Molecular Dynamics Assessment of Modelled IgG Binding Receptor Protein (1PGB)

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Citation

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Abstract

Protein structure prediction is one of the most interesting and challenging area in Structural Biology due to its functional importance. In this paper an attempt has been made to minimize the gaps between insilico and wet lab determination of three-dimensional structure of a protein by molecular modelling and simulation technique. The working concept has been presented through small, typical B1 domain of protein (IgG binding receptors protein) case study. Insilico molecular model of IgG binding receptors protein has been prepared through MODELLER. Energy minimization and molecular dynamics calculations were done through GROMACS using OPLS force field and validation has been done through PROCHECK and ERRAT programs (97.04%). RMSD, RMSF, Phi (f) / Psi (), B-factor and ASA analysis, were calculated for modelled structure. The performance of prediction has been assessed by error estimation in Phi/Psi values.

INTRODUCTION

Protein structure prediction is very important due to its functional importance. Tertiary structure is the native state, or folded form, of a single protein chain in three dimensional space i.e. functional forms [12]. Primarily, tertiary structure of protein is defined through three dimensional coordinates. Another way to define the tertiary structure is as a sequence of backbone torsion angles phi () and psi () [3]. Protein structure determination in laboratory conditions is a time taking and not an economic process. Traditionally X-ray crystallography has been most dominant techniques for resolving protein structures followed by subsequent steps i.e. isolation, purification, crystallization, diffraction diagram and electron density map etc. More recently, NMR technology have been developed and refined to solve larger proteins structures in solution. Thus, both approaches have enormous experimental costs with intensive computer based process [4]. Molecular dynamics simulation approaches can expedite the structure prediction with lowest error and data loss. Various theoretical works has been done in the protein structure prediction with various parameters i.e. force fields temperature, pressure, deviation, fluctuations, unfolding, or interaction with other molecules etc. with different simulation approaches [5]. Before wetlab determination of proteins structure, insilico molecular modelling, energy minimization, quality checking and verification (i.e. RMSD, RMSF , Phi/Psi values, B-factor analysis, ASA analysis, dihedral angle distribution, analysis of Harmonic bond distribution, analysis of mean square displacement) should be performed[67].

MATERIAL AND METHODOLOGY

Here, we demonstrated the examples of small IgG binding receptor protein which having B1 domain of protein G. These proteins allow the pathogenic bacterium to evade the host immune response by coating the invading bacteria with host antibodies, thereby contributing significantly to the pathogenicity of these bacteria etc.

MODELLING OF IGG BINDING RECEPTORS PROTEIN

To accomplish the goal of study, modelling of IgG binding receptors proteins were performed using MODELLER. A template search has been performed through BLASTP programs [8]. Global alignment method was used for comparison between the target-template sequences [9]. Alignment file for MODELLER was prepared by CLUSTALW [10]. Energy minimization of generated 3D-model was done through GROMACS (OPLS force field) by using Steepest Descent and Conjugate Gradient Algorithms [7].

QUALITY ANALYSIS AND MOLECULAR DYNAMICS STUDIES

Parameters like covalent bond distances, bond angles, stereo

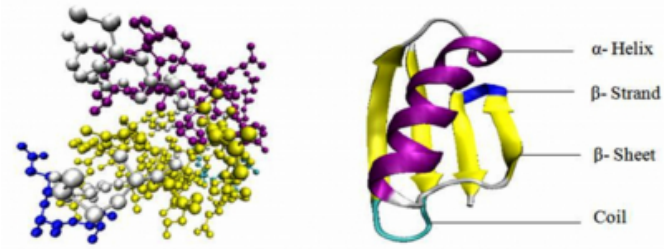
chemical validation, atom nomenclature were validated by Ramachandran plot by using PROCHECK software and overall quality factor of non-bonded interactions between different atoms types were measured by ERRAT program [11].Molecular dynamics and simulation studies have been performed through GROMACS simulation software. Various algorithms and model has been used for calculation of RMSD (root-mean-square deviation) and RMSF (Root Mean Square Fluctuation), ASA analysis, B-factor analysis of modelled structure [12].

RESULTS AND DISCUSSIONS

For modeling of IgG binding receptors protein, template search were performed with the BLASTP and 2ZW0 were found as suitable PDB template. -sheet has dominance in model. Modeled structure of protein is shown in figure-1.

