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Homology modeling of β-ketoacyl synthase-II *from Neisseria meningitidis*

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Abstract: Fatty acid biosynthesis is indispensable for bacterial growth. Enzymes involved in fatty acid biosynthesis are attractive targets for discovery of novel antibacterial agents. β-ketoacyl synthase-II (FabF) catalyses condensation reaction for chain elongation in fatty acid biosynthesis pathway. Three dimensional structural models of two paralogous FabF enzymes from *N. meningitidis* (NMFabF-1 and NMFabF2) have been constructed using knowledge-based homology modeling. Detailed analysis of models and their active sites revealed considerable differences in ligand binding pockets of the two enzymes. The ligand binding cleft of NMFabF-1 homology model resembles exclusively with the typical bacterial β-ketoacyl synthase. The active site of NMFabF-2 model has several amino acid substitutions which would result in formation of comparatively smaller ligand binding pocket. Based on these observations, we predict that NMFabF-1 would catalyze short chain fatty acid elongation reactions whereas NMFabF-2 would be involved in elongation of comparatively longer fatty acid chains.

Keywords: Fatty acid metabolism, novel drug targets, comparative modeling, structural bioinformatics.
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INTRODUCTION

Fatty acid synthesis (FAS) systems are essential for cell growth and viability. The organization of this system is strikingly different between prokaryotes and eukaryotes[1]. In eukaryotes, FAS system is composed of a single polypeptide chain containing seven different catalytic sites while the FAS components exist as separate entities in prokaryotes and each enzyme protein catalyzes a defined reaction in fatty acid biosynthesis pathway[2]. Due to the differences in arrangement and structures of FAS enzymes in prokaryotes and eukaryotes, bacterial FAS enzymes are attractive targets for novel antibacterial drug discovery. Three β-ketoacyl-acyl carrier protein synthases catalyze condensation reactions for chain elongation in fatty acid biosynthesis pathway namely KAS-I, -II, and –III[3]. KAS-III enzyme catalyzes the initial condensation of acyl CoA and malonyl CoA[4]. KAS-I and KAS-II use acyl ACP rather than acyl CoA as the primer for subsequent condensation. Considerable differences exist in their substrate specificities and sensitivities to cerulenin[5,6]. Another fatty acid condensing enzyme called KAS-IV or FabJ has been reported[7]. The KAS-IV is sensitive to cerulenin and has substrate specificity for C4:0- and C6:0-ACP.

Neisseria meningitidis is a gram-negative β-proteobacterium which belongs to Neisseriaceae, a well-known bacterial family[7,8]. *N. meningitidis* is an important and devastating human pathogen which represents the worldwide cause of epidemic meningitis and severe sepsis[1,3-5]. Based on the differences in capsular polysaccharide structures, 13 serogroups of *N. meningitidis* have been identified whereas, only six serogroups (A, B, C, W-135, X, and Y) are recognized to cause most life-threatening disease[3,6]. No effective vaccines against *N. meningitidis* serogroup B has been established yet. Novel and effective therapeutic agents against this pathogen are needed to combat its emerging resistance.

The genome of *Neisseria meningitidis* encodes two paralogous genes of β-ketoacyl-acyl carrier protein synthases (termed as NMFabF-1 and NMFabF-2: TIGR ids; NMB0219 & NMB1703; http://www.jcvi.org/cms/research/projects/cmr/overview/). We have constructed 3D homology models of these paralogous enzymes using crystal structure coordinates of *E. coli* FabF complexed with cerulenin as a template.

MATERIALS AND METHODS

The amino acid sequences of *N. meningitidis* NMFabF-1 and NMFabF-2 was retrieved from Comprehensive Microbial Resource database (http://www.jcvi.org/cms/research/projects/cmr/overview/) and SWISSPROT database[12]. Sequence homology searches were carried out against non-redundant PDB sequences[13] by PSIBLAST[14] and FASTA[15]. Multiple sequence alignment of FabF proteins from different bacteria including *E. coli* was carried out using CLUSTALX[16], T- Coffee and 3D-Coffee[17]. Homology models of both *N. meningitidis* FabF paralogous proteins (NMFabF-1 and NMFabF-2) were built in complexed with cerulenin using *E. coli* FabF crystal structure coordinates [PDB id; 1B3N] as template. All steps of homology model building, refinement and energy calculation were carried out by the protein structure-modeling program MODELLER[18]. The programs PROCHECK[19] and PROSA[20] employed for the assessment of reliability of the homology models. WebLab Viewer[21] and DSVisualizer (www.accelrys.com) used to analyze 3D strutures.

