Homology Modeling and Docking Study of β-Ketoacyl Acyl Carrier Protein Synthase III from *Enterococcus Faecalis*

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β-Ketoacyl acyl carrier protein synthase (KAS) III is a particularly attractive target in the type II fatty acid synthetic pathway, since it is central to the initiation of fatty acid synthesis. *Enterococcus faecalis*, a Gram-positive bacterium, is one of the major causes of hospital acquired infections. The rise of multidrug-resistant of most bacteria requires the development of new antibiotics, such as inhibition of the KAS III. In order to block the fatty acid synthesis by inhibition of KAS III, at first, three dimensional structure of *Enterococcus faecalis* KAS III (efKAS III) was determined by comparative homology modeling using MODELLER based on x-ray structure of *Staphylococcus aureus* KAS III (saKAS III) which is a gram-positive bacteria and is 36.1% identical in amino acid sequences with efKAS III. Since His-Asn-Cys catalytic triad is conserved in efKAS III and saKAS III, substrate specificity of efKAS III and saKAS III and the size of primer binding pocket of these two proteins are expected to be similar. Ligand docking study of efKAS III with naringenin and apigenin showed that naringenin docked more strongly with efKAS III than apigenin, resulting in the intensive hydrogen bond network between naringenin and efKAS III. Also, only naringenin showed antibacterial activity against *E. faecalis* at 256 μg/mL. This study may give practical implications of flavonoids for antimicrobial effects against *E. faecalis*.

Key Words : KAS III, *Enterococcus faecalis*, Fatty acid synthesis, Antibiotics, Flavonoids

Introduction

Fatty acid synthesis (FAS) system is essential for cell growth and viability. The organization of this system is strikingly different between prokaryotes and eukaryotes.[1] In eukaryotic FAS I including animals and humans, seven different catalytic sites are present on a single polypeptide chain. It is noteworthy that many eukaryotic multi-enzyme complexes are multifunctional proteins in which different enzymes are linked covalently. In contrast, in prokaryotic FAS II including bacteria and plants, the FAS components exist as discrete proteins, so each of reaction is catalyzed by distinct mono-functional enzymes.[2] Because of these differences in organization and structure of enzymes make these systems attractive targets for antibacterial drug discovery.[3]

The β-ketoacyl-acyl carrier protein synthase (KAS) catalyzes a condensation reaction in the biosynthesis of fatty acids.[4] In most bacteria, the chain elongation step of fatty acid biosynthesis is carried out by condensing enzyme superfamily, KAS I, II, and III.[3] The KAS III is the bacterial condensing enzyme in Gram-positive and -negative bacteria that initiates the FAS cycle by catalyzing the first condensation step between acyl-CoA and malonyl-ACP (Figure 1).[1,4] Two other bacterial condensing enzymes KAS I and KAS II functioning later in the FAS cycle, differ significantly from KAS III in that they use acyl-ACP rather than acyl-CoA as the primer for subsequent condensation. In various bacteria such as *E. coli* and *S. aureus*, KAS I is about 40% identical in amino acid sequence with KAS II, but KAS III shows no apparent overall sequence homology with either KAS I or KAS II.[5] The active site of KAS III contains a Cys-His-Asn catalytic triad.[6] These residues of active site are conserved in various bacterial KAS III molecules. KAS III, the most divergent member of the family of condensing enzymes, is a key catalyst in bacterial fatty acid biosynthesis and a promising attractive target for novel antibiotics.[7]

Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to old and new produced drugs are on the rise. The alarming increase of antibiotic-resistant bacterial pathogens points to the need for novel therapeutic approaches to combat infection. *E. faecalis* is a Gram-positive commensal bacteria inhabiting the alimentary canals of humans and animals, are now acknowledged to be organisms capable of

