Journal of Molecular Graphics and Modelling 29 (2011) 650–656

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Differences in active-site microarchitecture explain the dissimilar behaviors of PBP5 and 6 in *Escherichia coli*

Chiranjit Chowdhury, Anindya S. Ghosh*

Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal 721302, India

ARTICLE INFO

Article history:
Received 9 August 2010
Received in revised form
10 November 2010
Accepted 15 November 2010
Available online 24 November 2010

Keywords:
Penicillin-binding proteins
DD-carboxypeptidase
Escherichia coli
MODELLER
AutoDock

ABSTRACT

Out of the four DD-carboxypeptidases (DD-CPases) in *Escherichia coli*, only penicillin-binding protein (PBP) 5 performs physiological functions such as maintaining cell shape; its nearest homolog, PBP6, cannot perform such functions. Moreover, unlike PBP6, PBP5 efficiently processes both beta-lactam, and peptide substrates. The crystal structure of PBP5 reveals strong inter-residue hydrogen-bonding interactions around the active site, which favor its catalytic activity. However, the recently solved crystal structure of PBP6 cannot explain the reason for the observed functional discrepancies between the two proteins. Enzymatic analyses indicate that moving the morphology maintenance domain from one protein to another can alter the affinities and activities of PBP5 and 6 toward their substrates. To determine why the activities of these enzymes differ, we used molecular modeling, and docking analyses with substrate-mimetic ligands to estimate how amino-acid alterations in the morphology maintenance domain would affect the structure of PBP and hence its substrate specificity. The results obtained from kinetic analyses were directly correlated to the three-dimensional structures of the PBPs determined through *in silico* analyses, indicating a change in the active-site microarchitectures of PBP5 and 6 as a plausible cause of the difference in their biochemical behaviors.

1. Introduction

The most effective beta-lactam drug targets are the high-molecular-mass penicillin-binding proteins (HMM PBPs) [1]. The HMM PBPs are essential for cell survival and are involved in cell elongation, and septation, whereas the role of low-molecular-mass (LMM) PBPs is less clear, with the exception of PBP5 [2]. Of the seven LMM PBPs in *Escherichia coli*, PBP5 is the best studied with respect to structure, biochemistry, and physiology [3–6]. In contrast, little is known about the function of the nearest homolog of PBP5 in *E. coli*, PBP6. Although PBP5 and 6 share conserved signature motifs at and around their active sites and share substantial sequence identity [7], only the former exhibits a high level of DD-carboxypeptidase (DD-CPase) activity with pentapeptide substrates. PBP6 exhibits DD-CPase activity with smaller, artificial substrates but remains inactive toward larger pentapeptides [8]. Interestingly, PBP6 shows a higher rate of acylation by beta-lactam agents than does PBP5 [8]. To discover the functional domain responsible for DD-CPase activity, chimeric proteins were used that contained a stretch of 20 amino acids around the KTG motif of PBP5 (the morphology maintenance domain, or MMD) that had been swapped [5] with that of PBP6 and vice versa. Replacement of the PBP6 MMD with the PBP5 MMD (creating the chimeric PBP656) restored the DD-CPase activity, but the reverse chimera (PBP565) was inactive [8]. This difference indicated the possibility of structural discrepancies between the two proteins and the possibility of an involvement of the MMD in their structural alterations, although there were no significant changes in gross secondary structure as estimated through circular dichroism (CD) spectroscopic analyses [8]. Therefore, differences in the organization of active-site motifs of these PBPs might explain the variation in their biochemical functions. The crystal structures of soluble PBP5 and 6 are available; however, they do not explain the biochemical and physiological functions seen in PBP6 [6,9]. Moreover, there are no data that explain how the MMD alters the orientation of the active site, which may lead to different biochemical activities. Based on the findings of earlier studies [4,5,8,9], we performed homology modeling to elucidate the differences in microarchitecture seen at the active-site clefts of PBP6, 565 and 656. In addition, docking studies on substrate mimetic ligands and the PBPs focused on the site of the biochemical interactions and the relevant differences between PBP5 and 6.

In this study, we performed *in silico* analyses of PBP5 and 6, as well as their active-site chimeras, to elucidate the microarchitectural differences between these proteins, which may explain the differences in their biochemical behaviors.

* Corresponding author.
E-mail address: anindyain@yahoo.com (A.S. Ghosh).

1093-3263/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmgm.2010.11.009

2. Materials and methods

The primary amino acid sequences of PBP5 and 6 were obtained from the NCBI database (http://www.ncbi.nih.gov/). The amino acid sequences of the soluble (s) PBP6 and chimeric proteins used earlier by Chowdhury et al. [8] were used for model building. For modeling, the restraint-based modeling program MODELLER 9v1 [10] was used. The energy minimization (EM) of the model was performed using Discovery Studio software (Version 1.5; Accelrys Software Inc., San Diego, CA), and Gromacs v 3.3.1 [11] software was used for conducting the molecular dynamics (MD) simulation of all the soluble proteins.

2.1. Three-dimensional model building

Each of the target sequences was converted to the PIR format and used to search for potentially related sequences by using the "profile.build" command of MODELLER. The best template was chosen by comparing the root mean square (RMS) and distance root mean square (DRMS) deviations between the models' atomic positions and respective distances, the differences between their main-chain and side-chain dihedral angles, and the percentage sequence identities. The target sequences were aligned with their corresponding templates, and the three-dimensional (3D) model of each target sequence was calculated using MODELLER. The best model had the lowest value of the MODELLER objective function. Each model was further evaluated by analyzing its DOPE potential using MODELLER.