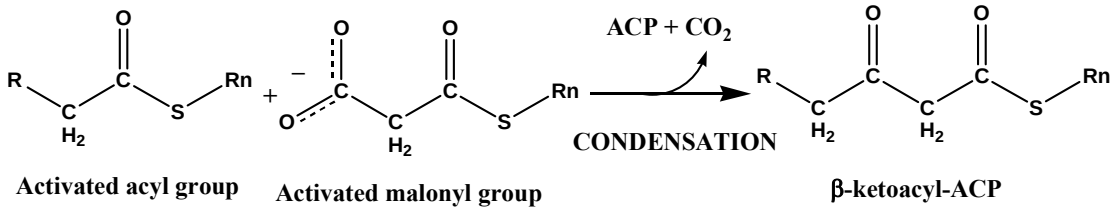

Figure 1: Schematic diagram showing the condensation reaction catalyzed by β-ketoacyl-ACP synthase II (FabF).

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Ec-FabF SKRRVVVTGLGMLSPVGNTVESTWKALLAGQSGISLIDHFDTSAYATKFAGLVKDFNCED
NMFabF1 YISAKEARRMDVFIHYGIAAALQAIADSGLDDVENLDKDRIGVNIGSGIGGLPGIEVTGK
Ec-FabF IISRKEQRKMDAFIQYGIVAGVQAMQDSGLEITEE-NATRIGAAIGSGIGGLGLIEENHT
NMFabF1 AVIEGGARKINPFFIPGSLINLISGHVTILKGYRGPSYGMVSACTTGAHAIGDSLRMIKY
Ec-FabF SLMNGGPRKISPFFVPSTIVNMVAGHLTIMYGLRGPSISIATACTSGVHNIGHAARIIAY
NMFabF1 GDADIMVAGGAEGAISTLGVGGFAAMKALSTRNDDPATASRPWDKGRDGFVIGEGAGILV
Ec-FabF GDADVMVAGGAEKASTPLGVGGFGAARALSTRNDNPQAASRPWDKERDGFVLGDGAGMLV
NMFabF1 LEELEHAKKRGAKIYAEIVGFGMSSDAYHITAPNE--EGPALAVTRALKDAGINPEDVDY
Ec-FabF LEEYEHAKKRGAKIYAELVGFGMSSDAYHMTSPPENGAGAALAMANALRDAGIEASQIGY
NMFabF1 VNAHGTSTPLGDANETKALKRAFGEHAYKTVVSSTKSMTGHLLGAAGGVEAVYSILAIHD
Ec-FabF VNAHGTSTPAGDKAEAQAVKTIFGEAASRVLVSSTKSMTGHLLGAAGAVESIYSILALRD
NMFabF1 GKIPPTINIFEQDVEAGCDLDYCANEARDAE-IDVAISNSFGFGGTNGTLVFKR
Ec-FabF QAVPPTINLDNPD--EGCDLDFVPHEARQVSGMEYTLCNSFGFGGTNGSLIFKK
NMFabF2  RRVAVTGIGGITAFGRDWQSIQAAFKAEKNAVKYMDWHERFPELEAQLGAPIENYAPPKH
Ec-FabF  RRVVVTGLGMLSPVGNTVESTWKALLAGQSGISLIDHFDT-SAYATKFAGLVKDFNCEDI
NMFabF2  WTRKQLRSMGRVSYLCVDAAEQALADAGLLGDESITDGRMGVACGSSSGSTKDIGDVGEL
Ec-FabF  ISRKEQRKMDAFIQYGIVAGVQAMQDSGL-EITEENATRIGAAIGSGIGGLGLIEENHTS
NMFabF2  LLTGTSRNFSANTYVRMMPHTTAANIGIFFGLKGRIIPTSSACSSGSQGIGYAYEAIKYG
Ec-FabF  LMNGGPRKISPFFVPSTIVNMVAGHLTIMYGLRGPSISIATACTSGVHNIGHAARIIAYG
NMFabF2  GLTDMMLAGGGEEFF-PSEVYVFDSLYAASRRNGEPEKTPRPYDANRDGLVIGEGAGIFV
Ec-FabF  GDADVMVAGGAEKASTPLGVGGFGAARALSTRNDNPQAASRPWDKERDGFVLGDGAGMLV
NMFabF2  LEELEHAKRRGAIIYAELVGYGANSDAYHISTPRP--DAQGAILAFQTALQHANLAPEDI
Ec-FabF  LEEYEHAKKRGAKIYAELVGFGMSSDAYHMT--SPPENGAGAALAMANALRDAGIEASQI
NMFabF2  GWINLHGTGTHHNDNMESRAVAAVFG---NNTPCTSTKPQTGHTLGAADAIEAAFAWGIA
Ec-FabF  GYVNAHGTSTPAGDKAEAQAVKTIFGEAASRVLVSSTKSMTGHLLGAAGAVESIYSILAL
NMFabF2  DRQSNPEGKLPPRLWDGQNDPNLPAINLTGSGSRWETEKRITASSSFAFGGSNCVLI
Ec-FabF  RD-----QAVPPTINLDNPDEGC-DLDFVPHEARQVSGMEYTLCNSFGFGGTNGSLI
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Figure 2: Pairwise sequence alignments of β-ketoacyl synthase-II from *E. coli (Ec-FabF)* and *Neisseria meningitidis* (Paralogue-I: NMFabF1 and Paralogue-II: NMFabF2). Red colored residues are involved in catalysis. Blue colored residues form the binding pocket for the hydrocarbon tail of the acyl intermediate.

