Journal of Computational Intelligence in Bioinformatics.
ISSN: 0973-385X Volume 4 Number 1 (2011) pp. 185—192
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http://www.ripublication.com/jcib.htm

Docking Study of MTHFR with FAD by Hex

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Abstract

In present study an *in silico* attempt was made to verify earlier wet lab reports of less affinity of A222V mutant MTHFR with its cofactor FAD by docking. Docking can assist in predicting protein-protein and protein-ligand interactions, and evaluating affinity of complexes. Docking was performed using wild and mutant MTHFR as receptor and FAD as ligand by Hex 5.1 software. 656 long amino acid sequence of MTHFR was extracted from Swissprot and submitted to ESyPred for structure prediction. ESyPred predicted wild as well as mutant MTHFR structure of only 292 amino acids long (i.e. of catalytic domain only). Docking between wild MTHFR-FAD and 677T mutant MTHFR-FAD were performed separately. The E-total (energy) was -375.98 in case of 222A MTHFR-FAD docking and -385.65 in case of 222V MTHFR-FAD docking. Stability of docked complex increased with decrease in energy value. Thus, *in silico* approach proved that the enzymatic activity of 222V mutant MTHFR is less and reduced due to lower affinity to FAD.

Keywords: Human methylenetetrahydrofolate reductase, FAD, molecular docking.

Introduction

Methylenetetrahydrofolate reductase (MTHFR) is one of the key enzymes in the folate and homocysteine metabolic pathway. The enzyme reduces the 5-10-methylenetetrahydrofolate to its biologically active form 5-methyltetrahydrofolate, and the latter then donates its methyl group for changing the homocysteine into methionine (Lucock, 2000). This reaction is important in one-carbon metabolism because methionine is the precursor of S-adenosylmethionine (SAM), the methyl group donor for DNA, protein, and lipid methylation. If there is a mutation in the MTHFR gene, the homocysteine level can be increased by decreased levels of 5-methyltetrahydrofolate and methionine. Over the past decade, there has been a growing body of evidence that even a moderately elevated serum homocysteine

concentration is associated with an increased risk of ageing-related diseases, such as atherosclerotic thromboembolic and neurodegenerative disorders, and also with early pathological events of life (Herrmann, 2001; Gueant et al., 2003).

The human MTHFR gene is 20 kb long (20,336bp) and mapped at 1p36.3 (OMIM 607093), having 11 exons. More than 40 polymorphisms have been described in MTHFR, but the most common and clinically important variants are C677T in exon 4 (Frosst et al, 1995) and A1298C in exon 7 (Weisberg et al, 1998). The C677 variant results from a single nucleotide substitution mutation at 677^{th} position of the gene, in which cytosine is replaced by thymine. At protein level this change makes substitution of alanine to valine at 222^{nd} amino acid. This change makes enzyme thermolabile with reduced enzymatic activity. This deficiency is inherited as an autosomal recessive trait. Individuals who are homozygous for the thermolabile variant of MTHFR (TT) have an increased risk of hyperhomocysteinemia and lower levels of folate in plasma and red blood cells (Molloy et al, 1997). C677T mutation has been reported to be a genetic factor for several disease like neural tube defects (Mills et al, 1995; Whitehead et al, 1995; van der Put et al, 1997), cardiovascular diseases (Frosst et al, 1995; Brilakis et al, 2003) and psychiatric disorders (Tan et al, 2004; Chen et al, 2005; Sazci et al, 2005) etc.

Docking is the process by which two molecules fit together in 3D space. The concept of docking is important in the study of various properties associated with protein-ligand interactions such as binding energy, geometry complementarity, electron distribution, hydrogen bond donor acceptor properties, hydrophobicity and polarizability. Since molecules in nature have a tendency to be found in their low energy form, the final configuration should also be of low energy (Pyne and Gayathri, 2005). In present study an *in silico* attempt was made to verify earlier wet lab reports of less affinity of clinically important A222V mutant MTHFR with its cofactor FAD.

