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RESEARCH ARTICLE

STRUCTURE PREDICTION AND ASSESSMENT OF BETA-LACTAMASE TEM-1 FROM S. *TYPHI* USING MOLECULAR DYNAMICS AND SIMULATION STUDIES

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ABSTRACT

Beta lactamase is one of the main reasons behind the development of antibiotic resistance among pathogenic bacteria against beta lactam antibiotics. *S. typhi*, Gram negative bacteria is main causative agent among human population in developing countries has developed resistance against β-lactam antibiotics which are already in their fourth generation. The availability of a good quality three dimensional structure of target protein is necessary for the development of therapeutic drugs. A good quality three dimensional structure of Beta lactamase TEM-1 from *S. typhi* was not available. In this study, primary, secondary and tertiary structures of Beta lactamase TEM-1 from *S. typhi* were predicted using Expasy's Protpram, PSIPRED and Homology modelling method respectively. The modelled structure was energy minimized and molecular dynamics and simulation studies were performed to investigate how the predicted model behaves structurally, dynamically and thermodynamically using Gromacs 5.1 molecular dynamics and simulation tool. The structure was predicted to be fast attaining the normal temperature (300 K), pressure (1 Bar) and density (1000 Kg/m^3) values during NVT (constant volume and normal temperature) and NPT (constant normal pressure and normal temperature) simulation steps within the given timeframe using OPLS-AA force field and SPC/E water model. The predicted structure was also validated using PROCHECK. The predicted model was found to be stereo-chemically stable in Ramachandran plot produced by PROCHECK as more than 93% residues were falling under core regions of the plot. The structure will aid in discovery of more efficient alternative therapeutic drugs against typhoid causing *S. typhi*.

INTRODUCTION

S. typhi; a Gram negative bacteria is the major causative agent of typhoid fever among human population in developing countries and is emerging as a major global concern among last two decades (Choudhary A. *et al.*, 2013). Indiscriminate use of antimicrobials has led to the development of Multiple Drug Resistance against β-lactam antibiotics which are already in their fourth generation. The World Health Organisation (WHO) in 1996 predicted approximately 33 million typhoid fever cases every year resulting in 5-6 Lac deaths and case fatality rates between 1.5-3.8% (Farooqui A. *et al.*, 2009). Beta lactamses TEM-1 (EC=3.5.2.6) are major cause of antibiotic resistance against penicillins, cephalosporins, and related compounds among bacterial species.

The availability of a good quality three dimensional structure of target protein is necessary for the development of therapeutic drugs. There are many approaches to generate a 3D structure of a protein such as homology modelling, threading (Dorn M *et al.*, 2014) and Ab-initio method (Samudrala R *et al.*, 1999). One of the most reliable and widely used methods for elucidating a protein's 3D structure is homology modelling which builds the structure based on the homology of the query sequence with the target protein whose three dimensional structure is already available (Meier A *et al.* 2015). The models generated using the modelling techniques are static but

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naturally all proteins are dynamic in nature. To study the dynamics of the protein one has to perform molecular dynamics studies of the given model. Molecular dynamics studies are performed to investigate how protein model behaves structurally, dynamically and thermodynamically in a force field. The predicted 3D structures of Beta lactamase TEM-1 from *S. typhi* are available on Protein Model Portal and Swissmodel repository based on templates TVFA (12% identity) and 1XPB (S235A mutant, 100% identity from 24-286 residue range) respectively. In this study the 3D structure of Beta lactamase TEM-1 of *Salmonella typhi* CT-18 was modelled based on the wild type Beta lactamase TEM-1 template from *E. coli* (PDB ID: 1ZG4) sharing 99.7% sequence identity with each other over the entire length. The present study is focussed on elucidating more accurate model of the protein using wild type and more identical template by homology modelling method and to study the behaviour of the model through molecular dynamics and simulation studies using Gromacs 5.1 molecular dynamics package. This will direct researchers to develop more efficient alternative therapeutic drugs against *S. typhi*.

METHOD

Data Collection

The amino acid sequence information of Beta lactamase TEM-1 (Uniprot ID: P62594) (Parkhill *et al.*, 2001) from *Salmonella typhi* was mined from Uniprot database (http://www.uniprot.org/uniprot/P62594).