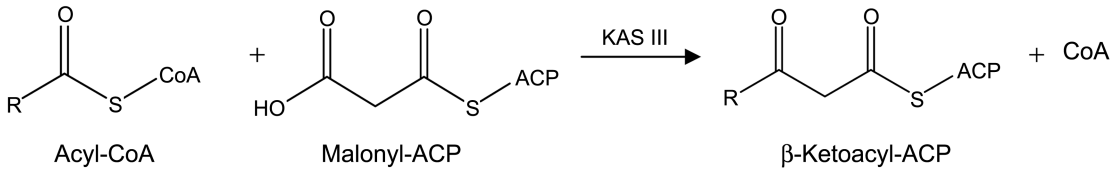

Figure 1. KAS III-catalyzed initiation reaction of fatty acid biosynthesis.

causing life-threatening infections in humans, especially in the nosocomial (hospital acquired) environment.[8] Many research groups investigate potential strategies, which could be alternative to antibiotic therapy against the human opportunistic pathogen *E. faecalis*. The 2.0 resolution crystal structure of saKAS III and the 1.6 resolution crystal structure of ecKAS III in complex with CoA have shown the atomic interactions between CoA and the important residues at the active site.[9] However, Crystal structure of efKAS III is not determined yet.

In this study, we targeted the efKAS III and proposed the three dimensional structure model, determined by comparative homology modeling. Crystal structure of saKAS III was used as a template protein for homology modeling. Since Inhibitor of efKAS III can be a good candidate of the new antimicrobial drugs, we studied interactions between the efKAS III and inhibitors, and MIC test were used to test availability of flavonoids as antibacterial agents.[10]

Methods

Comparative Protein Structure Modeling. The amino acid sequence of the efKAS III comprised of 321 amino acid residues was retrieved from Expasy.[11] We built structure of efKAS III using comparative homology modeling based on the x-ray structure of saKAS III. Sequence alignment of the efKAS III with the saKAS III was created with the Insight/ Homology module and adjusted to align key conserved residue as shown in Figure 2. The x-ray structure of saKAS III at 2.0 Å resolution (PDB entry 1ZOW) was used as a structural template. Based on the optimized alignment five comparative models of the target sequence were built by MODELLER,[12] applying the default model building routine 'model' with fast refinement. This procedure is advantageous because one can select the best model from several candidates. Furthermore, the variability among the models can be used to evaluate the reliability of the modeling. Energy minimization was performed using the consistent valence force field and the Discover program with steepest descent and conjugated gradient algorithms.[13] The qualities of these models were analyzed by PROCHECK.[14]

Ligand Docking. The various flavonoids were docked using AutoDock[15] to efKAS III structure determined by comparative homology modeling. The Lamarckian Genetic Algorithm (LGA) of the Autodock 3.05 was used for docking experiments. Distance-dependent function of the dielectric constant was used for the calculation of the energetic maps and all other parameters were used by default value.[16] We carried out 150 and 250 independent docking processes for each complex.

MIC Test. The minimum inhibitory concentrations (MICs) of the test compounds against *E. faecalis* were determined by a broth microdilution method. *E. faecalis* was grown to mid-log phase in Mueller-Hinton broth and then diluted 100-fold in the same medium.[17] A 20 μL aliquot of the diluted cell suspension (10^6 to 10^7 colony forming units) was used to inoculate each well of a 96-well plate containing 100 μL of Mueller-Hinton broth with the indicated concentration of inhibitors. The plate was incubated at 37 °C for 20 h. The MIC was defined as the lowest concentration of antibiotic giving a complete inhibition of visible growth in comparison to an antibiotic-free control well. The experiments were

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                    *        20         *        40         *        60
efKASIII : MKNYARISCTSRYVPENCVTNHQLSEMMDTSDEWIHSRTGISERRIVT-QENTSDLCHQVAKQLLE :   65
saKASIII : --MNVGIKGFGAYAPEKIIDNAYFEQFLDTSDEWISKMTGIKERHWADDDQDTSDLAYEASVKAIA :   64
ecKASIII : --MYTKIIGTGSYLPEQVRTNADLEKMVDTSDEWIVTRTGIRERHIAAPNETVSTMGFEAATRAIE :   64