Figure 1

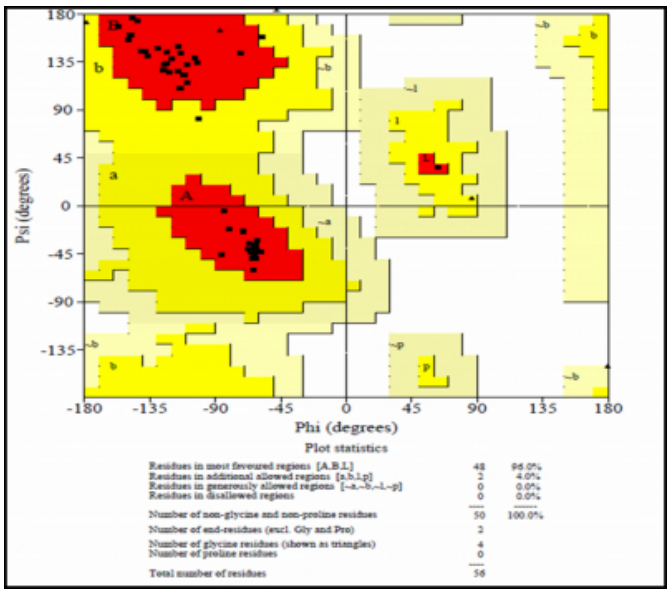
Figure 1: Three dimensional visualization of the modeled structure of protein through VMD software.



Energy minimization of generated 3D-model was done through GROMACS (OPLS force field by using steepest descent and conjugate gradient algorithms).The generated 3D model of target proteins was checked by Ramachandran plot (Figure-2) through PROCHECK program.

Figure 2

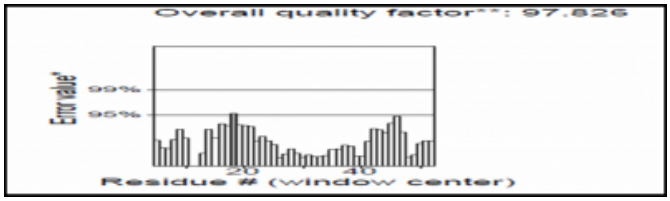
Figure 2: shows torsion angles of and in the generated models through Ramachandran plot.



In modelled structure, 96.03% residues were lies in most favoured region in Ramachandran plot. The overall quality factor for modelled structure was reported through structure validation server program ERRAT (Figure-3).

Figure 3

Figure 3: shows the overall quality factor of modelled protein through structure validation server ERRAT

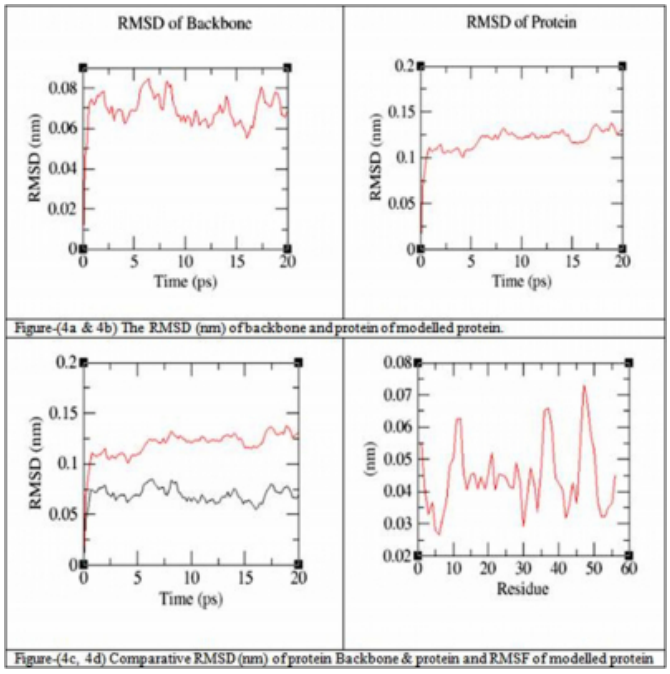


RMSD AND RMSF - RELIABLE INDICATORS TO CHECK VARIABILITY

The molecular dynamics simulations have provided significant new information on the nature of proteins. RMSD measures the accuracy whereas dynamic fluctuations (RMSF) of proteins around their average conformations play an important indicator of many biological processes such as enzyme activity, macromolecular recognition, and complex formations [13].

Figure 4

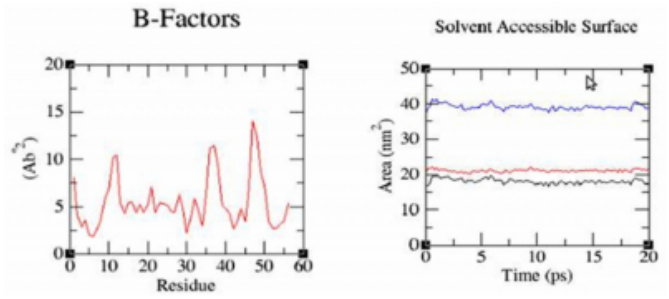
Figure 4: Analyzing structural variability of modelled protein structures using RMSD (a, b and c) and RMSF (d) after least square fit.



