Research Article

Molecular Docking Study on the Interaction of Riboflavin (Vitamin B₂) and Cyanocobalamin (Vitamin B₁₂) Coenzymes

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Cobalamins are the largest and structurally complex cofactors found in biological systems and have attracted considerable attention due to their participation in the metabolic reactions taking place in humans, animals, and microorganisms. Riboflavin (vitamin B₂) is a micronutrient and is the precursor of coenzymes, FMN and FAD, required for a wide variety of cellular processes with a key role in energy-based metabolic reactions. As coenzymes of both vitamins are the part of enzyme systems, the possibility of their mutual interaction in the body cannot be overruled. A molecular docking study was conducted on riboflavin molecule with B₁₂ coenzymes present in the enzymes glutamate mutase, diol dehydratase, and methionine synthase by using ArgusLab 4.0.1 software to understand the possible mode of interaction between these vitamins. The results from ArgusLab showed the best binding affinity of riboflavin with the enzyme glutamate mutase for which the calculated least binding energy has been found to be -7.13 kcal/mol. The results indicate a significant inhibitory effect of riboflavin on the catalysis of B₁₂-dependent enzymes. This information can be utilized to design potent therapeutic drugs having structural similarity to that of riboflavin.

1. Introduction

B₁₂ cofactors play important roles in the metabolism of microorganisms, animals, and humans. They are involved in the metabolism of almost every cell of the body specifically the DNA synthesis and regulation. The structure and reactivity of B₁₂ derivatives and structural aspects of their interactions with proteins and nucleotides are crucial for the efficient catalysis by the important B₁₂-dependent enzymes [1]. Biologically active cobalamins, adenosylcobalamin (AdoCbl), and methylcobalamin (MeCbl) are cofactors for many enzyme systems, containing a metal carbon bond involved in enzyme catalyzed reactions [2]. They catalyze enzymatic reactions which involve the making and breaking of the C-Co bond of these cofactors. The X-ray structures of B₁₂-enzyme complexes revealed that the B₁₂-cofactor undergoes a major conformational change on binding to the apoenzyme in AdoCbl and MeCbl containing enzymes such

as isomerases, eliminases, and methyltransferases [3]. A key step in the catalytic mechanism of coenzyme-B₁₂ containing enzymes is the homolysis of Co-C organometallic bond that leads to the intricate pathways of B₁₂ metabolic functions and the catalysis of related chemical reactions [4]. The Co-C bond undergoes homolytic cleavage in B₁₂-dependent enzymes more quickly as compared to that of the isolated cofactor in aqueous solution [5] which is a clue to the catalytic role of vitamin B₁₂.

Coenzyme B₁₂-dependent enzymes may bind to their cofactors in two possible modes, “base-on” or “base-off” modes. In base-on mode, the original 5,6-dimethylbenzimidazole base coordinates cobalt as the α -ligand at the lower side in the enzyme-coenzyme complex, while in base-off binding mode, the 5,6-dimethylbenzimidazole moiety is displaced and substituted by an exogenous ligand, such as histidine residue of the protein which coordinates the Co-atom as α -ligand, that is, base-off/His-on mode [6].

Cobalamins in the +3 oxidation state exist usually in the base-on form with the axial ligand X (Ado, CH₃, or CN) coordinated on the β side (upper side) of the octahedral cobalt compound, while in the +2 oxidation state, it has no β ligand and is called cob(II)alamin or B12r and in the +1 oxidation state it has been assigned a name co(I)alamin or B12s, in the base-off form along with the absence of β ligand [7].

The objective of the current study was to evaluate the binding affinity of riboflavin with B₁₂ coenzymes by *molecular docking* technique to find out its effect on the inhibition or acceleration of enzyme activity. This depends on the interaction of the functional groups of riboflavin with those amino acid residues of B₁₂ enzymes that are present in the active site cavity or take part in enzyme catalysis indirectly.

