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Insilico Structure Prediction of 6 Phosphofructo-2-Kinase/Fructose-2,4 Bisphophatase-4, a Novel Protein in Colon Cancer

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Abstract

Colorectal cancer, more commonly known as colon cancer, is a common form of cancer; It is the third most common type of cancer and the second most lethal. It is fairly common due to the numerous causes and contact with foreign substances. In this paper, a bioinformatics and molecular modeling approach was adopted to explore properties and structure of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 (PFKFB-4). 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB) is a bifunctional enzyme, which is responsible for maintaining the cellular level of fructose-2,6-bisphosphate, a powerful allosteric activator of glycolysis. PFKFB-4 isozyme in the human breast and colon malignant tumors as compared to corresponding non-malignant tissue counterparts. Over expression of PFKFB-4 transcript levels in breast and colon malignant tumors correlates with enhanced expression of PFKFB-3, hypoxia-inducible factor (HIF)-1alpha and known HIF-1 dependent genes glucose transporter 1 (Glut1) and vascular endothelial growth factor (VEGF). The role of functional interactions between the phosphorylation of PFKFB3 and activated glycolysis in human cancer cells.

Three dimensional (3D) structures for this protein were not available as yet at Protein Data Bank. Therefore, homology models for these PFKFB-4 proteins were developed. For the modeling, template protein was obtained by National Center for Biotechnology Information (NCBI) database, template protein pdb|1BIF|chain A having identity (93%), E value (4e-108) and alignment Score (385). The modeling of the 3D structure of these proteins shows that models generated by MODELLER, a program for comparative

modeling. The models were validated using protein structure checking tools PROCHECK and SAVES Server. From Ramachandran plot analysis it was found that the portion of residues falling into the most favoured regions was (92.4%). The predicted 3-D model may be further characterized and analyzed using other techniques. We concluded that PFKFB-4 stimulate colon cancer cell proliferation, migration and angiogenesis. And we also discovered a ligand which can be used to further implement in future drug designing.

Keywords: 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4, Colorectal cancer Homology model, Ramachandran plot, PROCHECK.

Introduction

Colon cancer is the cancer that occurs in colon or rectum. The rectum is the passage way that connects the colon to the anus. Colorectal cancer is one of the most common cancers in Western society. Although it is not as common in India, the incidence of colon cancer has been rising in both men and women [1]. Cancer of colon and rectum is third leading causes of cancer in males and fourth leading cause in females. Most colorectal cancer develops first as colorectal polyps which are growth inside colon or rectum that may later become cancerous. For the year 2001, it was estimated that the incidence of colorectal cancer in India would be 18,427 in men and women 13,092 in women [1]. Some of the characteristics of cancer cells are high rates of cell proliferation, cell survival, and the ability to invade surrounding tissue. Dynamic changes in the cytoskeleton are necessary for cell motility and cancer cells are dependent on motility for invasion and metastasis. Cancer cells maintain a high glycolytic rate even in the presence of oxygen, a phenomenon first described over 70 years ago and known historically as the Warburg effect [2].

The major regulatory step in glycolysis involves phosphofructokinase-1 (PFK-1) activity, which is controlled by the intracellular ratio of ATP to AMP. High levels of ATP inhibit PFK-1 activity. The intracellular allosteric regulator, fructose 2,6-bisphosphate (F2,6BP), in turn, is a potent activator of PFK-1 [3]. Fructose 2,6-bisphosphate increases the affinity of PFK-1 for fructose 6-phosphate and diminishes the inhibitory effect of ATP. The discovery of F2,6BP has led to the concept that intracellular F2,6BP levels regulate the glycolytic rate in proliferating cells by coupling hormonal signals with metabolic demand. At least four genes (PFKFB1, PFKFB2, PFKFB3, and PFKFB4) have been identified that encode PFK-2/FBPase [4]. Distinct properties characterize these isoforms, including the ratio of their kinase/phosphatase activities, their response to protein kinases, and their tissue expression profiles [5–7]. Fructose 2,6-bisphosphate (F2,6BP) is a potent activator of phosphofructokinase, which is a rate-limiting enzyme of glycolysis. The concentration of F2,6BP depends on the activity of the bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase). Four genes encoding PFK-2/FBPase have been identified and termed PFKFB1 to PFKFB4. PFKFB3 protein is expressed in high levels in human tumors in situ [8]. Accordingly, PFKFB3 protein has the highest kinase/phosphatase activity ratio of all the PFK-

2/FPBase isoforms discovered to date, which is consistent with its role as a powerful activator of glycolysis [9].