RESULTS AND DISCUSSION

β-ketoacyl (ACP) synthase II (FabF) is involved in fatty acid biosynthesis and catalyzes the condensation of acyl groups derived from acyl-ACP with malonyl-ACP (Figure 1). The amino acid sequence of FabF from *E. coli* aligned well with both of the *N. meningitidis* FabF paralogues and selected as template for homology modeling. The NMFabF-1 (gene-product of open reading frame No. NMB0219) has 74% while NMFabF-2 (gene-product of open reading frame No. NMB1703) has 53% sequence similarity with *E. coli* FabF (Figure 2).

E. coli FabF enzyme has been reported to exist as a homodimer. Each monomeric protein consists of two mixed five-stranded β-sheets surrounded by α-helices. The fold can be described as consisting of five layers α-β-α-β-α[22]. Both sequences of *N. meningitidis* FabF aligned full length with *E. coli* FabF. The overall fold of both *N. meningitidis* FabF structures is also the same (Figure 3). The crystal structure of the *E. coli* FabF has Leu342 in disallowed region (as indicated by Ramachandran plot), therefore the equivalent Leu residue in NMFabF-1 and NMFabF-2 models also appeared in disallowed region. The structural superposition of Cα traces of *E. coli* FabF and *N. meningitidis* NMFabF-

1 and NMFabF-2 models show RMS deviation of 0.2 Å and 0.4 Å respectively. The catalytically important residues (Cys163, His303, and His340) are conserved in both *N. meningitidis* FabF enzymes and are positioned appropriately in the active site compared with the crystal structure (Figure 4). The residues forming the binding pocket for the hydrophobic tail of acyl intermediate are totally conserved in the NMFabF-1 model (Figure 4a) but the differences lies in the ligand binding residues of NMFabF-2.