The changes in structural conformation were monitored in terms of RMSD and RMSF. Figure -4(a, b,) shows RMSD for modelled protein after 20 ps time when calculated for Protein-Protein was 0.123 and for Backbone-Backbone was 0.068. Figure-4c shows the comparative RMSD of protein and backbone. Figure-4d shows the RMSF fluctuations upto 20ps (0.025 to 0.071).

Figure 5

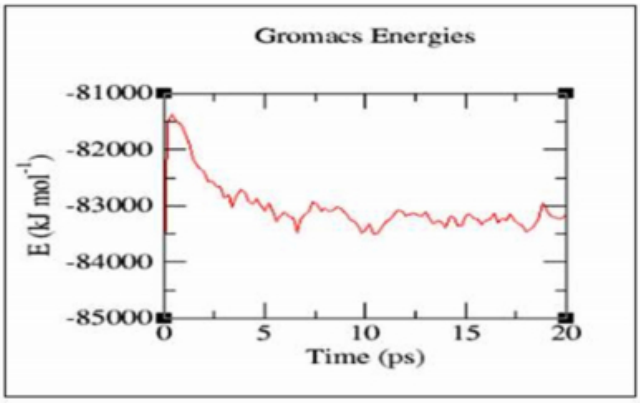
Figure 5(a & b): B-Factors Analysis and Solvent Accessible Surface of modelled protein.



Theoretical temperature factor for amino-acid residues backbone atoms (in Ab02) computed from RMSF as B=8/3π rmsf2 and averaged over last 20ns (figure-5a). Graph shows higher fluctuations in structural elements transitions regions which exposed to solvent that are not involved in binding sites and average B-factor is 5(Ab02) [14]. Solvent accessible surface area (ASA) is a direct measure of interaction of solute and solvent, which in a simple way relate to the hydrophobic energy in the empirical calculations [15]. Figure-5b shows the total SAS (blue), hydrophilic (red) and hydrophobic (black).

Figure 6

Figure 6: Protein stability analysis through RMSD and Energy data in 20ps time.



Protein stability analysis has been performed through RMSD and energy data in 20ps simulation time. It is clear from figure-6 that protein is most stabilized around 13ps during simulation and it is again confirmed by RMSD of protein (Figure- 4b). Most stable protein conformation has been exposed in term of Phi/Psi values.

COMPUTATION OF PHI/PSI FOR MODELLED PROTEIN

In the present study, tertiary structure prediction of protein has been made through backbone torsion angles phi (□)/psi (□). The combination of □ and □ angles fully determine the backbone configuration of a protein. More than 27% Phi and 33% Psi data of residues are missing in x-ray crystallographic structure of IgG binding receptors protein (PDBID-1PGB). Computation of Phi and Psi angles has been done for entire chain (56 residues) of modelled protein for the most stable conformation. A error estimation have been made for generated data (Phi/Psi values from modelled protein) with wetlab data (Phi/Psi values from PDB data). Error evaluation in Phi values (1.2%) and Psi value (5.3%) has been done.

CONCLUSION

In this paper an attempt has been made to minimize the gaps between insilico and wet lab determine of three-dimensional structure of a protein by molecular modeling and simulation technique. To achieve the goal, we modelled the IgG binding receptors protein by using MODELLER (Figure-1) and

validated by PROCHECK and ERRAT programs. In modelled protein, 96.03% residues were lying in most favoured region in Ramachandran plot (Figure-2) which was good indicator of fitness of stereochemical quality of protein structures. The overall quality factor for modelled protein (95.098%) was reported through structure validation server ERRAT (Figure-3). Energy minimization and molecular dynamics calculations were done through GROMACS using OPLS force field. RMSD for modelled protein after 20 ps time when calculated for Protein-Protein was 0.123 and for Backbone-Backbone was 0.068(Figure- 4a & 4b). An average RMSF fluctuation was reported from 0.025 to 0.071(Figure-4d). Computation of Phi and Psi angles has been done for entire chain (56 residues) of modelled protein for the most stable conformation. A error estimation have been made for generated data with wetlab data. Error in Phi values (1.2%) and Psi values (5.3%) prediction were reported.

FUTURE DIRECTIONS

The present model for tertiary structure prediction can be applied for those proteins which have difficult and erroneous structural estimation prior to the wet lab assessment. The work will contribute positively toward the reduction of errors in results and also helpful in minimization the labour cost. All these analysis and predictions are made on the basis of bioinformatics tools & techniques by statistical analysis.

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