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INSIGHT FROM THE ACTIVE SITE PREDICTION OF THE HUMAN RAC-ALPHA SERINE/THREONINE PROTEIN KINASE MODEL.

VIDYANATH CHAUDHARY*[1], SHAKILA AFROZ[2], KAILASH SINGH[1] AND VINEET KUMAR MISHRA[1]

[1]Department of Biotechnology, Faculty of Science, Jamia Hamdard, New Delhi, India
[2]Department of Biochemistry and Biotechnology, Faculty of Basic Medical and Pharmaceutical Science, University of Science and Technology (USTC) Chittagong, Bangladesh

ABSTRACT

RAC-alpha serine/threonine-protein kinase is an enzyme encoded by AKT1 gene in human. The activation inhibits the phenomenon of apoptosis, one of the major causes for promoting cancer. In experiment with mice lacking Akt1, they become resistant to cancer. There is no sufficient information regarding RAC-alpha serine/threonine-protein kinase in Homo sapiens. Therefore, we describe the development of RAC-alpha serine/threonine-protein kinase model in human. We further document the predicted active sites in the structural model with solvent exposed ASA residues. During this study, the model was built by CPH program and validated through PROCHECK, Verify 3D, ERRAT and ProSA for reliability. The active sites were predicted in the model with further ASA analysis of active site residues. The discussed information thus provides insight to the predicted active site of RAC-alpha serine/threonine-protein kinase in human.

KEYWORDS: **Homology Modeling, Active Site, Ligand Binding, ASA**

VIDYANATH CHAUDHARY
Department of Biotechnology, Faculty of Science, Jamia Hamdard, New Delhi, India

Corresponding author

INTRODUCTION

RAC-alpha serine/threonine-protein kinase, one of the members of protein kinase family, is an enzyme [EC: 2.7.11.1] in human encoded by AKT1 gene. This protein kinase possess a catalytic subunit which transfers the gamma phosphate to one or more amino acid residues in a protein substrate side chain from nucleotide triphosphates (often ATP), resulting in a conformational change in their structure altering protein function [1]. The activation of this protein kinase occurs through phosphatidylinositol 3-kinase [2] and is abrogated by mutations in the pleckstrin homology domain of AKT1 [3]. The activation of serine/threonine kinase AKT1 inhibit the phenomenon of apoptosis by phosphorylating and inactivating components of the apoptotic machinery, one of the major causes for promoting cancer. The serine-threonine protein kinase AKT1 is on inactive state in serum-starved primary and immortalized fibroblasts [2]. In experiment with mice lacking Akt1, the mice display a 25% reduction in body mass, indicating that Akt1 is critical for transmitting growth promoting signals [4]. Mice lacking Akt1 are also resistant to cancer. There is no sufficient information regarding RAC-alpha serine/threonine-protein kinase in Homo sapiens. So, the present study deals with prediction of active site in the protein model with their further ASA analysis of active site residues.

METHODOLOGY

COMPUTATIONAL METHODS

The computational methods of 3D-model building involved template selection, building of the model and evolution of the structure. The sequence of RAC-alpha serine/threonine-protein kinase of Homo sapiens was retrieved from NCBI database (Accession: NP_005154.2).

TEMPLATE SELECTION

The PDB (Brookhaven Protein Databank) database was extensively screened using BLAST (Basic Local Alignment Tool) server, developed and maintained in Adam Godzik's laboratory at the Burnham Institute, to find out the related homologues of the query sequence. PDB database was searched by PSI – BLAST using a profile generated from Non-Redundant protein database for proteins exhibiting similarity to the unknown structure.

MODEL BUILDING

The program CPH models-3.0 server [5] was used to build the model as PDB file of RAC-alpha serine/threonine-protein kinase of Homo sapiens according to the homology modeling method. The PDB of RAC-alpha serine/threonine-protein kinase of Homo sapiens was submitted to the PyMOL version 1.3(http: //pymol. sourceforge.net/) to obtain the 3D- structure.

EVALUATION AND VALIDATION OF MODEL

The steriochemical quality of the models was verified with the program PROCHECK [6] in order to select the best model. The 3D- profiling of the residue was done by Verify3D Structure Evaluation Server [7, 8], ERRAT [9] and "ProSA" (https://prosa.services.came.sbg.ac.at/prosa.php) [10]. ProSA is a tool widely used to check 3D models of protein structures for potential errors.

PREDICTION OF ACTIVE SITE

One of the keenest areas in the bioinformatics is the active sites of the protein and its size. This was used to find out the ligand binding efficiency with the predicted model. Active site of RAC-alpha serine/threonine-protein kinase was predicted by CASTp (http://sts.bioengr.uic.edu/castp/calculation.php/) [11].

ACCESSIBLE SURFACE AREA (ASA) ANALYSIS

POPS (http://mathbio.nimr.mrc.ac.uk/~ffranca/POPS) is a new method based on an empirically

parameterisable analytical formula to calculate solvent accessible surface areas of protein [12].

DISCUSSION

TEMPLATE SELECTION

The pleckstrin homology domain of the human protein kinase B (accession ID: 1P6S) was selected from RSCP PBD and was compared against complete Non-Redundant Protein Database using The NCBI PSI-BLAST and RAC-alpha serine/threonine-protein kinase of Homo sapiens absent in PBD is used as subject (Accession: NP_005154.2)

MODEL BUILDING

The hypothetical protein model of RAC-alpha serine/threonine-protein kinase in Homo sapiens is stored as PDB output file by CPH models-3.0 server from its FASTA sequence. The three dimensional structure of RAC-alpha serine/threonine-protein kinase was generated by using PyMOL program (figure 1A).