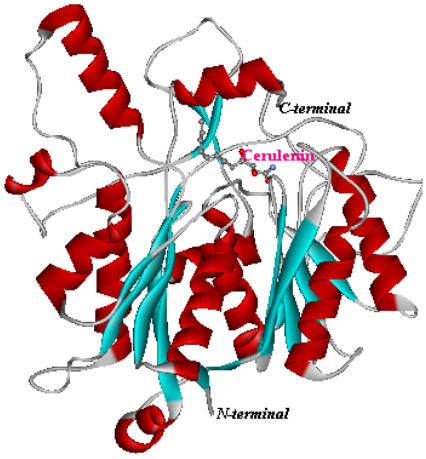

Figure 3: Homology model of *N. meningitides* β-ketoacyl (ACP) synthase-II (FabF).

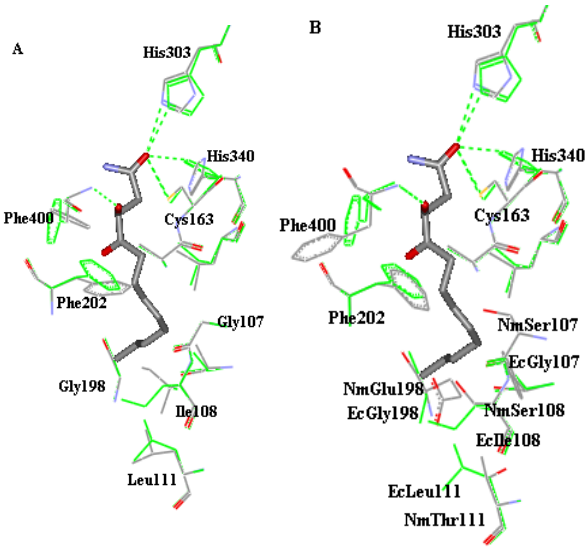

Figure 4: Comparison of active site residues of the crystal structure of *E.coli* (FabF) with *N. meningitides* FabF-1 (NMB0219)(A) and FabF-2 (NMB0219)(B); the paralogous genes.

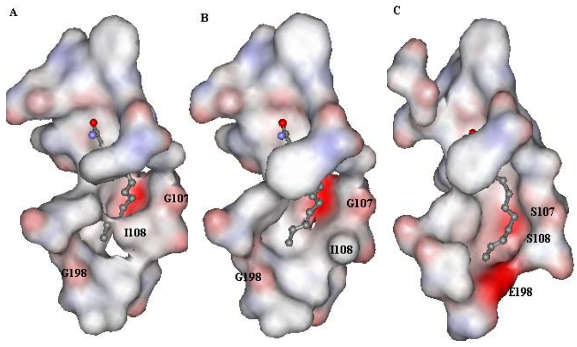

Figure 5: Comparison of surface potential of *E.coli* FabF active site (B) with *N. meningitides* FabF-1 (A) and FabF-2 (C) active sites with bound cerulenin inhibitor.

Five of the conserved hydrophobic residues at ligand binding pocket are substituted in the NMFabF-2 model i.e. Gly107→Ser, Ile108→Ser, Leu111→Thr, Ala193→Phe and Gly198→Glu [Figure 4b]. These substitutions are predicted to form a narrower ligand binding pocket in NMFabF-2 compared to 'typical' FabF enzymes (e.g. *E. coli* FabF). The comparison of molecular surface representation of active sites revealed topological similarity between *E. coli* FabF and NMFabF-1 whereas active site cavity of NMFabF-2 has variation in the distribution of surface electrostatic potential and the volume of ligand binding pocket (Figure 5).

According to Inokoshi et al[23], the exchange of Gly107 to Ser makes the FabF enzyme resistant to cerulenin, a FabF inhibitor. In addition to Gly107→Ser mutation, NMFabF-2 has four supplementary mutations.

Furthermore, replacement of five small hydrophobic residues with hydrophilic residues make the active site cavity charged and narrower compared to *E. coli* FabF and therefore cerulenin inhibitor cannot be accommodated by the active site due to certain steric clashes.

Based on these observations we speculate that (a) NMFabF-2 might be responsible for the elongation of short fatty acids whereas FabF-1 catalyzes the long chain fatty acid elongation; (b) the NMFabF-2 would be resistant to the well known FabF inhibitor, cerulenin.

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