Molecular docking techniques are used to predict how a protein interacts with small vitamin-like molecules. This ability governs a significant part of the protein's dynamics which may enhance/inhibit its biological function [8]. Two factors are of paramount importance in molecular docking studies: optimizing the candidate ligand for the correct native conformation in the presence of which it can achieve a best fit orientation to bind with a protein of interest, and the conformational flexibility of ligand and protein [9]. Thus, the accurate prediction of the binding modes between the ligand and protein is of fundamental importance in modern structure-based drug design. Computer-based molecular modeling aims to speed up drug discoveries by predicting potential effectiveness of ligand-protein interactions prior to laborious experiments and costly preclinical trials.

Numerous software packages have been developed with the implementation of various molecular docking algorithms based on different search methods [10]. The present work of molecular docking has been done using commercially available software, ArgusLab 4.0.1. It is a molecular modeling, graphics, and drug designing program based on genetic algorithm. It is implemented with exhaustive search methods, the Argus Dock docking engine and AScore scoring function [11]. It is also capable of performing molecular geometry calculations and molecular structure visualization.

2. Materials and Methods

2.1. Computational Methodology

2.1.1. Data Set. Three-dimensional (3D) experimentally known protein-ligand complexes were obtained from Brookhaven Protein Data Bank (PDB) [12] (<http://www.rcsb.org/>). These were the structures of enzymes from three major enzyme families with bound coenzyme B₁₂: glutamate mutase from isomerases [13], diol dehydratase from lyases [14], and methionine synthase from transferases [15] having PDB codes 1CCW, 1EGM, and 3IV9, respectively.

2.1.2. Input File Preparations for Energy Minimization of Protein. For each of the protein-ligand complexes chosen for the study, a "clean input file" was generated by removing water molecules, ions, ligands, and subunits not involved

in ligand binding from the original structure file. Water molecules were removed because ArgusLab sometimes failed to dock the compounds having water molecules at their binding sites [16]. All hydrogen atoms in the protein were allowed to optimize. The hydrogen locations are not specified by the X-ray structure but these are necessary to improve the hydrogen bond geometries, at the same time maintaining the protein conformation very close to that observed in the crystallographic model. The resulting receptor model was saved to a PDB file. Minimization was performed by geometry convergence function of ArgusLab software performed according to Hartree-Fock calculation method.

2.1.3. Ligand Input File Preparation and Optimization. Ligand input structure was drawn using Marvin Sketch software. The structure was cleaned in 3D format and energy was minimized using Marvin sketch software. The resulting structure was then saved in "mdl mol" and "sdf" file formats for molecular docking studies

2.2. Docking Methodology. After the preparation of the protein and ligand, molecular docking studies were performed by ArgusLab 4.0.1 to evaluate the interactions.

2.2.1. ArgusLab 4.0.1. ArgusLab is implemented with shape-based search algorithm. Docking has been done using "Argus Dock" exhaustive search docking function of ArgusLab with grid resolution of 0.40 Å. Docking precision was set to "Regular precision" and "Flexible" ligand docking mode was employed for each docking run. The stability of each docked pose was evaluated using ArgusLab energy calculations and the number of hydrogen bonds formed [17].

2.2.2. Molecular Docking Study. To perform docking one first needs to define atoms that make up the ligand and the binding sites of the protein where the ligand should bind. The prepared 3D structures of lccw, legm, and 3iv9 proteins were downloaded into the ArgusLab program and binding sites were made by choosing "Make binding site for this protein" option. The ligand (cleaned riboflavin molecule) was then introduced and docking calculation was allowed to run using shape-based search algorithm and AScore scoring function. The scoring function is responsible for evaluating the energy between the ligand and the protein target. Flexible docking was allowed by constructing grids over the binding sites of the protein and energy-based rotation is set for that ligand's group of atoms that do not have rotatable bonds. For each rotation, torsions are created and poses (conformations) are generated during the docking process [11]. For each complex, 10 independent runs were conducted and one pose was returned for each run. The best docking model was selected according to the lowest AScore calculated by ArgusLab, and the most suitable binding conformation was selected on the basis of hydrogen bond interactions between the ligand and protein near the substrate binding site. The lowest energy poses indicate the highest binding affinity as high energy produces the unstable conformations.