            *        80         *       100         *       120         *
efKASIII : KSGKQASEIDFILVATVTPDFNMPSVACQVQGAIGATEAFAFDISAACSGFVYALSMAEKLVLSGR :  131
saKASIII : DAGIQPEDIDMIIVATATGDMPFPTVANMLQERLGTGKVASMDQLAACSGFMYSMITAKQYVQSGD :  130
ecKASIII : MAGIEKDQIGLIVVATTSATHAFPSAACQIQSMLGIKGCPAFDVAAACAGFTYALSVADQYVKSGA :  130

                 140         *       160         *       180         *       2
efKASIII : YQTGLVIGGETFSKMLDWTDRSTAVLFGDGAAGVLIEA-AETPHFLNEKLQADGQRWAALTSGYTI :  196
saKASIII : YHNILVVGADKLSKITDLTDRSTAVLFGDGAGAVIIGEVSEGRGIISYEMGSDGTGGKHLYL---D :  193
ecKASIII : VKYALVVGSDVLARTCDPTDRGTIIIFGDGAGAAVLAA-SEEPGIISTHLHADGSYGELLTLPNAD :  195

                 00         *       220         *       240         *       260
efKASIII : NESPFYQGHKQASKTLQMEGRSIFDFAIKDVSQNILSLV-----TDETVDYLLLHQANVRIIDKIA :  257
saKASIII : ------K----DTGKLKMNGREVFKFAVRIMGDASTRVVEKANLTSDDIDLFIPHQANIRIMESAR :  249
ecKASIII : ------RVNPENSIHLTMAGNEVFKVAVTELAHIVDETLAANNLDRSQLDWLVPHQANLRIISATA :  255

                    *       280         *       300         *       320         *
efKASIII : RKTKISREKFLTNMDKYGNTSAASIPILLDEAVENGTLILGSQQRVVLTGFGGGLTWGSLLLTL-- :  321
saKASIII : ERLGISKDKMSVSVNKYGNTSAASIPLSIDQELKNG--KLKDDDTIVLVGFGGGLTWGAMTIKWG- :  312
ecKASIII : KKLGMSMDNVVVTLDRHGNTSAASVPCALDEAVRDG--RIKPGQLVLLEAFGGGFTWGSALVRF-- :  317
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Figure 2. Sequence alignment of template protein (saKAS III) and target protein (efKAS III).

Table 1. RMSD and energy of five efKAS III models predicted by MODELLER

	RMSD with saKAS III	Energy (kcal)
efKAS III 1	0.29	1812.72
efKAS III 2	0.30	1857.76
efKAS III 3	0.36	2071.25
efKAS III 4	0.34	2006.53
efKAS III 5	0.28	1779.42

replicated at least three times to verify the methodology reproducibility when using the above-mentioned conditions.

Results and Discussion

Five models of efKAS III were generated by MODELLER. Energy and RMSD for five models were listed in Table 1. The five generated models of efKAS III are represented in Figure 3(A). Among these five efKAS III models, the lowest energy structure was efKAS III 5 shown in Figure 3(B). In order to select the best model, we checked the structural validity of efKAS III by PROCHECK. The torsion angles of φ and ψ in the generated models are represented in Ramachandran plot as shown in Figure 4. These torsion angles of 88.9% of the residues had values within the most favored regions and only 0.3% of the residues had values within disallowed regions and the overall G-factor[18] is 0.15 as shown in Table 2. The overall G-factor is a measure of the overall normality of the structure and low G-factors indicate that residues have unlikely conformations. The overall value is obtained from an average of G-factors for all residues in structure. X-ray structure of saKAS III has a resolution of 2.0 and a G-factor of 0.26 Å. In Ramachandran plot, the stereochemical quality of a protein model can be judged by the use of φ, ψ scatter plots, with incorrect structures generally having a much larger fraction of residues lying in disallowed regions.[19] Since our model of efKAS III has only 0.3% of its residues in disallowed regions, it can be said that our efKAS III structure satisfies criteria of a good model.

Although catalytic mechanisms of KAS III in various bacteria are very similar, they have appeared significantly different substrate specificities in various species. KAS III in *E. coli*, a Gram-negative bacterium, can utilize primarily short straight-chain acyl-CoA, while KAS III in *S. aureus*, a Gram-positive bacterium, make use of straight- and branched-chain acyl-CoA primers.[20] This might result from the difference in structures of KAS III of both bacteria.