Materials and Method

Protein interactions are the basis of life processes at the cellular level,and during last thirty years molecular docking is emerged as a powerful tool to understand the protein-protein interactions. It is utilized for the prediction of protein-ligand complexes which is composed of two components: a search algorithm that creates possible protein-ligand complex geometrics, and thus performs the process of “pose generation” and a scoring function that predicts the binding affinity of the ligand to the protein based on the complex geometry.

In present study we used biological databases- UniProtKB/ Swiss-Prot and PDB, bioinformatic tools- ESyPred3D, YASARA (version 10.1.8), Rasmol (version 2.7.5) and Hex (version 5.1). UniProtKB/ Swiss-Prot database was used for the amino acid sequence of MTHFR enzyme (http://www.uniprot.org/). UniProtKB/Swiss-Prot is a curated protein sequence database which strives to provide a high level of annotation. UniProt knowledgebase (UniProtKB) is the central access point for extensive curated protein information, including function, classification and cross reference. PDB (Protein Data Bank)(http://www.pdb.org/pdb/home/home.do), is the single worldwide archieve of structural data of biological macromolecules, established in Brookhaven National Laboratories (BNL) in 1971. It contains structural information of the macromolecules determined by X-ray crystallography and NMR methods etc.

ESyPred3D (http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred/) (Lambert et. al., 2002) is a new automated homology modelling program. The final three dimensional structures are built using the modelling package MODELLER. YASARA (Yet Another Scientific Artificial Reality Application) is a molecular graphics, modelling and simulation program. It is an interactive real-time molecular dynamics program, creates a new level of interaction with the 'artificial reality'. Rasmol (Raster Display of Molecules) is a molecular graphics program intended for the structural visualization of protein, nucleic acids and small biomolecules. The program reads in molecular coordinate files and interactively displays the molecule on the screen in variety of representations and colour schemes. Hex 5.1 (Ritchie, 2008) is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate protein- ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. It is docking and superposition program to use Spherical Polar Fourier (SPF) correlations to accelerate the calculation and has built in graphics to view the results.

Result and Discussion

MTHFR is a 77 kDa enzyme with 40 kDa catalytic and 37 kDa of regulatory domains. MTHFR enzyme functions as a homodimer in which each subunit is composed of an N-terminal catalytic domain, which binds FAD and 5, 10-methylenetetrahydrofolate (THF) and a C-terminal regulatory domain that contains the binding site for the allosteric regulator SAM. The enzyme activity of 222V mutant MTHFR is reduced approximately 50% in homozygous and ~ 30% in heterozygous and wet lab experiments already proved that binding affinity of A222V mutant MTHFR with FAD is less than the wild MTHFR-FAD binding affinity. The 222V mutant enzyme is called as "thermolablile" because the activity of the encoded enzyme is reduced at 37^0C or more (Kang et al, 1991). The decrease in its enzymatic activity is due to dissociation of dimer into monomers and loss of FAD-binding capacity (Yamada et al., 2001).

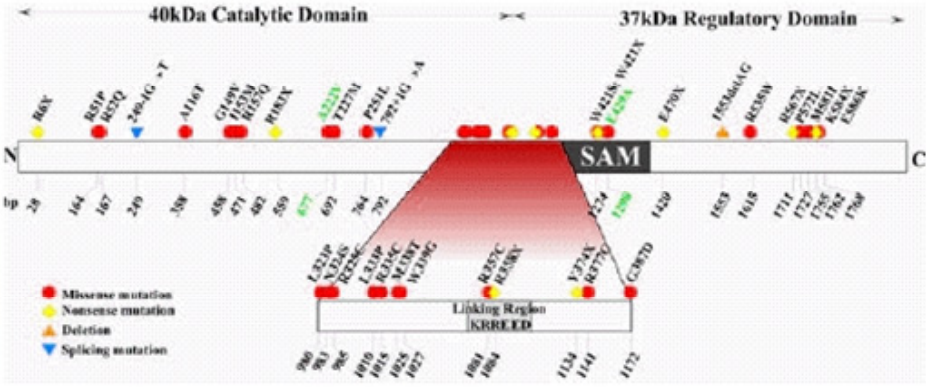

Figure 1: MTHFR protein showing two domains-40kDa catalytic domain and 37kDa regulatory domain and a small linking region.