TABLE 1: Binding energies of riboflavin coenzyme B₁₂ containing enzyme complex by AScore scoring function of ArgusLab.

S. no.	Enzyme name (pdb code)	AScore (kcal/mol)
1	Glutamate mutase (lccw)	-7.13
2	Diol dehydratase (legm)	-6.98
3	Methionine synthase (3iv9)	-6.07

FIGURE 1: Minimized structure of riboflavin. Atoms in red are oxygen, blue are nitrogen, grey are carbon, and white are hydrogen. The number of hydrogen atoms is indicated in light green color.

3. Results and Discussion

Minimized structure of riboflavin is given in Figure 1. Docking studies of the compound riboflavin with each of the three enzymes having PDB codes *lccw*, *legm*, and *3iv9* were carried out by ArgusLab 4.0.1. The least binding energy exhibits the highest activity which has been observed by the ranking of poses generated by AScore scoring function of ArgusLab and is given in Table 1.

List of hydrogen bonds between riboflavin and coenzyme B₁₂-dependent enzymes is given in Table 2. The best fitted poses adopted by riboflavin docked into enzymes *lccw*, *legm*, and *3iv9* are shown in Figures 2, 3, and 4, respectively.

In the present study, cyanocobalamin coenzyme was taken as an active ligand instead of cocrystallized inhibitor D-tartaric acid, to bind with the compound riboflavin in order to examine a possible mode of interaction between these two vitamins in an enzyme system. The docked binding mode of riboflavin was manually inspected in order to verify that it effectively binds to the catalytic site. The docking results of riboflavin with each of the individual enzymes are as follows.

3.1. Glutamate Mutase. The compound riboflavin interacted with enzyme glutamate mutase (in complex with coenzyme B₁₂ and the inhibitor, D-tartaric acid) by least binding energy of -7.13 kcal/mol. In Figure 2 riboflavin seemed to bind at the lower axial end of the coenzyme B₁₂ with the base 5,6-dimethylbenzimidazole (DMB) by replacing water

FIGURE 2: Docking of riboflavin into enzyme glutamate mutase [13]. Riboflavin and protein binding sites are shown in element colors in wireframe and thick stick models, respectively, whereas cyanocobalamin is represented in pink stick model. Inhibitor TAR is seen in green color in thick stick.

molecules. The oxygen (O2) of carbonyl group of riboflavin binds with nitrogen atom (NH) of amino acid residue Gly120. The hydrogen bond distance between these groups is 2.52 Å. Another hydrogen bond was formed between the nitrogen atom (N1) of riboflavin and NH of amino acid Thr121 at a distance of 2.72 Å. No interaction was observed between the riboflavin and cocrystallized inhibitor, D-tartaric acid. In glutamate mutase, the elongation of Co-N bond probably contributes to the weakening of Co-C bond in the coenzyme [13]. Therefore, the docked position and conformation of riboflavin revealed that the compound may inhibit the usual catalytic process of the enzyme by altering the conformational change in the nucleotide base, which plays an important role in initiating the Co-C bond cleavage and thus hinders the continuous progress of radical reactions. The relative conformation and arrangement of cofactor and substrate also play a part in this aspect. The calculated AScore shows a significant affinity of riboflavin towards the enzyme glutamate mutase.

3.2. Diol Dehydratase. In Figure 3, deep analysis of the docked structure of riboflavin revealed that the molecule seemed to bind in between the enzyme's active site, that is, the E-subunit and B₁₂-cofactor binding domain as indicated by the presence of amino acid residues Thr222, Val300, Phe374, and Gln336. These amino acid residues have been found to form the hydrophobic contacts with the inhibitor 1, 2-propanediol and play an important role in holding the substrate in the active site [14]. Three hydrogen bonds were formed with this enzyme; the nitrogen atom (N5) of riboflavin interacted with the sulphur (SH) of amino acid residue Cys302. The hydrogen bond distance between these groups was found to be 2.50 Å. Another two hydrogen bonds

TABLE 2: List of hydrogen bonds between riboflavin and coenzyme B₁₂ containing enzymes.

