International Journal of Computational Bioinformatics and In Silico Modeling
Vol. 2, No. 3 (2013): 110-114
Research Article
Open Access

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ISSN: 2320-0634

Insights from the analysis of phosphorylation sites in the CK2 alpha catalytic subunit

Priyanka Dhar[1]*, Abhijit Datta[2] and Ravi B. Srivastava[1]

[1] *Defence Institute of High Altitude Research, Defence Research and Development Organisation, Leh-Ladakh, C/o 56 APO, Pin 901205, India;*
[2] *DBT Centre for Bioinformatics, Presidency University, Kolkata 700073, India.*

*Corresponding author: Priyanka Dhar, e-mail: priyankodrdo@gmail.com

Received: 08 April 2013 Accepted: 22 April 2013 Online: 01 May 2013

ABSTRACT

Protein kinase CK2 is a critical regulator of several cellular and molecular signaling pathways. CK2 is unanimously distributed in eukaryotes and subsists in tetrameric complexes comprising two catalytic alpha and two regulatory beta subunits. Several reports have confirmed that protein kinase CK2 has great significance in diverse biological courses, particularly in cellular growth and proliferation in normal and disease conditions. However, *in silico* analysis of phosphorylation sites in the catalytic alpha subunit of CK2 remains to be elucidated. We described the distribution of predicted (neural network predictions for serine (S), threonine (T) and tyrosine(Y)) STY phosphorylation sites in CK2 alpha subunit of 15 multicellular and 2 unicellular organisms. We also showed the clustering of CK2 alpha subunit in these organisms using a phylogram. This data showed the prevalence of CK2 alpha subunits with potential STY phophorylation sites in several eukaryotic unicellular and mulicellular organisms that could be significant in the context of enigmatic protein kinase activities of CK2, enzyme-substrate interactions, further ligand-binding studies and new therapeutic interventions.

Keywords: CK2 alpha subunit, protein phosphorylation sites, phylogeny

INTRODUCTION

The protein kinase activity was revealed by Burnett and Kennedy in 1954 after the discovery of protein phosphorylation [1-3]. The kinase activity was down to the unusual protein kinases, called casein kinases. It is also referred to as casein kinase I (CK1) and casein kinase II (CK2). The physiological role of CK2 remains enigmatic [4]. It plays crucial role in universal processes such as synthesis of rRNA and tRNA [5], cell survival [6], apoptosis [7], transformation [8] and other cellular processes [9-14] with an enormous number of substrate specificity [15].

The regulations of most fundamental cellular processes are predominantly dependent on the reversible phosphorylation of proteins [16, 17]. The human genome encodes several hundred distinct protein kinases [18, 19], that one third of all cellular proteins appear to be phosphorylated at numerous distinct sites [20, 21]. Each protein kinase may phosphorylate 10-12 proteins within a cell in general. However, some protein kinases, are exquisitely specific and phosphorylate plausibly only one or two diverse protein targets. In contrast, several other protein kinases display a large range of specificity and can phosphorylate hundreds of distinct proteins within cells. Protein kinase CK2 represents a small family of closely linked protein kinases that corresponds to the second class of kinases [6, 10, 11, 13, 14, 22-25]. The structural, regulatory and enzymic characteristic of CK2 and its role in cell survival and death has been studied extensively [12]. In eukaryotic organisms, protein kinase CK2 is distributed universally where it often exists in tetrameric complexes consisting of two catalytic alpha subunits having the kinase domain and two regulatory beta subunits. Distinct isoenzymic forms of the catalytic subunit of CK2 have been also recognized in various organisms, [26-30]. The crystal structure of human protein kinase CK2 and the properties of the CK2 holoenzyme were reported [31]. CK2 is also known to possess dual specific kinase activity [32, 33]. A large number of substrates are

phosphorylated by CK2 including Cdc2, BRCA1 and p53 that play crucial role in cell cycle regulation [34-36]. Reticence of CK2 activity might result in altered cell cycle progression [37-39]. The aim of the present investigation was to identify the distribution of predicted (neural network predictions for serine (S), threonine (T) and tyrosine(Y)) STY phosphorylation sites in CK2 alpha subunit of 15 multicellular and 2 unicellular organisms. We also showed the phylogenetic evolutionary relationship of CK2 alpha subunit from different organisms.