In recent years, Bioinformatics has greatly accelerated the understanding for the protein structures, functional complexity and their biological relationships. A large number of computational tools are available from different sources for making predictions regarding the identification and structure prediction of proteins. The major drawbacks of experimental methods that have been used to characterize the proteins of various organisms are the time frame involved, high cost and the fact that these methods are not amenable to high throughput techniques. *Insilico* approaches provide a viable solution to these problems. The amino acid sequence provides most of the information required for determining and characterizing the molecule's function, physical and chemical properties. Computationally based characterization of the features of the proteins found or predicted in completely sequenced proteomes is an important task in the search for knowledge of protein function [10].

In this paper the *Insilico* analysis and homology modeling studies on 6 phosphofructo-2-kinase/fructose-2,4 bisphophatase-4 protein was reported. Three dimensional structures for this protein were yet not available. Hence to describe the main aim of this study is to predict three dimensional structural features and to understand molecular function, the model structure establish some important analysis for this protein.

Materials and Methods

The methodology of homolog modeling included retrieval of target sequence, template selection, target-template alignment, model building and evaluation of the modeled structure.

Retrieval of Target Sequence

The amino acid sequence of the 6 phosphofructo-2-kinase/fructose-2,4 bi sphophatase-4 (PFKFB-4) (Accession No. NP_004558 and Gene ID: 4758902) [11] was obtained from National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), a publicly available database. It was ascertained that the three-dimensional structure of the protein was not available in Protein Data Bank (PDB) [12]. Hence the present exercise of developing the 3D model of the novel protein (PFKFB-4) was undertaken. This protein sequence was retrieved in FASTA format and used for further analysis. The protein consists of 469 amino acids.

Template Searching

An attempt was made to find a suitable template protein for the modeling of the target protein. The template protein was searched through PDB database. From the homology searching 6-phosphofructo-2-kinasefructose-2,6-bisphosphatase bifunctional enzyme complexed with ATP-G-S and phosphate (1BIF) [13] of *Rattus norvegicus* was selected as template protein.

Sequence Alignment
Amino acid sequence alignment of target and template proteins was derived using the Protein-Basic Local Alignment Search Tool (BLASTp) [14] of NCBI. Default parameters were applied and the aligned sequences were inspected and adjusted manually to minimize the number of gaps and insertions.

Model
Once an initial target-template alignment was done, a variety of methods could be used to construct a 3D model (10 models) for the target protein. The modeling of the three dimensional structure of the protein was performed by MODELLER 9v8 [15].

Evaluation of Refined Model
In the next step of model refinement we selected 5 models which were having the minimum dope (Discrete Optimized Protein Energy) score.

In the last step of homology modelling the structures of the model was subjected to a series of tests for testing its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the Psi/Phi Ramachandran plot obtained from PROCHECK [16] analysis, for all the selected models this is done. The Swiss-PdbViewer [17] energy minimization test was applied to check for energy criteria in comparison with the potential of mean force derived from a large set of known protein structures. Ramachandran Plot [18] analysis is studied in depth and the model having best results for Ramachandran Plot is been selected for further analysis. Then the quality of the refined structure is tested using Verify_3D [19] tool of SAVES NIH [20] web server for Bioinformatics.

Structure Submission
In general, 30% sequence homology is required for generating useful models. The best possible structure was obtained and submitted. The best 3D model is having 92.4% residues in most favoured region in Ramachandran Plot. With 0% residues in disallowed region.

Result and Discussion

The 3D structure of target protein 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 (PFKFB-4). (Figure 2) was built by homolog modelling based on the template obtained from protein data bank. In our study, based on the result (PFKFB-4) obtained from MODELLER, PROCHECK and Verify_3D SAVES server. These target protein sequence was retrieved from the NCBI, database. A, pdb|1BIF|A, that is a high resolution structure of 6-phosphofructo-2-kinasefructose-2,6-bisphosphatase bifunctional enzyme complexed with ATP-G-S and phosphate of *Rattus norvegicus* was selected as template. By using BLAST, for template protein 1BIF; E value (4e-108), identify (93%) was obtained. MODELLER was used for building the model. With the help of MODELLER software 10 models were generated, stereochemical parameters of the proteins like main-and side chains data of PFKFB-4 was considered for determining the quality of the model which were

generated by using PROCHECK 3.0. The main chain parameters like Ramachandran plot quality; peptide bond planarity, C-alpha Chirality, over-all G factor and the bad contacts per 100 residues are found to be within the limits for the model. The side chain parameters are in better range and within the limits for PFKFB-4. After comparing all parameters best model was obtained. Refined model was analyzed by different protein analysis programs including PROCHEK tool for the evaluation of the Ramachandran plot quality.

