Research Article

Insights into the structure and 3D spatial arrangement of the b-ketoacyl carrier protein synthases

Dimitrios Vlachakis[1], Spyridon Champeris Tsaniras[2] and Sophia Kossida[1]

[1]Biomedical Research Foundation of the Academy of Athens, 11527, Athens, Greece
[2]Department of Physiology, Medical School, University of Patras, Rio, Patras, Greece

Received on September 17, 2013; Accepted on October 7, 2013; Published on October 17, 2013

Correspondence should be addressed to Dimitrios Vlachakis; Phone: +30 210 6597647, Fax: +30 210 6597545, Email: dvlachakis@bioacademy.gr

Abstract

The b-ketoacyl carrier protein synthases (the KAS enzymes) are key enzymes that can be used as potential anti-Plasmodium drug targets. In bacteria, three KAS enzymes have been identified (KAS I, KAS II and KAS III), whilst in Plasmodium a KAS I/II and KAS III enzyme has been reported. The protein has a total of four active sites, which have been found to be different to each other, rather than four copies of the same active site. The active sites differ not only in the type of interaction they establish with the ligand, but, in the case of Cerulenin as a ligand, the active sites of the KAS I/II enzyme also differ in the number of residues involved in the ligand protein interaction. This is very interesting biochemically, because these differences imply that the affinity of each active site for binding to the ligand might be different as well.

Introduction

Malaria is a parasitic disease transmitted by mosquitoes that can be lethal if left untreated (Figure 1). The real cause of malaria, a single-cell parasite called plasmodium, was discovered in the end of the 19th century (Komaki-Yasude *et al*. 2013). The parasite can be transmitted from an infected person to another through the bite of a female Anopheles mosquito (Olupot-Olupot *et al*. 20183). Today, there is an urgent need for new drugs since most known antimalarial drugs are rapidly losing their effectiveness, as the rate of drug resistance increases. *P. falciparum* is a member of the protistan phylum Apicomplexa (mostly parasites, the Apicomplexa are named after a characteristic apical complex of microtubules within the cell). A curious feature of the apicomplexan parasites is that these single cell organisms harbour a plastid; otherwise better known as an organelle found in plants and algae and classified into leucoplasts, chromoplasts and chloroplasts (Menard *et al*. 2013). This association, which is symbiotic today, is thought to have arisen via the engulfment of a cyanobacterium-like prokaryotic cell by an ancestor of the parasite (McFadden & Roos 1998).

The b-ketoacyl carrier protein synthases (b-KAS) are key regulators of fatty acid biosynthesis (Klein *et al*. 2013), which has emerged as a target for the development of therapeutic agents (Komaki-Yasude *et al*. 2013). Thiolactomycin and cerulenin are two natural ligands, capable of binding to the active site of b-KAS enzymes and inhibit their function (Komaki-Yasude *et al*. 2013). A variety of different structures of these enzymes, from different origins, have already been determined from X-Ray and NMR studies. An extensively studied source of proteins is the *E. coli* bacterium. The latter is a type of bacterium commonly found in the intestine of animals and humans. *E. coli*, the whipping boy of microbiology and genetics labs around the world, is the most encountered bacterium in the clinical laboratory. As part of the normal flora of the human intestinal tract, *E. coli* plays a crucial role in food digestion by producing vitamin K from undigested material in the large intestine. It is a very versatile bacterium, being a very strong strain in terms of survival, requiring only basic nutrients and replicating very fast. As a result, a lot of research has been done on it including X-ray and NMR studies of its KAS proteins.

There are two main fatty acid synthesis systems (Komaki-Yasude *et al*. 2013). The type I system, also known as the associated system, is found in the most complex organisms, such as mammals. This system composes a unique and multifunctional polypeptide. The other, the type II or dissociated system, is

found in lower organisms such as bacteria and plants. This involves the cooperation and coordination of a variety of different enzymes, each having a unique task in the elongation of the fatty acid chain.