MATERIALS AND METHODS

Dataset

CK2 alpha subunit protein sequences were obtained from GenBank (release 184.0, June, 2011) [40]. The dataset consists of CK2 from 15 multicellular and 2 unicellular organisms (Table 1).

Table 1. CK2 alpha subunit sequence dataset from GenBank (release 184.0, June, 2011).

GenBank accession	Organisms
914049|gb|AAB34248.1|	*Danio rerio*
162463354|ref|NP_001105632.1|	*Zea mays*
23092648|gb|AAN11415.1|	*Drosophila melanogaster*
415716|gb|AAA74462.1|	*Rattus norvegicus*
55977123|sp|P68400.1|	*Homo sapiens*
14532298|gb|AAK66566.1|	*Trypanosoma brucei*
14150749|gb|AAK54616.1|	*Nicotiana tabacum*
151943124|gb|EDN61459.1|	*Saccharomyces cerevisiae*
32400946|gb|AAP80679.1|	*Lilium davidii*
3413816|emb|CAA04753.1|	*Mus musculus*
192910694|gb|ACF06455.1|	*Elaeis guineensis*
88319941|ref|NP_777060.2|	*Bos taurus*
50355952|ref|NP_001002242.1|	*Gallus gallus*
112983288|ref|NP_001036956.1|	*Bombyx mori*
20336346|gb|AAM18184.1|	*Ciona intestinalis*
12697581|dbj|BAB21591.1|	*Oryza sativa* Indica
12697575|dbj|BAB21588.1|	*Oryza sativa* Japonica

Phosphorylation sites

Phosphorylation sites in CK2 alpha subunit were located using the NetPhos 2.0 server. It is a prediction server for predicting probable phosphorylation sites at serine, threonine and tyrosine residues in protein by an artificial neural network method in independent sequences with 69-96% range of sensitivity [41, 42].

Phylogeny

Phylogenetic analysis of CK2 alpha subunit obtained from the 17 organisms was performed. Multiple sequence alignments were performed using EBI ClustalW2 [43, 44]. It is a general purpose multiple sequence alignment program for DNA or proteins that attempts to calculate the preeminent match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. First, the protein sequences in FASTA format was submitted to ClustalW. Slow, but accurate alignment method was used to perform the pairwise alignments to generate the guide tree. The Gonnet Multiple alignment protein sequence comparison matrix series was used to score the alignment. Gap open, Gap extension and Gap distances were 10, 0.20 and 5 respectively. No End Gaps, Iteration and Num Iter were of the default values. The clustering was performed by Neighbour-joining (NJ) method and ClustalW alignment format with residue numbering (Aln w/numbers) to generate the multiple sequence alignment. The order in which the sequences appear in the final alignment was determined by the guide tree.

RESULTS AND DISCUSSION

Several phosphorylation sites were detected in the CK2 alpha subunit indicative of the fact that it may also be regulated by the mechanism of protein phosphorylation. The three most abundant residues that are most frequently phosphorylated were serine, tyrosine and threonine. Serine phosphorylation sites were found to be highest (with 9 serine phophorylation sites) in *Gallus gallus* and *Cioana invtestivalis* where as it was lowest (with 1 serine phophorylation site) in *Oryza sativa* Japonica group. Phosphorylation sites at the threonine residue was highest (with 6 threonine phophorylation sites) in *Gallus gallus* and it was found to be lowest (with 0 serine phophorylation site) in *Oryza sativa* Japonica group. The tyrosine phosphorylation sites were highest (with 9 tyrosine phophorylation sites) in *Lilium davidii* and it was lowest (with 3 tyrosine phophorylation sites) in *Nicotiana tabacum* and *Oryza sativa* Japonica group. A total of 21 (maximum) phosphorylation sites was detected in *Gallus gallus* and only 4 (minimum) phosphorylation sites was detected in *Oryza sativa* Japonica group (Figure 1).