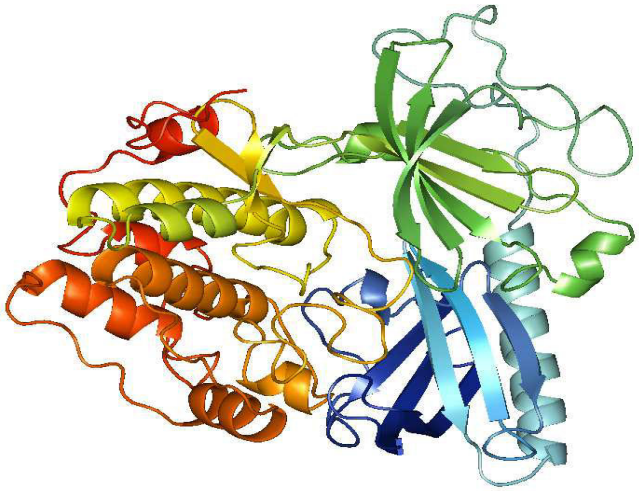

Figure 1A
3-D model of RAC-alpha serine/threonine-protein kinase of Homo sapiens by PyMOL

EVALUATION AND VALIDATION OF MODEL

To verify the predicted structure, validation was carried out with PROCHECK program. Ramchandran plot obtained by PROCHECK shows a good distribution of 226 residues with 88.8% of the total amino acids in most favored regions and remaining other i.e. 11.2% of amino acids, in allowed regions including disallowed region with 0.0% which satisfy the sterochemical and geometrical parameters of the model. The compatibility of an atomic model (3D) with its own amino acid sequence (1D) is analyzed by Verify3D. VERIFY_3D shows 86.34% of the residues had an averaged 3D-1D score greater than 0.2 indicating a good environment profile of the model. Similarly, ERRAT2 shows 93.119 overall quality factors indicating good resolution structure. The value of z-score -8.29 predicted by ProSA is in a range characteristic for native proteins indicating very less erroneous structures.

PREDICTION OF ACTIVE SITE

A total of 46 active sites were evaluated in the structure through CASTp software with ideal parameters. All pockets were characterized to find out its residues around probe radius of 1.4Å and among them, largest active site has an area of 3439 Å and volume of 5741.2 Å. The green color (figure 1B) shows the largest active site position in the protein which lies between amino acid 14 and 358.

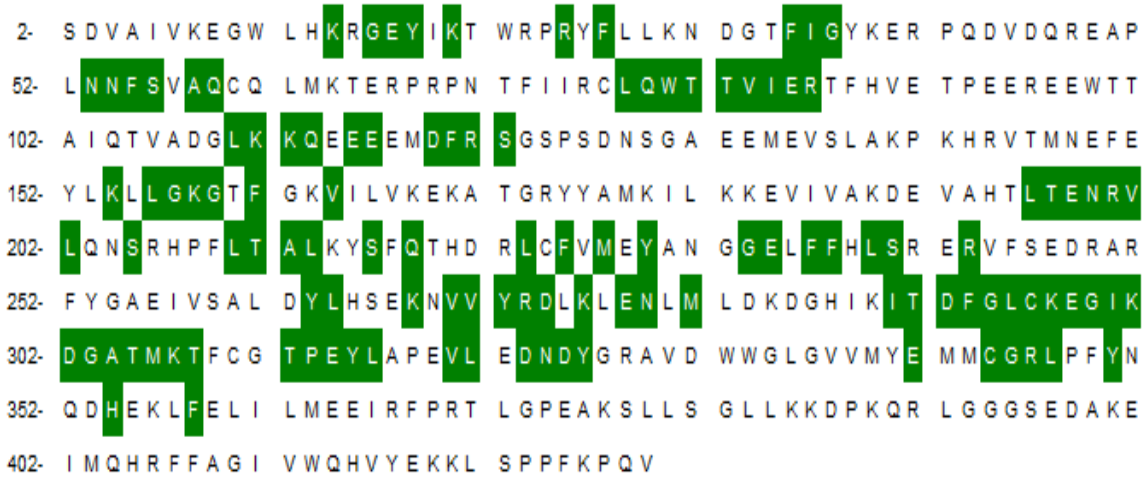

Figure 1B
Largest active site position of RAC-alpha serine/threonine-protein kinase of Homo sapiens in green color predicted by CASTp.

ACCESSIBLE SURFACE AREA (ASA) ANALYSIS

Accessible surface area (ASA) analysis of the predicted model showed the active site amino acids with low ASA value are buried inside the catalytic cleft and with high ASA values are on the surface of the cleft. Some of the ligand binding residues and metal ion binding residues were found to have high ASA values (Ser2, Gln43, Arg48, Glu49, Arg67, Glu114, Glu115, Glu116, Glu117, Met118, Arg121, Arg241, Lys307, Arg367, Arg370) and some others were found to have low ASA values (Gly37, Ala177, Ala317, Ala329, Gly334, Gly336, Gly393). All the active site amino acids are hydrophobic in nature except Met118 which is hydrophilic. The largest active site predicted in the model has high ASA value at Glu115, Glu116, Arg121, and Lys307 indicating their location on the surface of the cleft and hydrophobicity.

CONCLUSION

The three-dimensional structure of RAC-alpha serine/threonine-protein is not available as yet at PDB. So, we develop model by CPH program and validated by PROCHECK, Verify 3D, ERRAT and ProSA. Active sites in the model were predicted for binding ligand and metal ion residues. Further analysis of ASA in the model showed that most of active site amino acids are on the surface of the cleft and are accessible to solvent indicating hydrophobic nature. This analysis highlights solvent exposed active site amino acid residues with their nature in binding ligand and metal ion.

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