Primary structure prediction

Various physico-chemical properties such as amino acid composition, molecular weight, number of positive and negative ions, theoretical Isoelectric point (pI), half life, aliphatic index, instability index (Guruprasad K. *et al.*, 1990), Extinction coefficient (Gill SC and PH., 1989), Grand Average Hydropathicity (GRAVY) (Kyte and Doolittle, 1982) associated with the primary structure of P62594 were predicted by analysing the amino acid sequence of the protein using Expasy's ProtParam server (http://web.expasy.org/protparam/).

Secondary structure prediction

The secondary structure of TEM Beta lactamase was predicted using PSIPRED (Buchan D. W. *et al.*, 2013) server (http://bioinf.cs.ucl.ac.uk/psipred/). The secondary structure was compared with the modelled tertiary structure to find the structural correspondence of the amino acid residues in various structural domains of the model generated by Modeller 9.15.

3D structure prediction, Energy minimization and Molecular dynamics Studies

The template for the structure modelling was selected by performing Blastp search keeping PDB as target database. It was found that Beta lactamase TEM-1 (PDB ID: 1ZG4) from *E. coli* shared the 99.3% identity with the queried protein. The 3D structure of the protein was determined using Modeller 9.15 modelling tool (Eswar N. *et al.*, 2006). Energy minimization using steepest decent method and molecular dynamics studies of all the predicted structures were performed with Gromacs 5.1 (Van Der Spoel D. *et al.* 2005) using OPLS-AA force field and SPC/E water model at standard temperature and pressure parameters. The energy plots of the simulations were plotted using Grace plotting tool. The active site of P62594 was predicted using PROSITE.

Structure analysis and visualization

All the visualizations were performed using DeLano Scientific PyMol 3D molecular viewer. The RMSD analysis of the 3D structure was done with the template and the energy minimised structure to find the accuracy of the model. The predicted structure before energy minimisation was compared with the energy minimised structure and template structure to identify any significant distortion in the active site region of the protein. The steriochemical stability of the predicted structure was studied with PROCHECK (Laskowski R. A. *et al.*, 1993), QMEAN Z-score (Benkert P. *et al.*, 2011) and Dfire (Zhou and Zhou 2002) parameters at Swissmodel server (Schwede T. *et al.*, 2003).

RESULTS AND DISCUSSION

Primary structure prediction

The primary structure analysis by Expasy's ProtParam (Table 1) showed that P62594 contain 286 amino acids residues with estimated molecular weight of 28907.0. The theoretical pI (pH at which protein remain stable) was predicted to be 5.46 which is less than pI=7 which implies that the protein can be considered as acidic in nature.

Table1 Various physico-chemical properties of Beta lactamase TEM-1 from *S. typhi* predicted using Expasy Protparam tool.

Primary structure prediction of Beta lactamase TEM-1 from *S. typhi*	
Parameters	**Predicted value**
Molecular weight	28907.0
Theoretical pI	5.46
Number of positive residues	29
Number of negative residues	36
Half life mammalian reticulocytes (*in vitro*)	3.5 hrs
Half life yeast (*in vivo*)	10 mins
Half life *E. coli* (*in vivo*)	10 hrs
Extinction coefficient	28085-27960
Instability index	39.65
Aliphatic index	92.05
GRAVY index	-0.253

pI- Isoelectric point; GRAVY- Grand Average Hydropathicity

This estimate can be used to develop the buffer system for the protein of interest. The total number of positive (R+K) and negative residues (D+E) were calculated to be 29 and 36 respectively. The half life can be defined as the time taken to degrade a protein to its half concentration after is synthesis. The half life of P62594 for three model organisms (humans, yeast and *E. coli*) was estimated to be 3.5 hrs for mammalian reticulocytes *in vitro*, 10 mins for yeast *in vivo* and more than 10 hrs for *E. coli in vivo*. The extinction coefficient indicates

the amount of light absorbed by a particular protein at 280 nm measured in water in a spectrophotometer during its purification. The extinction coefficient for P62594 was estimated to be ranging between 28085-27960 based on the cystine composition.

>sp|P62594|BLAT_SALTI Beta-lactamase TEM OS=*Salmonella typhi* GN=bla PE=3 SV=1

MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLG
ARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAV
LSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMT
VRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGD
HVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLT
GELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIAD
KSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDE
RNRQIAEIGASLIKHW

FASTA sequence of TEM Beta lactamase of S. typhi (P62594) in FASTA format retrieved from Uniprot database

Statistical analysis of 12 unstable and 32 stable proteins which revealed that there is significant difference in occurrence of certain dipeptides in unstable proteins as compared to stable proteins (Guruprasad K. *et al.*, 1990).