The phylogram generated by the multiple sequence alignment (EBI, ClustalW 2) showed that the alpha subunit of CK2 in *Homo sapiens* & *Bos taurus*; *Oryza sativa* Indica & *Oryza sativa* Japonica; *Trypanosoma brucei* & *Saccharomyces cerevisiae*; *Drosophila melanogaster* & *Bombyx mori* were originated from the same node (Figure 2).

Protein phosphorylation regulates the function of proteins by phosphoregulation which is one of the most important determinants of signaling systems. It is an extremely complex procedure on a proteome-wide scale due to a great number of modifying proteins, a large number of sites on substrate proteins that are modified by these enzymes, and the vibrant nature of

protein expression during different cellular programs. The critical biological processes are regulated by many of these phosphorylation sites and may provide evidence for diagnostic or therapeutic targets for molecular medicine. Hence, the identification and detection of phosphorylation sites on a wide diversity of cellular proteins are remarkably essential to understand the signaling proteins and pathways concerned in disease state progression. Thousands of novel *in vivo* phosphorylation sites are detected by the mass spectrometry. In the last two decades, researchers have developed several tools for the experimental and computational identification of sequence and structural motifs which are responsible for encoding the kinase-substrate interaction residues and the phosphorylated amino acid itself [45]. The results obtained from the proteomic analysis revealed that the most abundant phosphorylation site in the CK2 alpha subunit was at the serine residue with over 100 phosphorylation sites. The protein from *Oryza sativa* Japonica was found to possess the least number of phosphorylation sites while the largest number of phosphorylation sites was identified in *Gallus gallus*.