The active site of KAS III was divided into two regions, catalytic site and primer binding site as shown in Figure 5(A). The mechanism of action of KAS III involves a so-called catalytic triad composed of an asparagine, a histidine, and the catalytic cysteine residues in catalytic site.[6] The catalytic triad of saKAS III comprises Cys112, His238, and Asn268 and these residues are conserved in efKAS III (Cys113, His246, and Asn276). Condensing enzymes, such as KAS III, catalyze carbon-carbon bond formation by condensing an acyl primer with an elongating carbon source often attached to a holo-ACP,[21] so primer binding site of KAS III is important to defining of substrate specificities. In previous research by Qiu *et al*.,[9] critical factor of substrate binding is related on the size of pocket rather than difference of residues. It has been reported that upon substrate binding, amino acids in the primer binding site in saKAS III are shifted and primer binding site of saKAS III is larger than ecKAS III. As shown in Figure 5A and 5B, primer binding

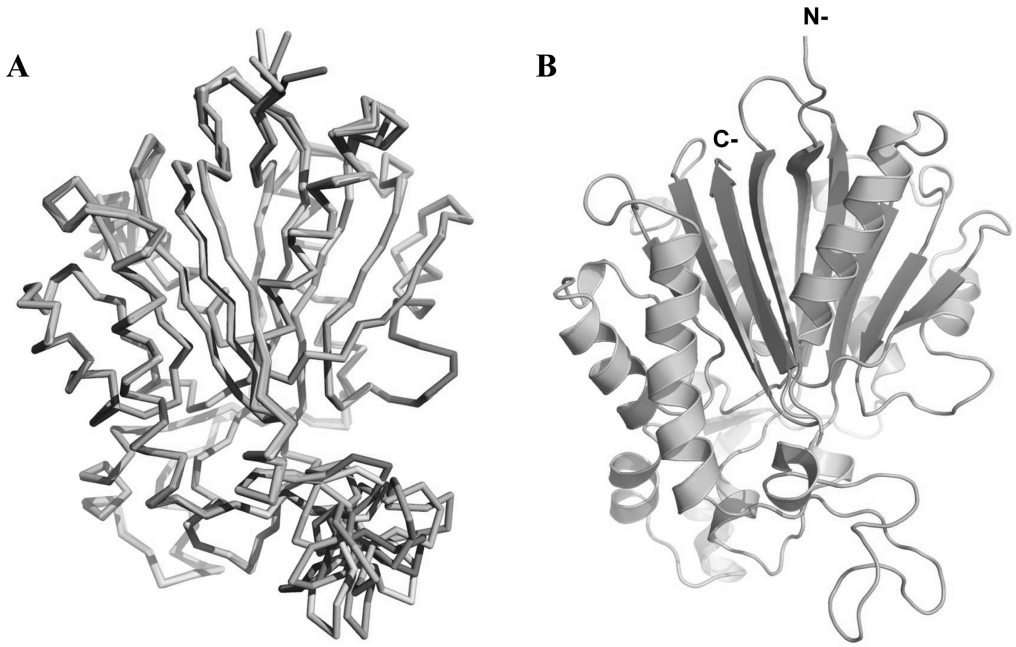

Figure 3. (A) Ribbon representation of five efKAS III structures determined by MODELLER. (B) Representation of three dimensional structure of efKAS III.

