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Comparative modeling of Anhydro-N-Acetylmuramyl-L-Ala Amidase from *Neisseria meningitidis*: implications for structure-function relationship

*Yasmeen Rashid[1,2] and M. Kamran Azim[1]**
[1]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan
[2]Department of Biochemistry, Dow University of Health Sciences (OJHA Campus), Karachi, Pakistan

Abstract: Anhydro-N-Acetylmuramyl-L-Ala Amidase (AmpD) is a bacterial amidase involved in the recycling of cell wall fragments in gram negative bacteria. It specifically cleaves the amide bond between the lactyl group of the N-acetylmuramic acid and the α-amino group of alanine in degradation products. The protein fold of AmpD comprised of three β-sheets, four α-helices and one 3^{10}-helical turn. Here, we present structural bioinformatics of AmpD from *N. meningitidis.* The solution structure of *Citrobacter freundii* AmpD (PDB id; 1J3G) was used as a template to construct the homology model, with which *N. meningitidis* AmpD sequence showed 68% similarity. Structural comparison pointed out that majority of amino acid residues interacting with Zn++ and the peptide moiety of the substrate are conserved in both AmpD proteins. In *N. meningitidis* AmpD protein model, the catalytically important Tyr63 residue was found to be substituted by Phenylalanine amino acid. Supported by this structural standpoint, we predict that *N. meningitidis* AmpD would contain lower amidase activity compared to AmpDs from other bacteria.

Key words: Meningitis, structural bioinformatics, AmpD, peptidoglycan recycling.
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***Author for Correspondence:** kamran.azim@iccs.edu

INTRODUCTION

Peptidoglycan recycling is a process in which the cell captures and reutilizes the turnover products. Many enzymes involved in this recycling phenomenon have been reported; seven of them are evolved to carry out recycling of murein amino acids[1]. Anhydro-N-Acetylmuramyl-L-Ala Amidase (AmpD) is one of such candidates known to specifically cleave anhMurNAc-L-Ala bond. It cleaves anhMurNAc-tripeptide at a rate of 10,000 times faster than that of MurNAc-pentapeptide and UDP-MurNAc-pentapeptide[2]. Therefore, AmpD specifically cleaves anhydrous moieties without demolishing UDP-MurNAc-pentapeptide[1]. The solution structure of *Citrobacter freundii* AmpD revealed a crucial zinc atom bound at the active site[3,4]. Three amino acid residues i.e. His34, His154 and Asp164 were found to be coordinated with zinc ion. Alanine scaning of these residues showed a lack of Zinc-ion binding to the active site and consequently no amidase activity. Site directed mutagenesis studies proved Tyr63 and Lys162 to be involved in substrate binding[4]. We have carried out structure-function relationship studies on AmpD from *N. maningitidis* using sequence comparison and knowledge-based protein modeling techniques.

MATERIALS ANDMETHODS

The amino acid sequences of AmpD protein was retrieved from SWISSPROT[5] database. Sequence homology searches were carried out against PDB[6] and SWISSPROT by PSIBLAST[7] and FASTA[8] programs. The homology model of AmpD protein in complexed with Zinc atom was built using *Citrobacter freundii* AmpD (PDB id; 1J3G) structure as template. All steps of homology model building and refinement including energy calculation were carried out by the protein structure-modeling program MODELLER[9]. The programs PROCHECK[10] and PROSA[11] were employed for the assessment of reliability of the homology models. Based on the optimized alignment ten comparative models of *N. meningitidis* AmpD enzyme were built until a stereochemically satisfied confident model was obtained. In order to analyze the three dimensional structure of the model and to perform structural investigations of the active site residues, the molecular modeling software DSVisualizer (http://accelrys.com/products/discovery-studio/index.html) and VMD[12] tools were used.

RESULTS AND DISCUSSION

Sequence analysis identified *N. meningitidis* gene product id NMB0668 showed as AmpD. The amino acid sequence alignment of AmpD from *C. freundii* and *N. meningitidis* showed 68% homology (Figure 1). The NMR structure of *C. freundii* AmpD (PDB id; 1J3G) selected as template to construct the *N. meningitidis* AmpD homology model. The overall fold of *N. meningitidis* AmpD protein homology model comprised of three β-sheets, four α-helices and one 3^{10}-helical turn (Figure 2). The AmpD model had good stereochemistry with no residue in disallowed region as well as an overall good energy profile (Figure 3).

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NMB_AmpD   12  GWLQSIRHTPSPNFSPRETGETVSLIVLHNISLPPFEYGTDAVEKLFANRLDPDGHPFFS  71
1J3G        6  GWLAEARRVPSPHYDCRPDDENPSLLVVHNISLPPGEFGGPWIDALFTGTIDPNAHPYFA  65

NMB_AmpD   72  LIHTLRVSSHFLIKRDGETVQFVSCDNMAYHAGVSSFGGREKCNAFSIGIELEGCDFEPF  131
1J3G       66  GIAHLRVSAHCLIRRDGEIVQYVPFDKRAWHAGVSSYQGRERCNDFSIGIELEGTDTLAY  125

NMB_AmpD   132 AEAQYRSLEALLDAI-CRHYP-VTAVTGHQDIAPGRKTDPGHFFDWRRIR  179
1J3G       126 TDAQYQQLAAVTNALITRYPAIANNMTGHCNIAPERKTDPGPSFDWARFR  175
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Figure 1: Sequence alignment of *N. meningitidis* AmpD (NMB_AmpD) with *C. freundii* AmpD (1J3G). Amino acid residues interacting with Zn++ and the peptide moiety of the substrate are shown in pink and blue color, respectively. Red color residues are critical for AmpD catalysis.