For present docking study we searched for human MTHFR protein structure in PDB database but we did not find any structure. The amino acid sequence of human wild MTHFR protein was obtained from UniProtKB/Swiss-Prot database, a 656 amino acid long sequence was retrieved for human MTHFR protein. The 656 amino acid long sequence was submitted to Expasy tool ESyPred for protein structure prediction. A 292 amino acids long protein structure was obtained in PDB format. Amino acid sequences of nine common MTHFR mutants (R51P, R52Q, R68Q, R157Q, A222V, T227M, P251L, L323P, and N324S) were submitted to EsyPred which again predicted only structure of 292 amino acids long i.e. of catalytic domain.

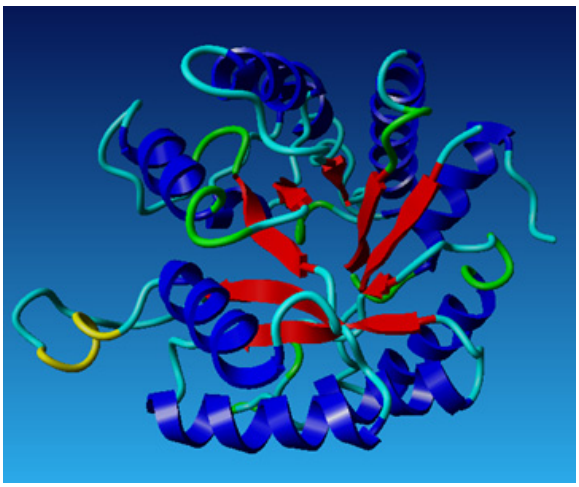

Figure 2: Predicted Structure of wild MTHFR by ESyPred3D.

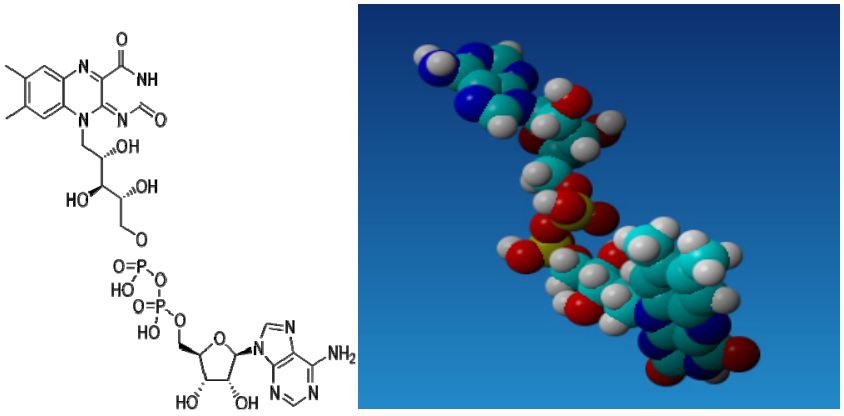

Figure 2: Structure of FAD taken fromPDBeChem.

Secondary structures were predicted in wild and clinically important mutant (A222V) of MTHFR protein by YASARA (version 10.1.8), which consisted of 49.7% helix, 15.4% sheet, 9.2% turn, 25.7% coil in wild and 51.4% helix, 15.4% sheet, 7.9% turn and 25.3% coil in mutant (A222V) MTHFR.

Table 1: Predicted secondary structures in wild and nine variants MTHFR sequences.

