CDK2 ACTIVATION AND INHIBITION BY PHOSPHORYLATION, A MOLECULAR DYNAMICS STUDY

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In human cell, cell cycle events are governed by several CDKs [1]. Cell-cycle dependent oscillations in CDK activity are induced by complex mechanisms that include binding to positive regulatory subunits and phosphorylation at positive and negative regulatory sites. For activation CDKs require binding to cyclins. CDKs obtain full activity at binding with adenosine triphosphate (ATP) by phosphorylation of a threonine residue in the CDK (Thr 160 in human CDK2) [2]. Activities of these enzymes are inhibited in several ways, for examples, (de)phosphorylation, interaction with various natural protein inhibitors [3]. CDK2 can be negatively regulated by phosphorylation on Tyr15 and to a lesser extent on Thr14 [4].

This work describes behavior of monomeric CDK2/ATP, CDK2/cyclinA/ATP complex, and pT160-CDK2/cyclinA/ATP complex (CDK2/cyclinA/ ATP complex phosphorylated on Thr160 residue of CDK2) using the molecular dynamics simulations with the Cornell et al. force field as implemented in the AMBER software package [5]. The next MD study was performed on pY15,pT160-CDK2/cyclinA/ATP system. The system was prepared from pT160-CDK2/cyclinA/ATP by phosphorylation of the Tyr15 residue of CDK2. Results of conformational behavior of ATP and key residues for activation in these complexes will be presented. Activation of CDK2 involves various conformational changes, including the reorientation of the phosphate part of ATP and key residues involved in ATP binding site. Transformation of conformation of ATP phosphate in the pT160-CDK2/cyclinA complex is important to form substrate binding site, and is thought to be critical for catalysis.

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MOLECULAR DYNAMICS SIMULATIONS OF DNA TRIPLEXES CONTAINING MODIFIED HOOGSTEEN STRANDS - POTENTIAL CANDIDATES FOR ANTIGENE THERAPY

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The concept of "antisense" and "antigene" nucleic acids represents a perspective approach in chemotherapy, promising to inhibit selectively unwanted gene expression by creation of a helical complex with target mRNA or DNA (carrying "sense" genetic information) [1]. The oligonucleotides with natural chemical composition have been, however, found as unsuitable for in vivo applications because of their insufficient resistance against nucleases. That is why numerous novel-type nucleotide analogs are designed, synthesized and tested [1-6].

A number of phosphonate-based mononucleotide analogs containing an O-(phosphono)methyl group instead of the natural phosphonomonoester one were found to be potent antivirals: this indicated enzyme stability of the phosphonate -O-P-CH_2-O- bond [7]. Several types of isopolar modified oligothymidylates and oligoadenylates (15 mers) with the phosphonate -O-P-CH_2-O- internucleotide linkage were prepared. The modified oligonucleotides were subjected to the study of their hybridization properties, resistance against nucleases, and the ability to elicit RNase H activity [2]. Impact of the internucleoside linkage modification by inserting a methylene group on the ability of the modified oligonucleotide to hybridize with a natural DNA and RNA strand was studied by fully solvated molecular dynamics (MD) simulations [3-6].

Triplex forming oligodeoxynucleotides have attracted a great deal of attention because of their potential use in gene therapy. In inter molecular triplexes, third strand of ODN binds to the major groove of the DNA. However, in general, the binding of a third-strand ODN to a target DNA duplex is thermodynamically weaker than duplex formation itself. Thus much effort has been made to increase the affinity of the third strand for its target. ODN analogues carrying various aminoalkyl linkers have been synthesized, some of which have been shown to increase the thermal stability of triplexes [8]. The thermal stabilization can be explained by an electrostatic interaction between the positively charged aminoalkyl residue of the nucleosides and a pro-R oxygen of a negatively charged phosphate at the second strand of the target DNA.

The present work deals with the phosphonate analog of the natural phosphodiester internucleoside linkage in conjunction with various aminoalkyl-linkers. Several triple helical structures consisting of a natural Watson-Crick duplex and a modified Hoogsteen thymidine strand were used as model systems. Impact of the sugar phosphate backbone modifications on the ability of the modified oligonucleotides to hybridize with a nautral duplex, was studied by molecular dynamics simulations. The nucleic acids were surrounded by a periodic box of ~10000 TIP3P water atoms. Fully solvated trajectories were computed using the AMBER 5.0 software package. The implemented