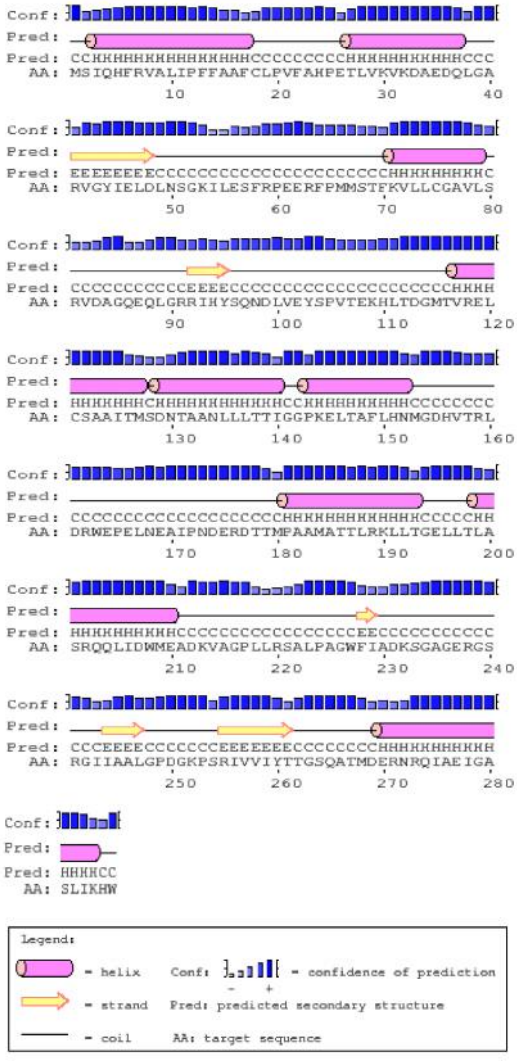

Fig 1 Secondary structure prediction of P62594 by PSIPRED showing various predicted structural domains.

Based on this information the information instability weight value was assigned to each of 400 hundred different dipeptides which were used to calculate instability index of the proteins. It was shown that the proteins with instability index of less than 40 were stable and value more than 40 were predicted to be unstable. The instability index of P62594 was predicted to be 39.65 indicating that the protein will be stable in test tube. The relative volume occupied by aliphatic side chains known as aliphatic index shows the thermal stability of the protein over wide range of temperatures. Higher value of aliphatic index indicates higher stability of the protein. The aliphatic index for P62594 is predicted to be 92.05. The GRAVY indices indicate the probability of the interaction of protein with water. The lower the value the higher will be probability that the protein will interact with water. The GRAVY index for P62594 was predicted to be -0.253. The GRAVY score shows that the protein will be interacting with water which indicates that the protein is antigenic in nature.

Secondary structure prediction

The secondary structure of a protein is composed of helices, sheets and coils. The prediction of secondary structure (fig. 1) of P62594 was performed with PSIPRED server which is based on the analysis of output obtained from PSI-BLAST (Position Specific Iterated - BLAST) by two feed-forward neural networks (Jones D. T., 1999). The results reveal that there is highest percentage of random coils than alpha helices and beta sheets; beta sheets having the least structural contribution.

Tertiary structure prediction, Energy minimisation and molecular dynamics

The tertiary structure of P62594 was modelled with homology modelling tool, modeller 9.15. The protein was queried in Blastp local alignment tool to find the homologous sequences using PDB as target database. The results showed that Beta lactamase TEM (*E. coli*) PDB ID: 1ZG4 shares 99.3% identity with the queried sequence (Fig. 2). So it was selected as the template for Modeller 9.15.

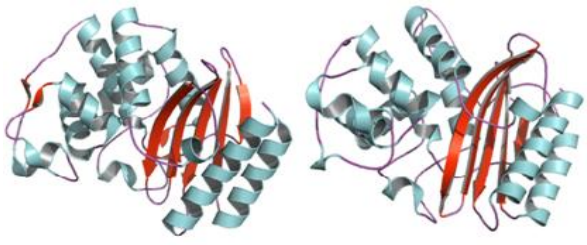

Fig. 2 Modelled Structure of Beta lactamase from *Salmonella typhi* (Uniprot: P62594) using Modeller 9.15 and Template Beta lactamase TEM from *E. coli* (PDB 1ZG4; 99.3% identity).

