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

force field doesn't contain force constants needed to describe the modified parts of the phosphonate analogs [9]. The completion was made on the base of ab initio calculations [3].

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FUNCTIONAL RECONSTITUTION OF PHOTOSYSTEM 2 INTO LIPOSOMES

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Most recent structural data on photosystem 2 (PS2), the first membrane protein complex in the photosynthetic electron transport chain, confirm that this complex exists as a functional dimer in the thylakoid membrane of cyanobacteria [1, 2]. Besides the membrane embedded part of this dimer with dimensions of 190 A x 100 A x 40 A, this complex also extends about 10 Ĺ out of the membrane in the stromal region and 55 A in the lumen; the latter is referred to as the oxygen evolving complex, harbouring the water-splitting site. Developing an approriate method to reconstitute dimeric PS2 into liposomes should finally help to answer the fundamental question concerning its structure-based function: Is a dimeric structure a prerequisite for optimal watersplitting activity (monomeric complexes, solubilized by detergent, are active, too, although at a lower level) and which is the impact of the lipid-phase-composition on the water-splitting activity? Here we present data on the orientation of reconstituted dimeric PS2 from the cyanobacterium *Thermosynechococcus elongatus* and also give indications for its activity within the liposomes and monomer-dimer distribution (by EM analysis).

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ELECTRON MICROSCOPY AND SINGLE PARTICLE ANALYSIS OF PHOTOSYSTEM II FROM RED ALGA *PORPHYRIDIUM CRUENTUM*

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Photosystem II (PSII) is a multisubunit pigment-protein complex embedded in the thylakoid membranes of higher plants, algae and cyanobacteria [1-3]. It performs series of photochemical reactions resulting in the reduction of plastoquinone, the oxidation of water, and the formation of a transmembrane pH gradient. The essential components of the PSII complex are intrinsic membrane proteins that are almost identical between cyanobacteria and higher plants: they include the D1 and D2 reaction center proteins, chlorophyll *a*-binding proteins CP47 and CP43, and subunits of cytochrome *b-559* (cyt *b-559*) and several low-molecular weight proteins with unknown functions [3, 4].

In addition, there are extrinsic proteins associated with PSII, which play important roles in maintaining the function and stability of the oxygen-evolving complex [5]. As both cyanobacteria and higher plants contain 33-kDa extrinsic subunit they differ in composition of the other lumenal subunits. While higher plants and green algae contain the 23 and 16 kDa extrinsic subunits, in cyanobacteria, these proteins are replaced by another two proteins coded by *psbU* and *psbV* gene (cyt c_{550} and 12-kDa subunit) [6]. Red algal PSII complex has a 20 kDa protein in addition to the three cyanobacterial proteins [7]. Recently, structure of the PSII complex isolated from two cyanobacterial strains has been presented [8,9]. These models give the idea of the