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An *in silico* approach to evaluate the polyspecificity of methionyl-tRNA synthetases

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ABSTRACT

Residue-specific incorporation is a technique used to replace natural amino acids with their close structural analogs, unnatural amino acids (UAAs), during protein synthesis. This is achieved by exploiting the substrate promiscuity of the wild type amino acyl tRNA synthetase (AARS) towards the close structural analogs of their cognate amino acids. In the past few decades, seleno-methionine was incorporated into proteins, using the substrate promiscuity of wild type AARSs, to resolve their crystal structures. Later, the incorporation of many UAAs showed that the AARSs are polyspecific to the close structural analogs of their cognate amino acids and that they maintain fidelity for the 19 natural amino acids. This polyspecificity helps to expand the use of this powerful tool to incorporate various UAA residues specifically through *in vivo* and *in vitro* approaches. Incorporation of UAAs is expensive, tedious and time-consuming. For the efficient incorporation of UAAs, it is important to screen substrate selectivity prior to their incorporation. As an initial study, using a docking tool, we analyzed the polyspecificity of the methionyl-tRNA synthetases (MetRSs) towards multiple reported and virtually generated methionine analogs. Based on the interaction result of these docking simulations, we predicted the substrate selectivity of the MetRS and the key residues responsible for the recognition of methionine analogs. Similarly, we compared the active site residues of the MetRSs of different species and identified the conserved amino acids in their active sites. Given the close similarity in the active site residues of these systems, we evaluated the polyspecificity of MetRSs.

1. Introduction

Aminoacyl tRNA synthetase (AARS) is the central component of the translational apparatus of the cell that exerts control over the accuracy of translation by charging the target tRNA molecule with its cognate amino acid [1]. In the past few decades, it has been reported that the endogenous AARS fails to discriminate between natural amino acids (cognate amino acid) and their close structural analogs, unnatural amino acids (UAAs). This substrate promiscuity of AARS towards these close structural analogs favors the residue-specific incorporation of UAAs into recombinant proteins [1,2]. Using this methodology, more than 100 UAAs were globally incorporated into proteins through *in vivo* and *in vitro* approaches [1,3,4]. This technique is referred to as residue-specific incorporation and facilitates the multi-site incorporation of UAAs. In the *in vivo* approach, auxotrophic cells were used to deprive the specific natural amino acid, which helps allowed efficient UAA incorporation in a controlled manner. A number of analogs of tryptophan, phenylalanine, tyrosine, proline and methionine have been incorporated into proteins with the help of their respective auxotrophs. Recently, a polyauxotrophic cell was used for multi-unnatural amino acid (MUAA) incorporation, in which two or more UAAs were simultaneously incorporated into the protein in a residue-specific manner [5]. These incorporated UAAs are used as probes of protein structure and function; they also expand the function and characteristic features of protein. Currently, analyzing its polyspecificity and developing mutant AARSs with broad substrate specificity for the efficient incorporation of UAAs are interesting targets in the protein-engineering field [6].

Methionyl-tRNA synthetase (MetRS) belongs to the family of class I AARSs that acylate methionyl tRNA with methionine [7]. The *in vivo* and *in vitro* incorporation of 25 methionine analogs into recombinant proteins was carried out using methionine auxotrophic cells and an *in vitro* translation system [8]. Among these methionine analogs, many UAAs have not been incorporated into proteins due to a lack of activity by the endogenous tRNA synthetase. On the other hand, testing the translational activity towards UAA is an expensive, tedious and time-consuming process. Therefore, it would be advantageous to develop an easy and efficient screening technique for identifying the best candidates

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for UAA incorporation. Recently, molecular docking was used as a tool for predicting the most suitable UAA substrate for endogenous tRNA synthetase [9–11]. Molecular docking also aids the development of AARSs with relaxed or altered substrate specificities by extending their activity towards non-reactive substrates [12]. Based on the docking score, docking simulation depicts the geometry of the compound and its binding affinity, which in turn provides information about sterically hindering residues.

This rational designing strategy will greatly enhance the growing number of interesting UAAs for incorporation into proteins. In this study, we carried out a bioinformatics analysis to assess the polyspecificity of wild type MetRS of *Escherichia coli* (*E. coli*) and to identify the crucial residues responsible for the polyspecificity of AARS. We also report the sequential and structural comparison of the catalytic cores of MetRSs belonging to different kingdoms of life and their conformational flexibility after substrate binding. Evaluating the polyspecificity of tRNA synthetase and expanding its substrate specificity by developing a mutant might help to improve the efficient incorporation of UAAs. Therefore, we utilized docking as a tool to analyze and sort the substrate specificity of endogenous MetRSs. Structural insight into the substrate recognition of MetRS and the key residues responsible for interaction and hindrance will help in the rational redesign of its substrate specificity. Based on the docking results, we sorted the substrate specificity of the MetRS with respect to its methionine analogs.