S. no	Enzyme name	No. of H-bonds	Amino acid residue atom	Ligand atom	H-bond distance (Å)
1	Glutamate mutase (1ccw)	2	Gly120 (NH)	(O2)	2.52
			Thr121 (NH)	(N1)	2.72
2	Diol dehydratase (1egm)	3	Cys302 (SH)	(N5)	2.50
			Ser301 (O)	(N1)	2.96
			Arg699 (N)	(O2)	2.82
3	Methionine synthase (3iv9)	2	His759 (NH)	(O4)	2.68
			His1145 (NH)	(O2)	2.72

FIGURE 3: Docking of riboflavin into enzyme diol dehydratase [14]. Riboflavin and protein binding sites are shown in element colors in wireframe and thick stick models, respectively, whereas cyanocobalamin is represented in pink color in thick stick.

were formed between the nitrogen (N1) of riboflavin and oxygen atom of Ser301 at a distance of 2.96 Å and between nitrogen of Arg699 and oxygen atom (O2) of riboflavin at a distance of 2.82 Å. The E-subunit comprises of active site of the enzyme which has been found to pack against the upper face of B₁₂ cofactor and may facilitate the transfer of 5'-adenosyl radical from cofactor to substrate. Therefore, it may be assumed that the riboflavin may interfere with the catalytic process of the enzyme by binding at the interface of cofactor B₁₂ and the enzyme's active site and thus inhibit the radical shuttling mechanism. The AScore value obtained for this complex was -6.98 kcal/mol which indicates that riboflavin significantly binds with the enzyme diol dehydratase.

3.3. Methionine Synthase. Figure 3 shows the binding of riboflavin near the cofactor making contact with amino acid residue His 759. The oxygen atom (O4) of the carbonyl group of riboflavin was hydrogen bonded to N-atom of the His 759 at a distance of 2.68 Å. Another hydrogen bonding

FIGURE 4: Docking of riboflavin into enzyme methionine synthase [15]. Riboflavin and protein binding sites are shown in element colors in wireframe and thick stick models, respectively, whereas cyanocobalamin is represented in pink color in thick stick.

was seen between the N-atom of His1145 and the carbonyl oxygen (O2) of riboflavin at a distance of 2.72 Å. The docking AScore of the ligand-protein complex was -6.07 kcal/mol. The crystal structure of methionine synthase revealed that the enzyme is present in "base-off" or "His-on" form with His759 as the lower axial ligand. His759 has been found to act as transient intermediate in the reductive reactivation and conformational transition of cobalt following methylation in methionine synthase-dependent enzyme catalysis [15]. Therefore, we may assume that riboflavin may inhibit the important catalytic step of the enzyme, that is, reactivation and methylation probably by making contact with the same amino acid residue (His759) that is involved in the reaction. The binding energy also shows a significant ligand-receptor complex with this enzyme.

4. Conclusion

By analyzing the docking results we hypothesized that riboflavin might have inhibitory activity against cobalamin coenzymes. The enzyme glutamate mutase has been found to be the most susceptible protein target for the studied ligand and the riboflavin showed the best binding affinity with this enzyme having least binding energy of -7.13 kcal/mol.

Glutamate mutase and diol dehydratase can be considered as important targets for the development of antibiotics. Other useful therapeutic drugs can also be synthesized to enhance the function of enzyme methionine synthase as it converts homocystein into methionine and requires methylcobalamin coenzyme for its function. Inactivation of this coenzyme leads to the elevated levels of homocystein in blood and urine and may result in clotting and in long-term damage to the arteries (stroke and heart attack). Therefore, the interaction of vitamin B₂ and B₁₂ and their role in important metabolic reactions in the body should be considered before preparing multivitamin complexes.

The fact that riboflavin participates in energy-based metabolic reactions may play an important role in enzyme catalysis which depends on a delicate energy balance for different reaction pathways. The local amino acids and substrates in the active sites of the enzymes may function in this respect.

Further experimental approaches can be adopted to probe the effect of structural alterations of flavin group of compounds in the catalytic properties of coenzyme B₁₂-dependent enzymes.

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