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A comparative study of structural and conformational properties of casein kinase-1 isoforms: Insights from molecular dynamics and principal component analysis

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HIGHLIGHTS

- *In silico* modeling of CK1 isoforms and their MD simulation.
- The conformational substates are explored to explain dynamical nature of proteins.
- PCA and potential energy surfaces of conformational subspaces are presented.

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ABSTRACT

Wnt signaling pathway regulates several developmental processes in human; however recently this pathway has been associated with development of different types of cancers. Casein kinase-1 (CK1) constitutes a family of serine–threonine protein kinase; various members of this family participate in *Wnt* signal transduction pathway and serve as molecular switch to this pathway. Among the known six isoforms of CK1, in human, at least three isoforms (*viz.* alpha, delta and epsilon) have been reported as oncogenic. The development of common therapeutics against these kinases is an arduous task; unless we have the detailed information of their tertiary structures and conformational properties. In the present work, the dynamical and conformational properties for each of three isoforms of CK1 are explored through molecular dynamics (MD) simulations. The conformational space distribution of backbone atoms is evaluated using principal component analysis of MD data, which are further validated on the basis of potential energy surface. Based on these analytics, it is suggested that conformational subspace shifts upon binding to ligands and guides the kinase action of CK1 isoforms. Further, this paper as a first effort to concurrent study of all the three isoforms of CK1 provides structural basis for development of common anticancer therapeutics against three isoforms of CK1.

1. Introduction

Wnt signaling pathway is well studied signaling for its role in early embryonic development of fruit fly, *Caenorhabditis elegans*, mouse and human. Moreover, this pathway has also been documented to play a major role in development of cancers of various origin (colon, ovarian, prostate, *etc.*), with temporally deregulated (or mutant) expression of components of *Wnt* pathway (Klaus and Birchmeier, 2008). One of the important players in *Wnt* signaling pathway is β-catenin which is under strict regulation of glycogen synthase kinase-3β (GSK-3β). The phosphorylated β-catenin is targeted for proteasomal degradation *via* ubiquitination. The presence of a *Wnt* factors (or a deregulatory signal) β-catenin degradation is inhibited and results in elevated level of cytosolic β-catenin. Once stable in the cytosol, β-catenin, translocates to the nucleus where it engages the transcription factors such as TCF and LEF. In human, transcriptions induced by TCF4 and LEF1 ultimately results in malignancy (Polakis, 2000; Jiang et al., 2013; Chen et al., 2010; Huang et al., 2011).

Casein kinase 1 (CK1) is an important family of highly conserved serine/threonine protein kinases among all the eukaryotes. Recently, CK1 has been studied as an attractive drug target for the development of anticancer therapeutics (Long et al., 2012a, 2012b).

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At least six isoforms of CK1 have been reported in human (i.e. α, γ1, γ2, γ3, δ and ε), which regulate several biological processes, including signaling pathways, circadian rhythms, RNA and DNA metabolisms, *etc.* (Amit et al., 2002; Etchegaray et al., 2009). At least three (i.e. α, δ, and ε) out of six have been reported for their key role in cell survival and carcinogenesis *via* direct or indirect stabilization of β-catenin in the *Wnt* signaling pathway (Knippschild et al., 2005). The biological function of CK1 isoforms depends on their 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; thus the receptor bound GSK-3β is unable for inhibitory phosphorylation of β-catenin which causes increased half-life of β-catenin (del Valle-Perez et al., 2011). Recently, Brockschmidt et al. (2008) and Rosenberg et al. (2013) have also shown highly increased level of CK1 δ/ε in pancreatic and breast cancer cell respectively. In all these studies a remarkably high level of nuclear β-catenin has been reported pointing towards an active role of different CK1 isoforms in carcinogenesis, through *Wnt*-signaling pathway.

The biological function of proteins directly depends upon their flexibility (McCammon, 1984; Henzler-Wildman and Kern, 2007). A crystal structure of protein represents its static average under a set of conditions, the proteins with dynamic personalities, thus the conformational changes observed during dynamics provide detailed structural as well as functional information. MD simulation is a standard computational method and is routinely used to access the molecular flexibility based upon the knowledge of the interaction potential for the particles (Karplus and Petsko, 1990; Karplus and McCammon, 2002). Here, we present a comparative study of three isoforms (α, δ and ε) of human CK1 with a particular emphasis on their dynamic etiquette under physiological condition in aqueous medium. Moreover, principal component analysis (PCA) of MD data was performed to reveal the conformational substates sampled during the dynamics of these kinases (Hayward and Go, 1995; Stein et al., 2006; Hayward and de Groot, 2008; Du et al., 2006; Li et al., 2009). Potential energy surfaces (PES), a contour map of potential energy as a function of conformational coordinates, were employed to confirm the conformational substates (Frauenfelder et al., 1991).