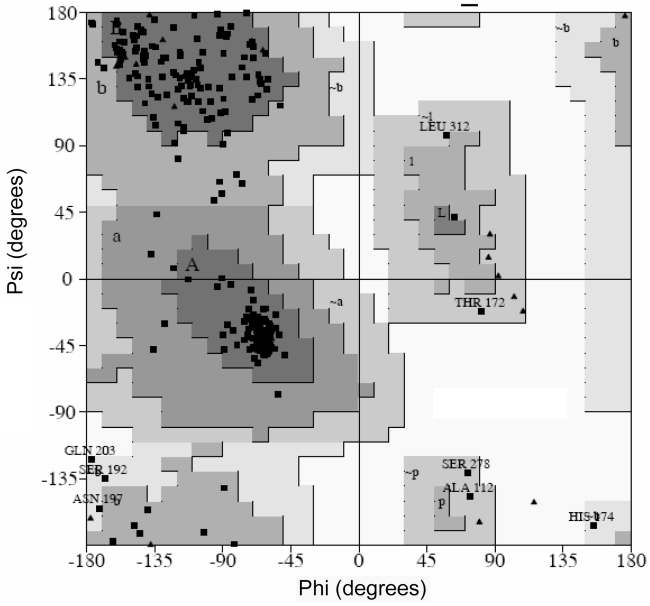

Figure 4. Ramachandran plot of efKAS III obtained by PROCHECK.

site of saKAS III and efKAS III are very similar and substrate specificity of efKAS III should be similar to that of saKAS III.

It is known that natural products have been a particularly rich source of antibacterial agents. Especially flavonoids, a group of polyphenolic compounds, are widely distributed through out the plant kingdom. Antibacterial activity has been displayed by a number of flavonoids.[22] In order to find specific natural inhibitors of efKAS III, we tried docking study for two flavonoids, naringenin (flavonones) and apigenin (flavones). Ligand docking study was carried out

Table 2. Quality of structures checked by PROCHECK

	Ramachandran plot quality (%)			Overall G-factor
	Core	Allowed	Disallowed	
efKAS III 1	88.9	10.8	0.3	0.15
efKAS III 2	88.3	11.4	0.3	0.09
efKAS III 3	88.9	10.8	0.3	0.14
efKAS III 4	88.9	11.1	0	0.17
efKAS III 5	87.5	12.5	0	0.10

for efKAS III with these flavonoids. Lowest models of efKAS III in complex with flavonoids are shown in Figure 6. As shown in figure 6(A), the side chains of Cys113, Phe308, Ser153 and Asn249 play important roles on hydrogen bonds with 7-, 4- and 4-hydroxyl groups of naringenin, respectively (Table 3). However, in case of apigenin, only side chain of Ser153 forms hydrogen bond with 7-hydroxyl group of apigenin. Double bond of C_2-C_3 position of C ring in apigenin provides a structural rigidity compared with naringenin and this rigidity interrupted a formation of H-bond between efKAS III and apigenin. To prove this result, we measured the antibacterial effects of these flavonoids against *E. faecalis* and other bacteria. Only naringenin showed antibacterial activity at the concentration of 256 μg/mL against *E. faecalis* and *S. aureus*, which are gram-positive bacteria. In case of *E. coli*, a gram negative bacteria, both flavonoids did not show antibacterial activity at >1024 μg/mL (Table 3). We expect that the outer membrane of gram-negative bacteria such as *E. coli* acts as a barrier against flavonoids. Previous research by Han *et al.*[23] reported that naringenin showed better antibacterial activity against gram-positive bacteria than gram-negative bacteria. Further study will be performed to prove these