2. Materials and methods

2.1. Active site comparison

The available MetRS crystal structures were retrieved from the Protein Data Bank (PDB); their sequence identities and active sites were compared. The key residues interacting with the co-crystallized substrate (methionine) and its analogs were carefully studied and compared with the active site residues of the MetRSs of other kingdoms. Further, the MetRS protein sequence of all species was retrieved from the National Center for Biotechnology Information (NCBI), and multiple sequence alignment was performed using MEGA5. The methionine-binding residues were compared and analyzed from this multiple sequence alignment.

Later, the conformational changes that occurred in the active site after binding of methionine and its analogs were analyzed by comparison of the closed and open conformations of the MetRS.

2.2. Homology modeling

As the *in vivo* incorporation of methionine analogs is well reported in *E. coli*, *Saccharomyces cerevisiae* and *H. sapiens*, we attempted to carry out docking studies in the MetRSs of these systems [13]. To further expand the study, the protein sequence for wild type MetRS of *S. cerevisiae* was retrieved from the NCBI (GenBank accession number: EDN61849), and the BLASTP algorithm against the PDB was carried out to identify the best template [14]. As we planned to model the protein with its substrate methionine, we chose 1F4L (28% identity) as a template instead of 1RQG (32% identity). Using the advanced modeling technique, the substrate methionine was modeled along with the protein sequence. Before constructing the model, a sequence alignment was generated using ClustalX; this was compared with the results obtained from the align2d tool of MODELLER and manually checked [15]. The overall structural and stereochemical qualities of the protein were assessed using the SAVES server [16]. Finally, the 3D structure was docked with the methionine analogs.

2.3. Preparation of ligands

Three-dimensional structures of methionine and the reported methionine analogs were retrieved from the Pubchem compound database (Fig. 1). Hydrogens and Kollman charges were added to the ligands using the Python Molecular Viewer and exported for the docking study as .mol2 files.

2.4. Molecular docking simulation

An initial docking calculation of methionine was performed for the apo- and holo-conformation of wild type MetRS (PDB IDs: 1PG2 and 1QQT) using GOLD [17–19]. Further docking calculations were performed for methionine and its analogs with the holo-form of the *Ec*MetRS and the *Sc*MetRS. Prior to the docking calculations, all hydrogens and Gasteiger charges were assigned to the 3D structures of the MetRS using the Python Molecular Viewer, and the non-polar hydrogens were added and merged. Prior to the protein preparation, co-crystallized methionine and adenylate were removed from the PDB structure file. The grid size was set to 10 Å centered on the *XYZ* coordinate of the methionine. A Lamarckian genetic algorithm was employed as a search parameter, and for each compound, a 50 GA run was carried out. For each GA run, 25,000,000 (long) evaluations were performed for a population size of 150. The operator weights for crossover, mutation and migration were set as the default parameters. The best-docked conformation was selected based on the interaction (hydrogen bond interaction with Leu13 and Asp52), the orientation in the active site and the docking score (GOLD fitness score). The docked complexes of best ranking solutions were exported in PDB format for further analysis.

2.5. Post docking analysis

The binding mode and interactions between the compounds and protein were analyzed using Pymol [20] and compared with the available methionine co-crystallized structures (PDB ID: 1F4L).

2.6. Virtually generated methionine analogs

To further extend our studies, we retrieved some isostructural methionine analogs from the Pubchem compound database using the Pubchem structure search with the help of the 2D smile of methionine. Amino acid-like isostructural analogs were then chosen for analysis, and we carried out the docking analysis with the *Ec*MetRS as described earlier.

3. Results and discussion

The main goal of this work is to study the polyspecificity of the MetRS towards methionine analogs and to compare the variations in the active site residues of the reported polyspecific MetRSs from *S. cerevisiae* and *E. coli*. This might help in the rational designing of new MetRS with broad substrate specificity, which can facilitate the incorporation of methionine analogs into recombinant proteins.

3.1. Active site of EcMetRS

The active site of the *Ec*MetRS catalyzes the charging of the appropriate tRNA with methionine. The methionine-binding site of the *Ec*MetRS is made up of a hydrophobic pocket enfolded by the residues Ala12, Leu13, Tyr15, Trp253, Ala256, Pro257, Tyr260, Ile297 and His301. Among these, His301 has been reported as contributing to the unambiguous recognition of the sulfur atom in methionine. Trp253 and Tyr15 control the conformational flexibility and help the binding of the methionine substrate [21].