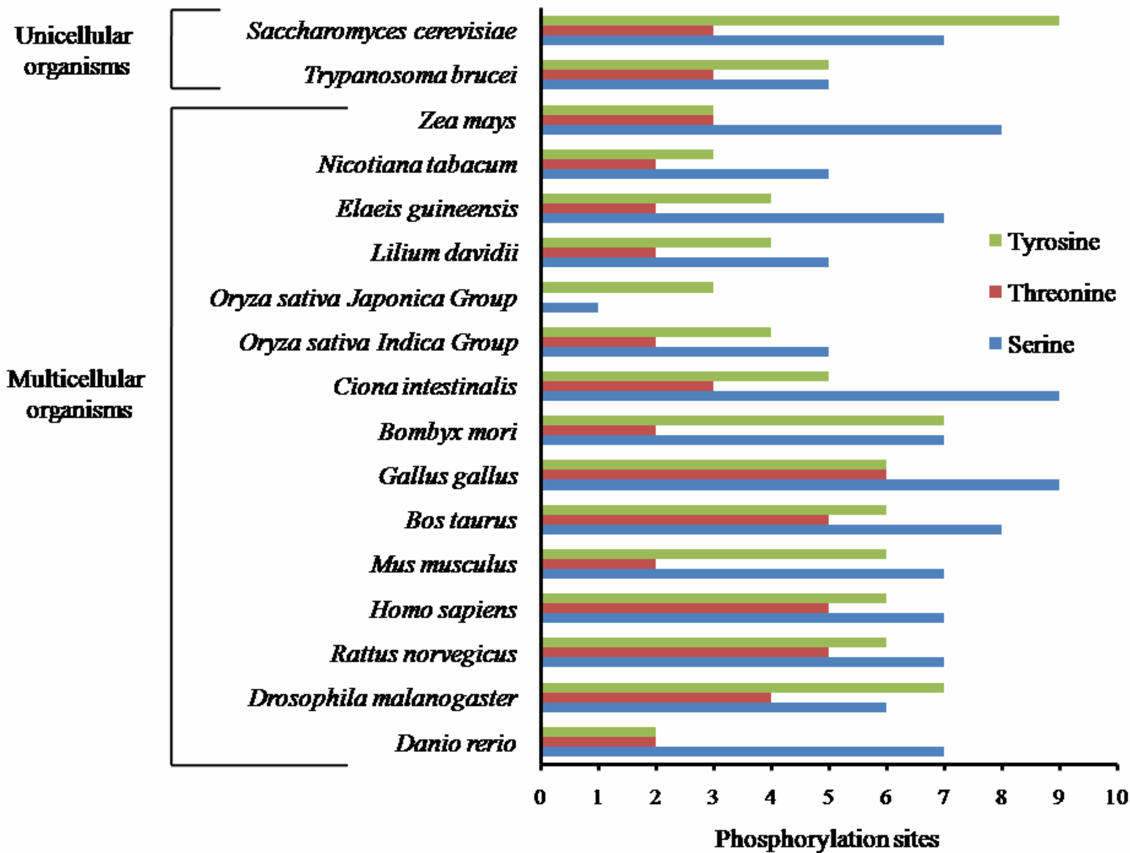

Figure 1. Distribution of phosphorylation sites (STY) in the CK2 alpha subunit in different eukaryotic species. Most abundant phosphorylation site in the CK2 alpha subunit was at the serine residue with 110 phosphorylation sites. Phosphorylation sites at threonine and tyrosine residues were found to be 51 and 86 respectively in all the 17 species. The phosphorylation sites are found to be prevalent in the animal group that the plant kingdom.

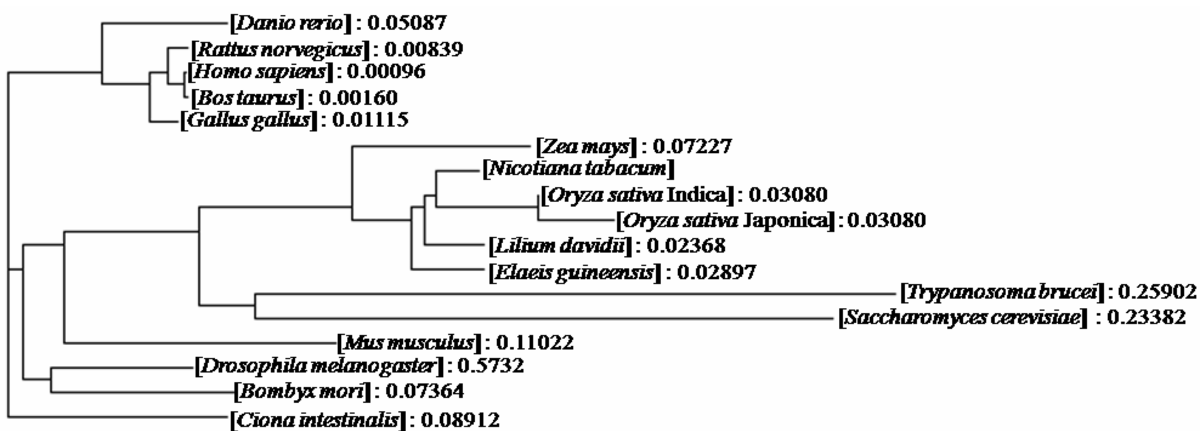

Figure 2. Phylogenetic tree depicting evolutionary relationships of CK2 alpha subunit between different organisms.

Phylogenetic analysis by multiple sequence alignment showed that the clusters of alpha subunit of CK2 from different organisms found to have strong homology and they also possess protein phosphorylation (serine/threonine kinase activity), ATP binding and nucleotide binding properties.

Experimental detection of protein phosphorylation sites is an arduous process and the prediction of phosphorylation sites using the primary sequences of a protein could be a useful alternative to provide guiding principle for further experimental design and elucidation of phosphoproteomic informations. The present study revealed the diverse phosphorylation sites (STY) in catalytic CK2 alpha subunit of different organisms with ATP and nucleotide binding properties that could be significant in the context of enigmatic protein kinase activities of CK2, enzyme-substrate interactions, further ligand-binding studies and new therapeutic interventions which will usher new era of cellular and molecular process regulation.

ACKNOWLEDGEMENTS

The authors are thankful to Defence Research & Development Organisation for supporting the research.

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