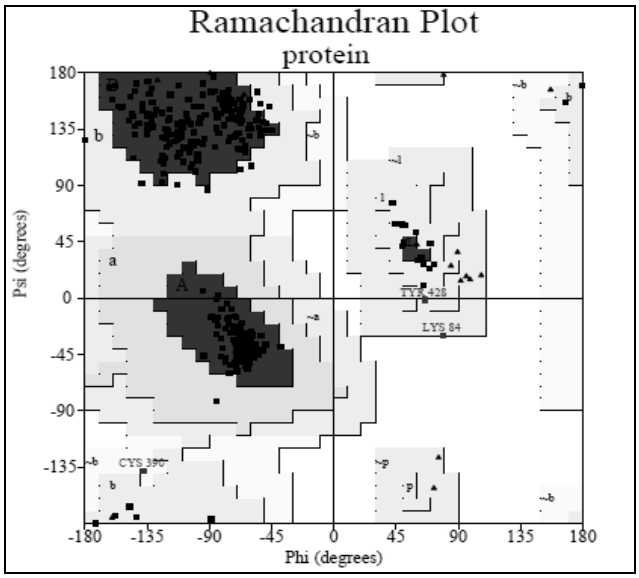

Figure 1: Ramachandran plot analysis of model using PROCHECK software. It shows the various residues falling in favoured allowed and disallowed region and the Glycine residues (390 residues are in favoured region, 27 in allowed region and 0 in disallowed region) so > 90% residues have allowed conformations.

The Ramachandran plot for PFKFB-4 using PROCHECK software revealed that among 469 residues, 390 (92.4%) were in favoured region, 27 (6.4%) were in allowed region and 0 (0.0%) were in disallowed region which proves that the predicted model is acceptable (Figure 1). Ramachandran plot for general, glycine, pre-proline and proline was also done and it showed the glycine, pre-Pro and proline of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 falling under allowed regions. From the tool VERIFY_3D and ERRAT [21] which is available at Structural Analysis and Verification Server (SAVES) it was shown that (80.64%) of the residues had an averaged 3D-1D score > 0.2 and overall quality factor (80.645).

The overall results provided the evidences that predicted 3-Dimensional structure of PFKFB-4 is acceptable with good quality. This structure (Figure 2; see PMDB ID - PM0076370 [22] for the corresponding coordinates in PDB format) was found to be satisfactory based on the above results.

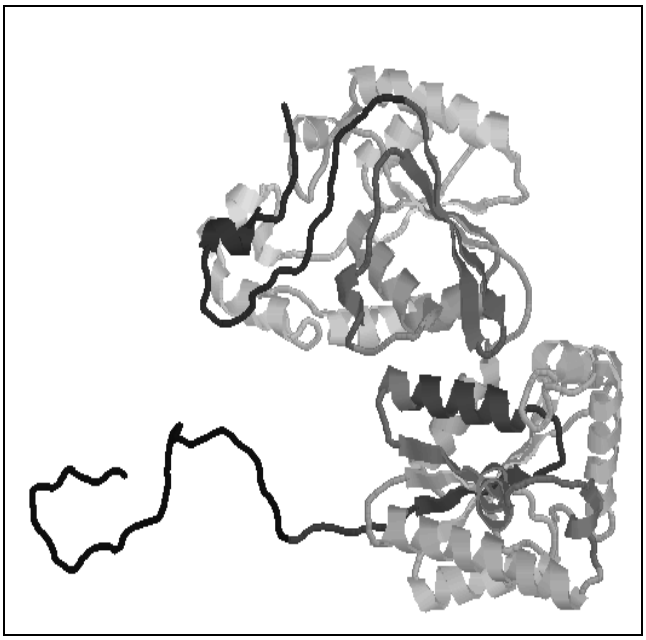

Figure 2: Ray diagram of 3D modelled structure of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4. In this model residues which are responsible for alpha helix and extended strand are 20 and 14 respectively.

Conclusions

Insilico study of protein, nucleic acid helpful in almost all research fields, it not only saves money but also saves valuable time. Bioinformatics is commonly used in Drug Design and Molecular Modelling. Homology Modelling is becoming a very useful technique in the field of bioinformatics because the knowledge of the three-dimensional structure of a protein would be an invaluable aid to understand the details of a particular protein. This model is successfully submitted in Protein Model Database (PMDB) the PMDB ID of the structure is – PM0076370. (Refer Figure 2). This 3D structure of PFKFB-4 may be further used in characterizing the protein in wet laboratory and understanding the mechanism how it's over expression. Such model can be effectively used to structural information and can be further implemented in future drug designing.

