Homology Modeling And Computational Assessment Of Class I Lysyl tRNA Synthetase Of Syphilis Causing Pathogen Treponema Pallidum

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Citation

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Abstract

Lysyl-tRNA synthetases (LysRSs) are fashionable amongst the aminoacyl-tRNA synthetases in being composed of unrelated class I and class II enzymes. The prominent role of aminoacyl-tRNA synthetases is to interpret the genetic code in terms of amino acids, providing the essential link between RNA and protein without which translation would be impossible. The recent genomic analysis showed that Class-I Lysyl tRNA synthetase of Archaea is showing similar homology with Treponema pallidum and other spirochete but not found in any eukaryota species. Because the Class-I Lysyl tRNA synthetase is constrained in Treponema pallidum, subsequently Class-I tRNA synthetase is considered to be a best drug target. The experimental structure for Lysyl Class I t RNA synthetase of Treponema pallidum is still unknown for the reason that Treponema pallidum could not be grown in vitro conditions. In present study aimed at to develop computational 3D model and structure assessment for Class-I Lysyl tRNA synthetase of Treponema pallidum.

INTRODUCTION

The computational approach is assumption that the potential target must play a prominent role in the pathogen's survival and constitute a critical component in its metabolic pathway. At the same time, this target should not have any well-conserved homolog in the host. Drug target identification is essentially subtractive because we use a subtraction template while comparing the two genomes under consideration. The focus is on the complement of the genome of the pathogen that is essential for it but is not present in human. Novel drug targets are required in order to design new defense against antibiotic sensitive pathogens. Multiple approaches to locate essential genes in a given organism exist, some of which focus on the concept that essential genes tend to be evolutionarily conserved over species (Itaya, 1995; Tatusov et al., 1997; Koonin et al., 1998; Kobayashi et al., 2003; Peeling RW and Hook EW, 2006).

Lysyl-tRNA synthetases (LysRSs) are fashionable amongst the aminoacyl-tRNA synthetases in being composed of unrelated class I and class II enzymes. The aminoacyl-tRNA synthetases have long been upheld as an Archaeal type of molecular evolution. This is because their products, aminoacyl-tRNAs, are essentially the same in all living organisms. The prominent role of aminoacyl-tRNA synthetases is to interpret the genetic code in terms of amino acids, providing the essential link between RNA and protein without which translation would be impossible. This highly conserved function has been assumed to place constraints on the evolutionary variation of aminoacyl-tRNA synthetases beyond those enforced on most other protein families.

Most aminoacyl-tRNA synthetases belong to one of two unrelated structural classes. The only widespread exceptions are the lysyl-tRNA synthetases, which are class I enzymes in certain bacteria and archaea but are otherwise members of class II. The class I lysyl-tRNA synthetase is found in a number of pathogenic bacteria (Borrelia burgdorferi, various Brucella and Rickettsia species, Treponema pallidum and Tropheryma whippelii) and is fundamentally different from the human class II enzyme, identifying the class I bacterial enzyme as a potential target for developing anti-infective agents. The aim of our work is to investigate structure/function relationships in the recently discovered class I-type lysyl-tRNA synthetases. The major goals include: i) Determining how class I and class II lysyl-tRNA synthetases recognize and discriminate between lysine and lysine analogues. ii) Identifying the protein-RNA

interactions that determine lysine tRNA recognition in vitro and in vivo. The results of these experiments will reveal how the same activity (attachment of lysine to lysine tRNA) can be achieved within two completely different structural frameworks. This, in turn, will provide a framework for developing therapeutics specifically targeted against the class I lysyl-tRNA synthetase (Peeling RW et.al., 1997).

The solved X-ray structure of Class-I Lysyl tRNA synthetase from Pyrococcus horikoshii, an Archaeal species considered as structure template for homology modeling of Class-I Lysyl tRNA synthetase of Treponema pallidum. The resultant homology model was subjected for structure assessment to evaluate 3D model accuracy. The resulting 3D model of Class I-type Lysyl tRNA synthetase of Treponema pallidum represents an essential support to further molecular docking studies on its structure-function relationships and as well as to design novel molecules useful to modulate its activity and to discover appropriate drug molecule(Terada et al, 2002).