	Helix	Sheet	Turn	Coil	3_{10} Helix
Wild/Normal MTHFR	49.7	15.4	9.2	25.7	0.0
R51P	49.7	15.4	7.9	25.3	1.7
R52Q	51.4	15.8	6.5	26.4	0.0
R68Q	49.0	15.4	6.5	27.7	1.4
R157Q	47.6	15.4	9.2	26.4	1.4
A222V	51.4	15.4	7.9	25.3	0.0
T227M	48.6	15.4	7.9	26.7	1.4
P251L	47.3	15.4	12.0	25.3	0.0
L323P	48.3	15.4	9.2	25.7	1.4
N324S	50.3	15.4	9.2	25.0	0.0

In predicted structure of wild MTHFR (222A) total number of amino acids, atoms and bonds were 292, 2304 and 2356 respectively and in 222V mutant MTHFR the number of atoms and bonds were 2306 and 2358 (RasMol, version 2.7.5).

Table 2: Estimation of total number of amino acids, atoms and bonds in predicted structures of wild and nine variants of MTHFR by RasMol.

	Groups	Atoms	Bonds
Wild/Normal MTHFR	292	2304	2356
R51P	292	2300	2353
R52Q	292	2302	2354
R68Q	292	2302	2354
R157Q	292	2302	2355
A222V	292	2306	2358
T227M	292	2305	2357
P251L	292	2305	2356
L323P	292	2303	2356
N324S	292	2301	2354

In present study docking was performed by Hex program, in which MTHFR was used as receptor and FAD as a ligand with default parameters. Docking between wild MTHFR-FAD and 677T mutant MTHFR-FAD were performed separately. The E-total (energy) was -375.98 in case of 222A (677C) MTHFR-FAD docking and -385.65 in case of 222V (677T) MTHFR-FAD docking. Stability of docked complex increased with decrease in energy value. Hence, *in silico* approach proved that the enzymatic activity of 222V (677T) mutant MTHFR is less and reduced due to lower affinity to FAD.

Table 3: Docking results of MTHFR variants with FAD.

Amino acid change	E-value
Wild/Normal MTHFR	-375.98
R51P	-365.22
R52Q	-385.20
R68Q	-388.11
R157Q	-358.33
A222V	-385.65
T227M	-355.01
P251L	-363.37
L323P	-360.99
N324S	-389.55

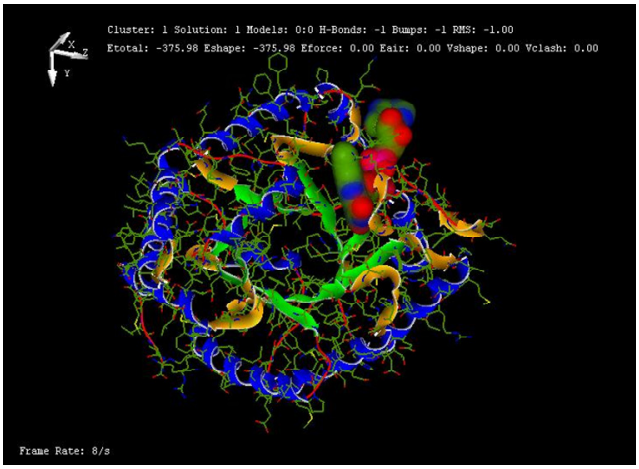

Figure 4: Docking of Wild MTHFR with FAD using Hex.

However, along with binding energies, several other physical parameters like electroststics, van der waals forces, hydrogen bonds, and hydrophobic and entropic effects influence the binding affinity, are also needed to be evaluated for calculating binding affinity of FAD with MTHFR.

Conclusion

In present study it is proved by docking that the 222V-FAD complex (with higher energy) is less stable than the 222A-FAD complex (with lower energy). Difference in binding energy of mutant-FAD complex may arise due to mutation, which can cause conformational changes in the mutant protein (222V MTHFR)that affect the binding affinity of ligand FAD, as it is known that binding energies reflects the binding affinity. MTHFR functions as dimer and due to less affinity to FAD, mutant MTHFR dissociated into monomers, which reduces its enzymatic activity.

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