The homology models of each sPBP that we constructed were improved through EM using the CHARMM version 22 program [12] in the Discovery Studio software suite (Version 1.5; Accelrys Software Inc., San Diego, CA). The models were improved based on the steepest descent energy minimization algorithm with a cutoff distance of 12 Å for non-bonded interactions, a solvent dielectric constant of 1, and a generalized Born solvent dielectric constant of 80 to achieve a reduction in the RMS gradient to under 0.1 kcal/mol Å. The models were further refined by adding explicit water molecules to the MD simulation.

Initially, the box edges of the cubic solid box around the protein lay at least 5 Å away from the protein surface. The box was allowed to fill with simple point charge (SPC) water molecules. Sodium ions (one for sPBP6, four for sPBP656 and three for sPBP565) were used as counterions, bringing the total charge of the system to zero. The 300-ps MD simulations were performed at 300 K using the particle-mesh Ewald method for calculating the electrostatic interactions using the Gromacs program. The conformation at 300 ps was chosen as the 3D structure for further study. PROCHECK [13] and Verify3D [14] (http://nihserver.mbi.ucla.edu/Verify_3D) software were used to evaluate the folding and stereochemistry of all the models.

Because the volume of the active-site groove influences the binding of the substrate and hence the catalysis, the volume of the groove associated with the active-site motifs of the model was measured by surface topography analysis (CASTp) [15].

We analyzed each protein model by the CASTp server (http://cast.engr.uic.edu). The CASTp server identified all the grooves and voids on the protein structures and analytically measured the volume and area of each groove and void. The volume of the groove associated with the signature motifs in each model was selected for further analysis.

2.2. Docking of the substrate mimetic ligand into the sPBPs

A detailed investigation of the mechanism of the DD-CPase reaction with PBP5 was previously described by Shi et al. [16], who docked acylated D-Ala-D-Ala into the PBP5 active site. However, the ligand used in that study was artificial and lacked peptidoglycans. Here, we docked both the artificial (Ac-L-Lys-D-Ala-D-Ala) and pentapeptide (L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala) substrates into the active-site clefts of the sPBPs. The docking studies were performed using AutoDock version 4 software (Scripps Research Institute, San Diego, USA) [17,18]. The peptidoglycan mimetic pentapeptide substrate was allowed to dock separately into the entire set of proteins to determine the substrate specificity and the nature of the interactions.

Before docking, the PDB files of the protein molecules were edited by removing the entire water molecules, adding hydrogen atoms (polar and non-polar), adding Gasteiger–Hückel charges and setting the number of torsions (single bonds were considered rotatable and peptide bonds non-rotatable). The grid parameters were set by considering the number of points (60, 60, 60) in the *x*, *y* and *z* coordinates, so that the grid box was positioned and centered around the active-site cleft. The spacing between the grid points was then adjusted to 0.375 Å. This allowed the pentapeptide substrate to dock in an orientation similar to the boronic acid inhibitor (which resembles the portion of the peptide substrate remaining after acylation and loss of the C-terminal D-alanine) which has been shown to bind to the active-site cleft of sPBP5 (PDB ID of sPBP 5 in complex with boronic acid inhibitor is 1Z6F) [19]. The docking parameters were set with a Lamarckian genetic algorithm with the number of energy evaluation set to 250,000. The grid map was generated by AutoGrid and the docking was performed by AutoDock module present in AutoDock 4 suite (http://autodock.scripps.edu/) Finally, the lowest energy docked conformations were combined with the sPBP molecule for further analysis.

3. Results and discussion

The 3D features of the models of sPBP6, 565 and 656 were obtained by homology modeling using MODELLER 9v1. 1Z6F chain A, the crystal structure of PBP5 of *E. coli* in complex with a boronic acid inhibitor [19] at a resolution of 1.60, was chosen as the best template for all the sPBPs. The best template and the target sequence were aligned using Clustal W software [20] to determine their percent identities. Pair-wise sequence alignment indicated that 1Z6F shared 64% identity with sPBP6, 66% with sPBP656, and 98% with sPBP565. With the help of the target sPBP sequences and alignment files, five models were generated by MODELLER, from which the best model was selected by picking the lowest value of the MODELLER objective function and the highest value of negative DOPE potential (a measure of the potential energy of the molecule). All the models had DOPE score profiles similar to that of their template, indicating folds and loops similar to those of their templates. To remove poor steric contacts and achieve a stable conformation, the energy minimizations and dynamics simulations were performed at 300 ps. Upon increasing the temperature, the potential energy initially rose rapidly and then relaxed again to the equilibrium at 300 ps. The average potential energies calculated for sPBP6, 656, and 565 were −1,109,370 kJ/mol, −1,630,870 kJ/mol, and −1,104,430 kJ/mol, respectively.

The final structure (Fig. 1A–C) was further checked using PROCHECK and Verify3D. The PROCHECK analysis indicated that 95.20% of the residues in the sPBP6 model were situated at the most favored region in the Ramachandran plot. The residues with an average 3D-1D score above 0.2 were 90.56%, as determined through the Verify3D. In sPBP656, 93.90% of the residues were placed in the most favored regions in the Ramachandran plot, and 96.44% of the residues had an average 3D-1D score greater than 0.2. In sPBP565, 93.60% of the residues were found in the most favored regions of the Ramachandran plot, and 93.61% of the residues had an average 3D-1D score greater than 0.2. All the protein models were deposited into the PMDB database under the accession num-