MATERIALS AND METHODS

The protein sequence of Class-I Lysyl tRNA synthetase of Treponema pallidum (O83650) was obtained from Swiss-Prot protein database (http://us.expasy.org/sprot). A pairwise comparision of target protein was done against Protein data bank (http://rcsb.org) using BLASTp search method (http://www.ncbi.nlm.nih.gov/blast/). Homology models were generated using MODELLER by satisfaction of spatial restraints (Andrew Šali et. al., 1993). The most reliable structure was chosen using chemical quality of the models by PROCHECK analysis (Laskowski RA. et. al., 1993). The predicted model quality assessment was checked using Ramachandran plot analysis (Ramachandran. G.N et al.,1963) for phi and psi tortional angles.

RESULTS

The results were obtained by the methods mentioned above are summarized. The data of BLAST search results showed that, structure template of Pyrococcus horikoshii (IRX) and target protein of Treponema pallidum (Class I lysyl tRNA synthetase) found to be the best homology among BLAST comparison hits. The overall sequence identity between structure template sequence and target protein was 40% and the E-value is 3e-110.

A group of 20 models of Class-I Lysyl tRNA synthetase of Treponema pallidum was modeled. The overall structural comparison of twenty models was well overlaid with native experimental structure. The graphical representation of predicted model and template structures is given in Figure 1.The result of predicted model of Ramachandran plot in PROCHECK illustrate 92.5 % residues in allowed region (Figure 2).

Figure 1

Figure 1: The diagrammatic representation of homology model of Class I-type Lysyl tRNA synthetase of (A) and experimental structure of Class I-type Lysyl tRNA synthetase (PDB:1IRX) of (B).

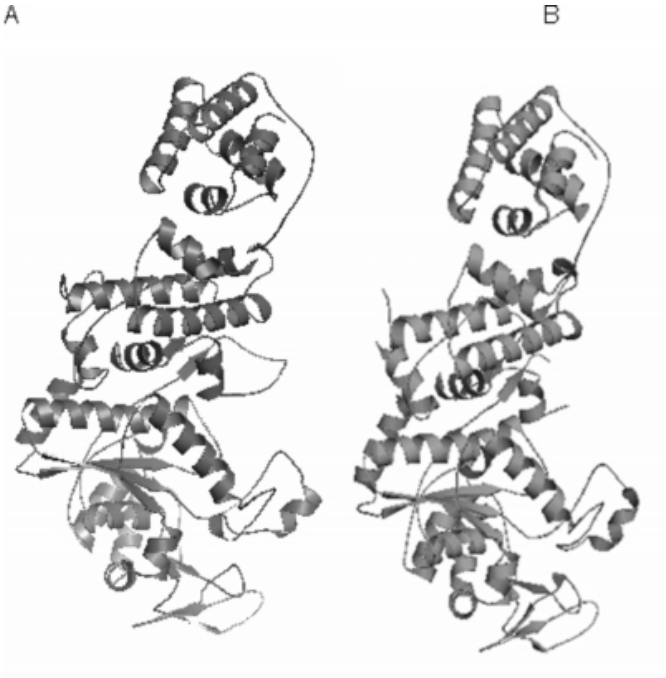
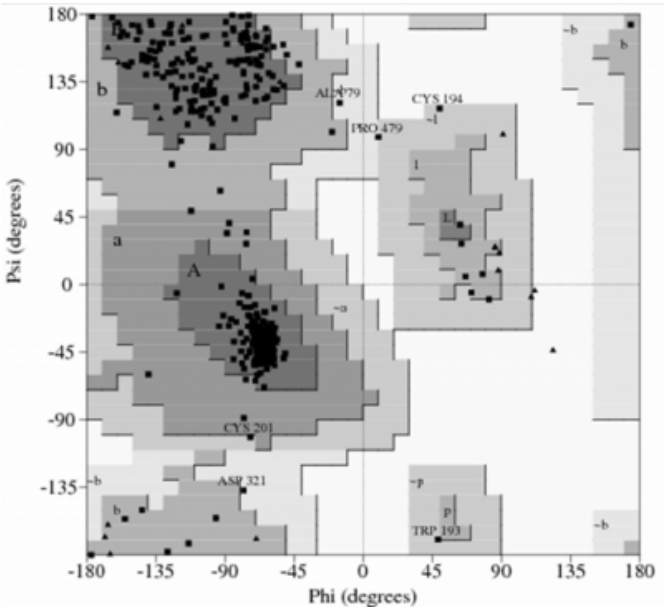