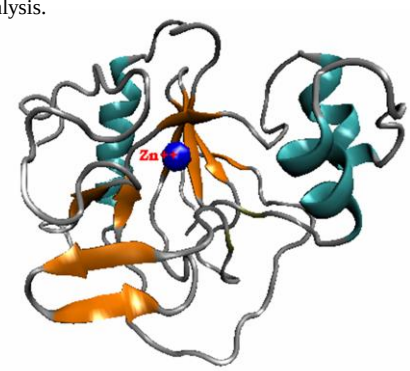

Figure 2: Homology model of *N. meningitidis* ampD complexed with Zinc ion. Alpha helices and beta sheets are shown in cyan and orange color, respectively. Zinc is represented as a blue-colored ball.

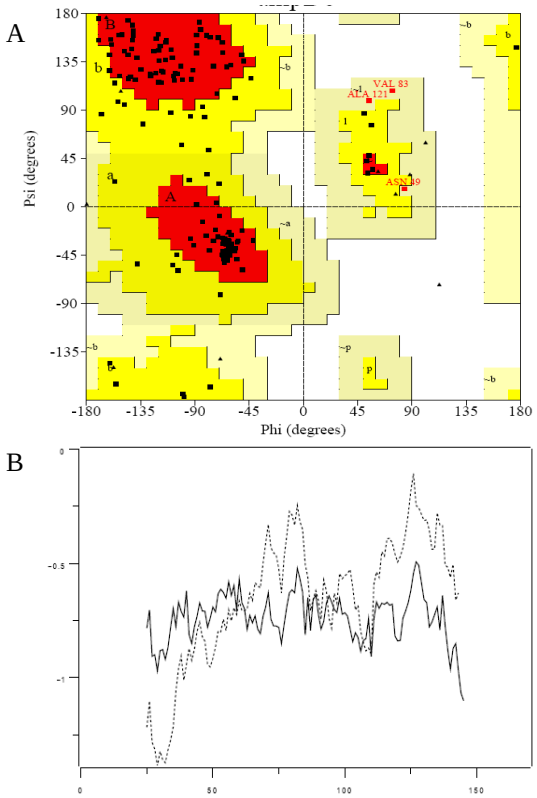

Figure 3: (A) Stereochemical analysis and (B) energy profile of *N. meningitidis* AmpD model using PROCHECK and PROSA. In the energy plot (B), *N. meningitidis* AmpD homology model is shown as dashed line and *C. freundii* AmpD structure (template) as solid line.

In the proposed AmpD protein model, the amino acid residues interacting with Zn^{++} ion and the substrate were analyzed carefully by selecting an area of 8Å around Zinc ion. Most of the interacting residues were conserved in *N. meningitidis* AmpD protein while three amino acid residues were observed as substituted (Figure 4). One of the amino acid substitutions i.e. Val33→Leu was conserved substitution which is not expected to affect the substrate-binding capability of the enzyme. In AmpD model, certain non conservative changes in amino acids i.e. Tyr63→Phe and Cys155→Gln can result in the alteration of substrate interaction.

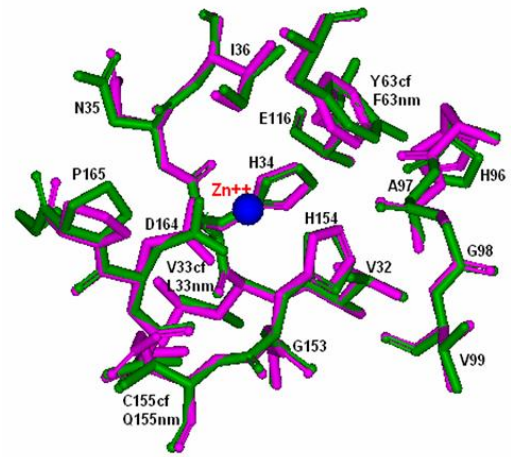

Figure 4: Superposed active site residues of *N. meningitidis* ampD (pink) and *C. freundii* ampD (green). Amino acid residues in an area of 8Å around the zinc atom (blue) are shown.

The *C. freundii* AmpD structure revealed there are two catalytically important residues i.e. Tyr63 and Lys162. Liepinsh et al.[3] indicated a key role for Tyr63 during amidase activity because mutation of Tyr63→Phe in AmpD abolished 90% activity. The lack or decrease in AmpD activity results in the intracellular accumulation of 1,6-anhydroMurNAc-tripeptides. These products allosterically activate AmpR, the transcriptional activator, which results in the expression of β-lactamase. Hence, mutations in AmpD provide a constitutive β-lactam resistance in bacteria[13]. In *N. meningitidis* AmpD Lys162, one of

the two critical amino acid residues was conserved where as catalytically important residue Tyr63 was substituted by Phenylalanine amino acid. Therefore, based on the information obtained from *N. meningitidis* AmpD protein model, we predict that *N. meningitidis* AmpD would contain lower amidase activity compared to AmpDs from other bacteria.

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