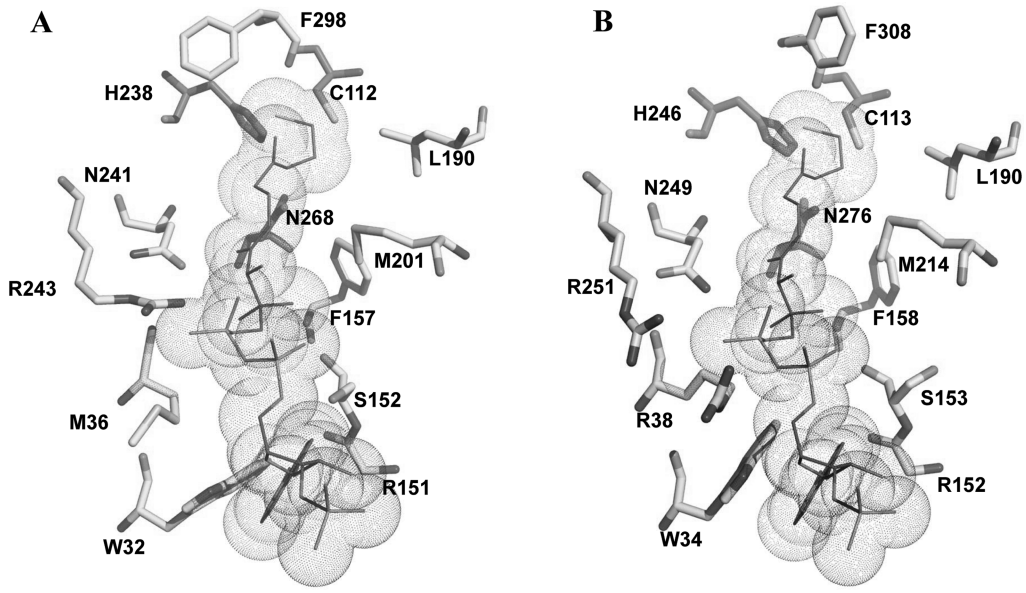

Figure 5. (A) Active sites and presumed binding pockets of saKAS III. (B) Active sites and presumed binding pockets of efKAS III. CoA molecule is depicted by space filling model using dots.

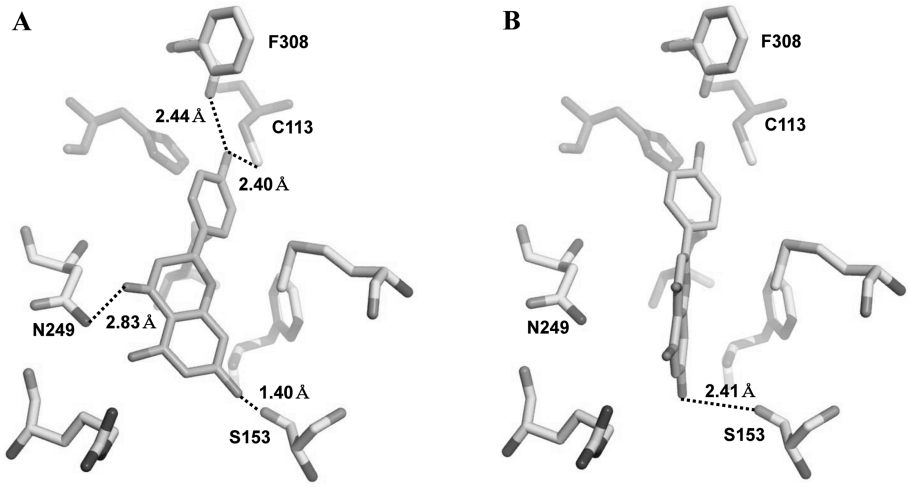

Figure 6. Docking models of flavonoids and efKAS III. (A) Docking model of naringenin and efKAS III. (B) Docking model apigenin and efKAS III.

Table 3. Antimicrobial activities of two flavonoids against *E. faecalis*, *E. coli*, and *S. aureus*

Antimicrobial agents	MIC (μg/mL)		
	E. faecalis	*E. coli*	*S. aureus*
Naringenin	256	> 1024	256
Apigenin	> 1024	> 1024	> 1024

Table 4. Hydrogen bond distances between flavonoids and efKAS III in docking models

Hydrogen bond between KAS III and naringenin	Distance (Å)	Hydrogen bond between KAS III and apigenin	Distance (Å)
Cys113 SH : naringenin 20O	2.44	Ser153 O : apigenin 30H	2.41
Phe308 O : naringenin 32H	2.40		
Ser153 O : naringenin 27H	1.40		
Asn249 NH : naringenin 11O	2.83		

possibilities.

In this study, three dimensional structure of efKAS III was determined by comparative homology modeling. From ligand docking study, naringenin provided proper binding model in active site of efKAS III with intensive hydrogen bond network, and naringenin showed antimicrobial activity against *E. faecalis* (MIC of 256 μg/mL). Purification of efKAS III is underway. Further studies using NMR spectroscopy will be used to develop and screen better inhibitors for efKAS III as potent antibiotics.

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