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Research article

Dynamic conformational ensembles regulate casein kinase-1 isoforms: Insights from molecular dynamics and molecular docking studies

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ABSTRACT

Casein kinase-1 (CK1) isoforms actively participate in the down-regulation of canonical Wnt signaling pathway; however recent studies have shown their active roles in oncogenesis of various tissues through this pathway. Functional loss of two isoforms (CK1-α/ε) has been shown to activate the carcinogenic pathway which involves the stabilization of of cytoplasmic β-catenin. Development of anticancer therapeutics is very laborious task and depends upon the structural and conformational details of the target. This study focuses on, how the structural dynamics and conformational changes of two CK1 isoforms are synchronized in carcinogenic pathway. The conformational dynamics in kinases is the responsible for their action as has been supported by the molecular docking experiments.

1. Introduction

The role of Wnt signaling pathway in carcinogenesis of various origins (colon, ovarian, prostate *etc.*), with temporal deregulations (or mutations) of different components, has recently been documented (Klaus and Birchmeier, 2008). One of the key regulators of the pathway is β-catenin; which is strictly regulated by glycogen synthase kinase-3β (GSK-3β). The presence of Wnt factors (or deregulatory signals) stabilizes β-catenin in the cytosol; the stable β-catenin translocates to nucleus and regulates the transcription of TCF/LEF-induced genes ultimately resulting in malignancy (Polakis, 2000).

In humans, CK1 constitutes a family of serine/threonine protein kinases (*viz.* α, γ1, γ2, γ3, δ and ε isoforms) which participate in various metabolic pathways (Amit et al., 2002; Long et al., 2012a). Recently, they have been studied as potential drug targets for the development of anticancer therapeutics (Long et al., 2012b). Among the six isoforms of CK1 at least two (*i.e.* α and ε) have been reported for their roles in cell survival and carcinogenesis via direct or indirect stabilization of β-catenin in the Wnt signaling pathway (Knippschild et al., 2005). The biological activity of CK1 depends on its special catalytic site which recognizes -Ser(p) XXSer/Thr- motif to phosphorylate target protein (Xu et al., 1995).

The coordinated action of CK1α and ε, over lipoprotein receptor-related protein 5 and 6 (LRP5/6), nucleates a binding site for GSK-3β at C-terminal domain of LRP5/6. The receptor bound GSK-3β is incapable for inhibitory phosphorylation of β-catenin; as an effect of this the half-life of β-catenin is increased (del Valle-Perez et al., 2011). Recently, Brockschmidt et al. (2008) and Rosenberg et al. (2013) have also shown highly increased level of CK1-ε in pancreatic and breast cancer cell respectively.

The proteins are the soft materials and its flexibility is critical for the biological functions. Molecular dynamics (MD) simulation is a standard computational tool to access the flexibility of biological molecules (Karplus and Petsko, 1990; Karplus and McCammon, 2002). The conformational selection model (Monod et al., 1965; Meireles et al., 2011) explains that all the conformations of proteins pre-exist in dynamical equilibrium prior to the ligand binding. Once the lingand binds to a conformation, and stabilizes it, shifting the equilibrium toward bound state conformations. Here, we present the comparative dynamic etiquette of two CK1 isoforms (α and ε) under physiological condition in an aqueous environment. Moreover, we also analyzed the conformational substates sampled during the dynamics. In order to substantiate the role of each substate, we performed molecular docking of several conformations from each substate of each isoform with ATP and ADP.

2. Materials and methodology

2.1. Protein structures and molecular systems

The native tridimensional structure of human CK1-α is unavailable in Protein Data Bank (PDB); however, that for CK1-ε

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it is found (id: 4HOK at 2.77 Å). The 3D structure of CK1-α was built by homology modeling using its sequence (UniProt id: P48729), and a template structure of apo form of CK1-δ (PDB id: 3UYS at 2.3 Å). The CK1-ε structure obtained from PDB is incomplete therefore, the missing residues were repaired using model refinement/loop modeling tool of Chimera 1.8.1. All the homology modeling and model repair experiments were performed by Modeller-9.10 (Sali and Blundell, 1993; Eswar et al., 2006). The quality of each newly modeled structure was estimated on the basis of Ramachandran plot (Laskowski et al., 1993) and *Z*-score (Sippl, 1993) analytics. To avoid any ambiguity and for simplicity these modeled structures are renamed as *alpha* and *epsilon* for α- and ε- isoforms respectively.

The united atom GROMOS 53a6 force field was used for preparing the topologies of each system using pdb2gmx tool of Gromacs 4.5.5 package (Oostenbrink et al., 2004; van der Spoel et al., 2005; Hess et al., 2008). Two proteins were kept in the center of cubic boxes with the minimum distance of 10 Å between wall and any atom of the protein; then each box was filled with extended simple point charge (SPC/E) water molecules (Berendsen et al., 1987; Mark and Nilsson, 2001). The systems were neutralized by adding appropriate numbers of sodium and chloride ions. Further, to pacify each system, energy minimization was performed with non-bonded cutoff of 9.0 Å for five thousand cycles of steepest descent without any restraints to the systems. Table 1 lists details of each system.