2. Materials and methodology

2.1. Protein structures

The native tertiary structure of human CK1-α was not found in Protein Data Bank (PDB), however, apoenzyme structures for δ and ε isoforms are available but incomplete (PDB code: 3UYS at 2.3 Å and 4HOK at 2.77 Å, CK1-δ and CK1-ε respectively). Starting from the sequence of CK1 α, (UniProt id- P48729), homology modeling was performed using a template of CK1-ε (PDB code: 4HOK at 2.77 Å) and the incomplete crystal structures of δ and ε isoforms were repaired using model refinement/loop modeling tool of Chimera 1.8.1. Modeler-9.10 (Sali and Blundell, 1993; Eswar et al., 2006) was used for all protein model construction experiments. Further, the quality of all the structures was estimated on the basis of Ramachandran plot and *Z*-score analytics. In order to avoid any ambiguity, these newly built structures were named as aCK1-M, dCK1-M and eCK1-M for CK1- α, -δ and -ε respectively.

2.2. Molecular systems

The pdb2gmx tool of Gromacs 4.5.5 package (van der Spoel et al., 2005; Hess et al., 2008) was used to prepare the systems with GROMOS 53a6 force field (Oostenbrink et al., 2004). Finally, each molecule was placed in the center of a cubic box with a minimum distance of 10 Å between its wall and any atom of the protein; then boxes were filled with extended simple point charge (SPC/E) water molecules (Berendsen et al., 1987; Mark and Nilsson, 2001). In order to maintain the physiological condition, *i.e.* to neutralize the total charge, appropriate numbers of sodium (Na^+) and chloride (Cl^-) ions were added to each system. Further, to pacify each of the three systems, energy minimization was performed with non-bonded cutoff of 9.0 Å. A total of five thousand cycles of steepest descent were carried out without any restraints to the system. The details of each system, used in molecular dynamics simulations, are tabulated in Table 1.

2.2.1. MD simulation

Systems were equilibrated in two steps, ahead of the production dynamics, of one nanosecond (ns) simulation time with time step of 2 fs (femtoseconds). The first phase of equilibration is a heating step of 100 ps (picoseconds), under canonical ensemble (NVT), using Berendsen thermostat (Berendsen et al., 1984) with temperature coupling time of 0.1 ps. This raised system's temperature to 300 K. Moreover, during NVT ensemble the initial velocities were assigned from the Maxwell's distribution of temperature. The second equilibration step is for remaining 900 ps of equilibration period, under isothermal–isobaric (NPT) ensemble. The Parrinello–Rahman barostat (Parrinello and Rahman, 1981), with pressure coupling time of 0.1 ps, was applied to maintain homogeneous pressure throughout systems. Linear Constraint Solver (LINCS) (Hess et al., 1997) algorithm was applied to preserve the length of all bonds and the long-range electrostatic interactions were treated by the particle mesh Ewald (PME) (Darden et al., 1993) method with the non-bonded cut-off distance of 9 Å, under periodic boundary conditions. The well equilibrated systems were then passed, to production run under NPT ensembl, for computing trajectories of 20 ns (nanoseconds). Snapshots at every 2 ps intervals were collected. All simulations were run on a Linux machine with an Intel Core-i5 processor.

2.2.2. Trajectory analysis

Gromacs 4.5.5 analysis tools were used for the analysis of trajectories, beginning with their stability evaluation. In order to ensure that all the three systems obeyed NPT ensemble throughout simulation period, variations in energies (potential, kinetic and total energy), temperature, pressure and density of systems were calculated. To explore the molecular plasticity, in three isoforms of CK1, root mean square deviation (RMSD) and root mean square fluctuation (RMSF) were estimated. The significant changes in elements of protein secondary structure, during evolution each

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

Name	Source	No. of residues	Total charge on protein	Volume (m^3)	Density (kg/m^3)	Number of Na^+ and Cl^-
aCK1-M	SwissProt id P48729	290	+15	5.35172×10^{-25}	984.374	32 and 47
dCK1-M	PDB id 3UYS	296	+13	6.39499×10^{-25}	1002.300	38 and 51
eCK1-M	PDB id 4HOK	296	+14	6.65850×10^{-25}	1028.140	40 and 54

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