Figure 2

Figure 2: The Ramachandran plot of Class I-type Lysyl tRNA synthetase predicted model of . The data were generated using PROCHECK. The dark grey area represents most allowed regions, whereas the medium gray areas represent allowed regions. Glycine residues are represented by triangles with other residues represented by squares.



The Best quality model would be expected to have above 90% (Ramachandran 1963). The Bond length and bond angles analysis of overall Treponema pallidum of Class-I Lysyl tRNA synthetase 3D model reported in Table.1. The obtained mean values of all bond lengths and bond angles are comparatively satisfied with small molecular experimental data proposed by Kabsch et al. (Kabsch et.al., 1983). The comparative modeling in biology is already rewarding and increasingly wide spread (Marc et.al., 2000).

Figure 3

Table 1: The Stereo chemical analysis report of Class I-type Lysyl tRNA synthetase of generated by PROCHECK: A) Stereo chemical parameter values of main chain. B) stereo chemical parameter values of main chain. C) The over all assessment of structure's quality using Morris et al (1992) stereochemical classification scheme. The number assigned under class column indicates the quality of structure.(1 being the best and 4 the worst score).

A)

STEREOCHEMISTRY OF MAIN-CHAIN

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. %-tage residues in A, B, L	468	92.5	83.8	10.0	.9	Inside
b. Omega angle st dev	518	4.0	6.0	3.0	-.7	Inside
c. Bad contacts / 100 residues	14	2.7	4.2	10.0	-.2	Inside
d. Zeta angle st dev	493	1.2	3.1	1.6	-1.2	BETTER
e. H-bond energy st dev	302	.7	.8	.2	-.3	Inside
f. Overall G-factor	520	.0	-.4	.3	1.2	BETTER

B)

STEREOCHEMISTRY OF SIDE-CHAIN

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. Chi-1 gauche minus st dev	70	7.5	18.1	6.5	-1.6	BETTER
b. Chi-1 trans st dev	176	8.7	19.0	5.3	-1.9	BETTER
c. Chi-1 gauche plus st dev	189	6.4	17.5	4.9	-2.3	BETTER
d. Chi-1 pooled st dev	435	7.6	18.2	4.8	-2.2	BETTER
e. Chi-2 trans st dev	115	9.3	20.4	5.0	-2.2	BETTER

C)

MORRIS ET AL. CLASSIFICATION

Parameter	Mean m	St.dev s	Classification 1	2	3	4	Value	Class
Phi-psi distribution	-	-	>75.0%	>65.0%	>55.0%	<55.0%	92.5	1
Chi-1 st.dev.	18.2	6.2	<12.0	<18.2	<24.4	>24.4	6.5	1
H-bond energy st dev	.87	.24	< .63	< .87	<1.11	>1.11	.75	2

In our study, we developed a knowledge based 3D model using computational approach. The structure assessment has been carried out to developed 3D model to evaluate the accuracy of the predicted 3D model folding. The model predicted through this method would helpful to study dynamic nature of Class-I t RNA synthetase in order to find structure and function determinant residues.

DISCUSSION

There is an urgent need to develop new classes of antibacterial agents to tackle effective drug targets in bacterial pathogens which are unable to grow in in vitro conditions (McDevitt D et. al.,2001). The main aim is to identify, exploit and analysis of new molecular drug targets at structural level. This computational approach will lead to the discovery and structural development of novel drug targets.

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