2.2. MD simulation

The MD simulations were performed using Gromacs 4.5.5. Ahead of the production dynamics, each system was equilibrated in two phases for 1 ns (nanosecond) with integration time step of 2 fs (femtoseconds). The first phase of equilibration was 100 ps (picoseconds) dynamics, to raise the system's temperature by 300 K, at NVT ensemble; the Berendsen thermostat (Berendsen et al., 1984) was used to maintain the constant temperature. During NVT ensemble the initial velocities were assigned from the Maxwell's distribution of temperature. The second equilibration step was also a dynamics of 900 ps maintaining homogeneous density in the system using NPT ensemble; the constant pressure of the system was maintained by Parrinello–Rahman barostat (Parrinello and Rahman, 1981). Linear Constraint Solver (LINCS) (Hess et al., 1997) algorithm was applied to preserve the length of all bonds. The long-range electrostatic interactions were treated by particle mesh Ewald (PME) (Darden et al., 1993) method with non-bonded cut-off distance of 9 Å, under periodic boundary conditions. Two systems were passed to production run, computing 30 ns (nanoseconds) trajectories under NPT ensemble.

2.2.1. Trajectory analysis

Production simulation of each system generated trajectories of 20 ns, each with snapshot at 2 ps intervals. It was ensured that each trajectory obeyed NPT ensemble during entire simulation period on the basis of variations in energies (potential, kinetic and total energy), as well as the temperature, pressure and density as function of time. Gromacs 4.5.5 analysis tools were used for the analysis of trajectories. The flexibility of the proteins was estimated on the basis of root mean square deviation (RMSD) of backbone atoms with reference to the respective initial structures and root mean square fluctuation (RMSF) of *alpha* carbon atoms (Cα) of each protein. The temporal changes in accessible surface area (SASA) and radius of gyration (*Rg*) were computed by g sas and g gyrate tools respectively.

2.3. Principal component analysis

Principal component analysis (PCA) was executed for both trajectories to study the collective motions in CK1 isoforms (Hayward and Go, 1995; Stein et al., 2006a; Hayward and de Groot, 2008; Amadei et al., 1993; Amadei et al., 1996). For all the PCA analyses g covar and g anaeig tools were used. The covariance matrices were constructed from the backbone atoms. Snapshots at every 2 ps were collected for visual inspection of concerted motions corresponding to each eigenvector in both molecules. Projections of eigenvectors to their respective trajectory, also known as principal components (PC), were obtained for essential dynamics sampling. For both trajectories it was observed that only first few PCs contribute significantly to the internal motions. Moreover, 1st and 3rd eigenvectors were selected for essential dynamics sampling since these eigenvectors comprised highest proportion of concerted motions throughout the molecules whereas the second eigenvector showed only jiggling of the C-terminus while the rest of the protein remained almost stable.

The free energy landscapes (FEL) were obtained by computing the probability distribution from the essential plane composed of first two eigenvectors. Free energies ΔG were calculated according to:

$$\Delta G = -k_B T \ln P(r)$$

where k_B is Boltzmann constant, T is absolute temperature, and P is probability distribution along coordinate r.

2.4. Molecular docking

We performed molecular docking for conformations at different time-frames of trajectories. A total of 38 conformations (nineteen for each) at different time points (*i.e.* from 2 to 20 ns at interval of 1 ns) were isolated from both trajectories. Each conformation was used for blind docking with ATP and ADP. All the input files for docking experiments were prepared by AutoDockTool 1.5.6 (Sanner, 1999) and the binding affinity, for each conformation with ATP as well as with ADP, were computed by AutoDock Vina 1.1.2 (Trott and Olson, 2010). The grid size (at the grid spacing of 1 Å) was adjusted for every round of docking study in such a way that it could cover the entire receptor. The exhaustiveness of global search was kept 20.

3. Result and discussions

3.1. Molecular structures

The homology modeling based generated inactive structure of *alpha* as well as repaired structure of *epsilon* were first subjected to the quality assessment. The Ramachandran plot analysis for both structures revealed the excellent distribution of amino acids over the allowed regions of the map (Supp. Fig. 1(a)). All amino acids of both structures were distributed over allowed and partially allowed regions. The *Z*-Scores were also excellent for both,

Table 1
Details of the systems used in molecular dynamics simulations.

Name	Source	No. of Residues	Volume (m^3)	Density (Kg/m^3)	Number of Na^+ and Cl^-
alpha	SwissProt id P48729	290	5.35172×10^{-25}	984.374	32 and 47
epsilon	PDB id 4HOK	296	6.65850×10^{-25}	1028.140	40 and 54