The modelled 3D structure of the target Beta lactamase TEM from *Salmonella typhi* CT 18 (Uniprot ID: P62594) and template Beta lactamase TEM from *E. coli* PDB: 1ZG4 (Stec B. *et al.*, 2005) were superimposed and calculated RMS= 0.127 (fig. 3) which shows the overall good quality of the modelled structure. The active site Ser 68 and Glu 166 of the target was determined from Scan PROSITE (http://prosite.expasy.org/scanprosite/) (de Castro *et al.*, 2006).

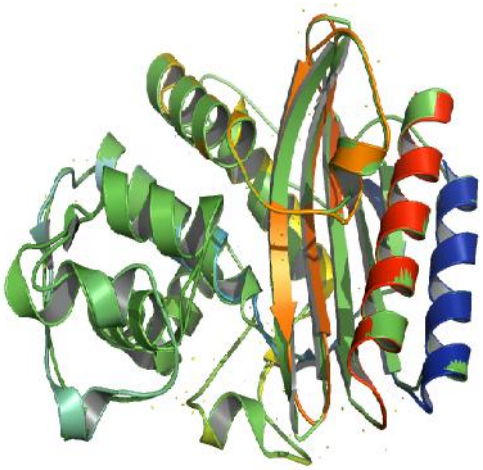

Fig. 3 Superimposed structures of 1ZG4 *(E. coli)* and TEM Beta lactamase *(S. typhi)* using PyMol visualization tool. Calculated RMS= 0.127.

The 3D modelled structure of the receptor was compared with the predicted secondary structure and the results revealed that both the predictions were consistent with each other.

The Ramachandran plot (fig. 4) of the model generated using PROCHECK shows that 93.6% of the residues are occurring under the core region which shows that the model is stereo-chemically stable.

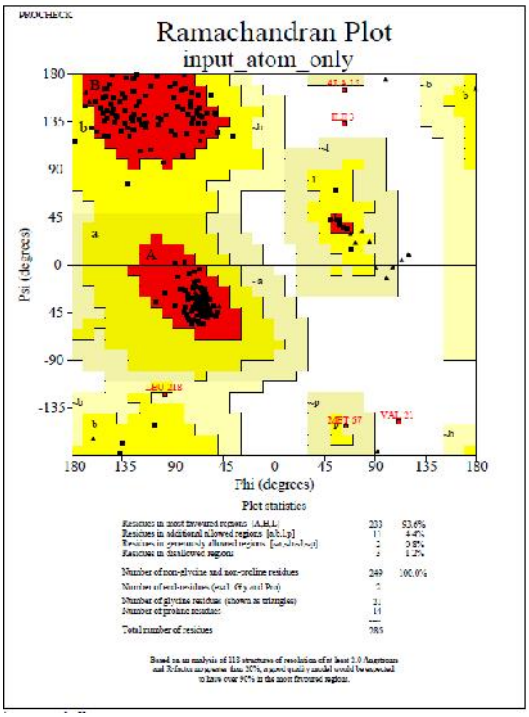

Fig 4 Ramachandran plot of P62594 generated using PROCHECK showing the occurrence of 93.6% amino acids in the core regions of the plot.

The QMEAN Z-score represents an measure of the absolute quality of a model by providing an estimate of the 'degree of nativeness' of the structural features observed in a model and by describing the likelihood that a given model is of comparable quality to experimental structures (Benkert P. *et al.*, 2011). Models of low quality are expected to have strongly negative QMEAN Z-scores (i.e. the model's QMEAN Z-score is several standard deviations lower than expected for experimental structures of similar size). QMEAN Z-score for the predicted Beta lactamase TEM-1 structure was predicted to be 0.22 which shows that the predicted structure is of high quality. The DFire is the statistical potential for all atoms based on the distance-scaled finite ideal gas reference state and is used to assess the non-bonded atomic interactions in the modelled protein. Lower energy values of DFire represent a model closer to the native conformations. The calculated DFire value for Beta lactamase TEM-1 is -394.32.

The modelled structure of Beta lactamase TEM-1 was subjected to molecular dynamics and various parameters were calculated (Table 2) to investigate the structure dynamics and thermodynamics of the model using Gromacs 5.1 molecular simulation tool. The energy of the model structure was minimised to the average of -1.05243e+06 KJ/mol with -133156 KJ/mol of total drift from the predicted model. The energy minimized system was then equilibrated for temperature, pressure and density parameters over 100 ps of simulation. The results show that the system reaches the target temperature of 300 K.