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References

[1] Mohandas K.M., Desai D.C., Epidemiology of digestive tract cancers in India. V. Large and small bowel. *Indian J Gastroenterol*; 18 (1999) 118-21.

[2] Van S. E., Jett M.F., Hue L. and Hers H.G., Control of liver 6-phosphofructokinase by fructose 2,6- bisphosphate and other effectors. Proc *Natl Acad Sci* USA; 78 (1981) 3483-6.

[3] Hue L., Rousseau G.G., Fructose 2,6-bisphosphate and the control of glycolysis by growth factors, tumor promoters and oncogenes. *Adv Enzyme Regu*l; 33 (1993) 97-110.

[4] Okar D.A., Manzano A., Navarro-Sabate A, Riera L., Bartrons R. and Lange A.J., PFK-2/FBPase-2: maker and breaker of the essential biofactor fructose-2,6- bisphosphate.*Trends Biochem Sci*; 26 (2001) 30-5.

[5] Manzano A., Perez J.X., Nadal M., Estivill X., Lange A. and Bartrons R. Cloning, expression and chromosomal localization of a human testis 6-phosphofructo-2- kinase/fructose-2,6-bisphosphatase gene. *Gene*; 229 (1999) 83-9.

[6] Heine-Suner D., Diaz-Guillen M.A., Lange A.J., and Rodriguez de Cordoba S., Sequence and structure of the human 6-phosphofructo-2-kinase/fructose-2,6- bisphosphatase heart isoform gene (PFKFB2*). Eur J Biochem*; 254 (1998) 103-10.

[7] Lange A.J., Pilkis S.J., Sequence of human liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. *Nucleic Acids Res*; 18 (1990) 3652.

[8] Bando H., Atsumi T., Nishio T., Niwa H., Mishima S., Shimizu C., Yoshioka N., Bucala R. and Koike T., Phosphorylation of the 6-Phosphofructo-2-Kinase/Fructose2,6-isphosphatase/PFKFB3 Family of Glycolytic Regulators in Human Cancer. *Clinical Cancer Research*, 11 (2005) 5784.

[9] Sakakibara R., Kato M., Okamura N, et al., Characterization of a human placental fructose-6-phosphate, 2-kinase/fructose-2,6-bisphosphatase. *J Biochem* (Tokyo); 122 (1997) 122-8.

[10] Sahay A, Shakya M., In silico Analysis and Homology Modelling of Antioxidant Proteins of Spinach. *J Proteomics Bioinform* 3 (2010) 148-154.

[11] Bobarykina A.Y., Minchenko D.O., Opentanova I.L., Moenner M., Caro J., Esumi H. and Minchenko O.H., Hypoxic regulation of PFKFB-3 and PFKFB-4 gene expression in gastric and pancreatic cancer cell lines and expression of PFKFB genes in gastric cancers *Journal Acta Biochim. Pol.* 53 (2006) 4, 789-799.

[12] Berman H.M., Westbrook J., Feng Z., Gilliland G., Bhat T.N., Weissig H., Shindyalov I.N., Bourne P.E., The Protein Data Bank. *Nucleic Acids Res*, 28 (2000) 1, 235–242.

[13] Hasemann C.A., Istvan E.S., Uyeda K., Deisenhofer J., The crystal structure of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase reveals distinct domain homologies., *Structure.* 15 (1996) ;4 (9):1017-29.

[14] Altschul S.F., Madden T.L., Schaffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25 (1997), 17: 3389-402.

[15] Sali A, Blundell T.L., Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol.* 234 (1993), 3:779-815.

[16] Laskowski R.A., Rullmannn J.A., MacArthur M.W., Kaptein R., Thornton J.M., AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *J Biomol NMR* 8 (1996): 477-486.

[17] Guex N., Manuel C. P., Data Modeling, Analysis and Classifi cation SWISSMODEL and the Swiss-Pdb Viewer: An environment for comparative protein modeling. *Electrophoresis* 18 (1997) 2714-2723.

[18] Ramachandran GN, Ramakrishnan C, Sasisekhran V., Stereochemistry of polypeptide chain confi guarations. *J Mol Biol* 7 (1963): 95-99.

[19] Lüthy R., Bowie J.U., Eisenberg D., Assessment of protein models with three-dimensional profiles. *Nature*.356 (1992) (6364):83-85.

[20] http://nihserver.mbi.ucla.edu/SAVES

[21] Colovos C., Yeates T.O., Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci.* 2(9) (1993):1511-1519.

[22] Castrignano T.`, Meo P. D'O. D, Cozzetto D., Giuseppe I., Tramontano T., A., The PMDB Protein Model Database. *Nucleic Acids Research*. 34 (2006): D306–D309.