simulation analysis shows that in CDK2-S16/ATP the distance between ATP and the residue 16 in the G-loop dramatically increased. The shift is equal 5 Å. It's known that the G-loop was changed during the early stage of inactive (CDK2/ATP), partly active (CDK2/cyclin A/ATP), and fully active (pT160-CDK2/cyclin A/ATP) CDK2 simulations in comparison with its conformation as found in the crystal structures [12]. The G-loop moves away from the ATP phosphate moiety binding site after the interaction of CDK2 with cyclin A and again after CDK2/cyclin A/ATP complex phosphorylation at the T160 site. The shift of the G-loop is equal to 3.5 Å (CDK2/cyclin A/ATP) and 8.6 Å (pT160-CDK2/cyclin A/ATP) in comparison with the G-loop position found in the CDK2/ATP system. It's interesting that we observe this shift in the mutant allele CDK2-G16S/ATP as well.

References