Table 2 Various molecular dynamics parameters for Beta lactamase TEM-1 from *S. typhi* calculated using Gromacs molecular dynamics tool

Gromacs Molecular Dynamics Parameters		
Parameter	**Average**	**Total Drift**
Potential (KJ/Mol)	-1.05E+06	-133156
Temperature (K)	299.784	1.01723
Pressure (Bar)	1.66243	4.39189
Density (Kg/m^3)	1021.37	1.23369

The average value of pressure during the equilibration phase remains around 1.66243 Bar. The density value was calculated to be averaging around 1021.37 Kg/m^3 which is comparable to the experimental value of water (1000 Kg/m^3). This shows that the system is stable over the entire course of simulation (fig. 5). The active sites of the model before and after molecular dynamics simulation were compared to identify any deviation from the native conformation using Pymol (fig. 6). The structural alignment of the active sites showed the RMSD of 0.252, which shows that there is no significant deviation of the active site residues after molecular dynamics simulation.

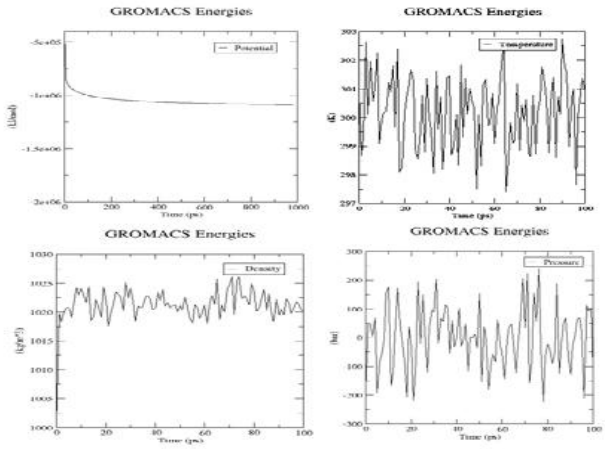

Fig 5 Molecular dynamics simulations over 100 ns showing the structural stability of the predicted structure of Beta lactamase TEM-1 from *S. typhi*.

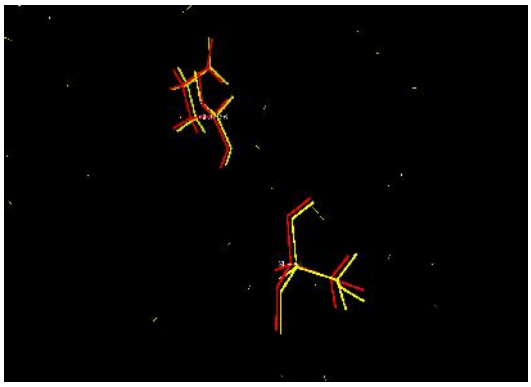

Fig 6 Comparison of the active site residues Ser 68 and Glu 166 of Beta Lactamase TEM-1 before (red) and after (yellow) molecular dynamics simulations.

CONCLUSION

The availability of reliable 3D structure of a molecular target is essential for the drug discovery. In this study, the primary, secondary and tertiary structures of Beta lactamase TEM-1 from *S. typhi* were predicted using Expasy's Protparam, PSIPRED and Homology modelling method respectively. The structure assessment of the predicted model was performed with QMEAN Z-score, D-fire and Ramachandran plot. The QMEAN Z-score for the Beta lactamase TEM-1 structure was predicted to be 0.22 which shows that the predicted structure is of high quality. Lower energy values of DFire represent a model closer to the native conformations. The calculated DFire value for Beta lactamase TEM-1 is -394.32. The Ramachandran plot showed that more 93.6% residues were falling under core region which means that the predicted structure is stereo-chemically stable. The structure, dynamics and thermodynamics of the modelled structure were investigated thorough molecular dynamics studies performed under OPLS-AA force field with Gromacs 5.1 showed that the modelled structure attained normal temperature and pressure values during NVT and NPT simulations under OPLS-AA force field and SPC/E water model. This showed that the predicted model was structurally, dynamically and thermodynamically stable. Comparison of the active sites of Beta lactamase TEM-1 showed that there is not much significant deviation of the structure from that of the original crystal structure. The structure provides a reliable platform for the designing specific antimicrobial drugs against antibiotic resistant *Salmonella typhi*.

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