Three different synthases have been found to regulate the pathways of the type II system (KAS I, II and III). According to work done by Zhang *et al.* (2000), the KAS enzymes are mainly known for catalysing the Claisen condensation reaction. This is done by the transport of an acyl primer to malonyl-ACP. The result is therefore a β-Ketoacyl-Acyl carrier protein elongated by two carbon units (Figure 1).

The β-Ketoacyl-Acyl Carrier Protein Synthase I (KAS I) plays a very important role in the elongation of fatty acids. Studies have shown that mutants lacking the KAS I enzyme face serious problems in growth and require exogenous unsaturated fatty acids. The function of KAS II is to mainly control the temperature - dependent regulation of fatty acid composition. Lack of KAS II will result in failure of the elongation of palmitoleate to cis-vaccenate. However, under standard culture conditions, growth is not suppressed (Komaki-Yasude *et al*. 2013). Finally, KAS III is responsible for controlling the rate of fatty acid synthesis, by catalyzing the first step in the pathway.

The β-ketoacyl synthase (KAS) components of fatty acids, together with polyketide synthase, create new carbon-carbon bonds by carrying out Claisen condensations. There are three main steps in these reactions that result in the elongation of specific primers. The first part involves the transfer of an acyl primer substrate from the acyl carrier protein (ACP) or CoA to a cysteine residue of the condensing enzyme. The second part consists of decarboxylation of an ACP-bound elongating substrate, so as to give a carbanion and, the last part, is the condensation of the carbanion with the carbonyl carbon of the enzyme-bound acyl primer. Three KAS isoenzymes participate in the synthesis of fatty acid carbon skeletons in plant plastids and *E. coli*, as part of multifunctional fatty acid synthase complexes, in which each protein is encoded by a discrete gene. Substrate specificities of KAS I, II and III overlap. KAS III is specialized in the first elongation step from C_2 to C_4 and uses a CoA-activated primer substrate. KAS I and II employ ACP-activated primer substrates. In plastids, KAS I extends C_4 to C_{16} whereupon KAS II carries out an additional step to give C_{18}. KAS proteins with 85% identity to KAS II, called KAS IV, found in the seeds of some plants, are implicated in the synthesis of intermediate chain lengths from C_8 to C_{14}. Classic studies in *E. coli* suggest that KAS I is unique in carrying out the first step of the unsaturated pathway whereas KAS II is unique in accomplishing the last one. In both plant plastids and *E. coli*, KAS I and II sequences show extensive homology, whereas KAS III sequences are more closely related to those of the polyketide chalcone synthase (CHS) enzymes. Disparities in substrate specificity are also reflected in differing sensitivities to antibiotics; for example, with cerulenin, which binds covalently to the active-site cysteine, KAS III is insensitive, KAS I is highly sensitive and KAS II is moderately sensitive. Inhibitors of the β-KAS I of *E. coli*, include thiolactomycin and the natural drug cerulenin (Figure 2).

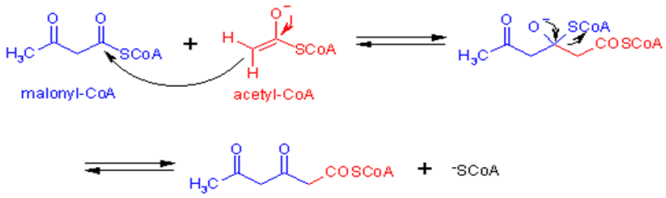

Figure 1. The Claisen reaction between acetyl-CoA and malonyl-CoA illustrates how b-keto esters are built up by nature using the enolate derived from acetyl-CoA as nucleophile. This reaction plays an important role in the biosynthesis of fatty acids.

The structure of b-ketoacyl carrier protein synthases is a dimeric structure with each of the monomers made up of a duplicated helix-sheet-helix motif (Figure 3). Cerulenin is an irreversible inhibitor of the b-ketoacyl carrier synthase proteins I and II. It establishes a covalent bond with the cysteine of the active site of the protein. It is not selectively antibacterial, as it has been found that it is a possible inhibitor of the condensation reaction, which is catalyzed by the multifunctional fatty acid synthase type I, that is found in

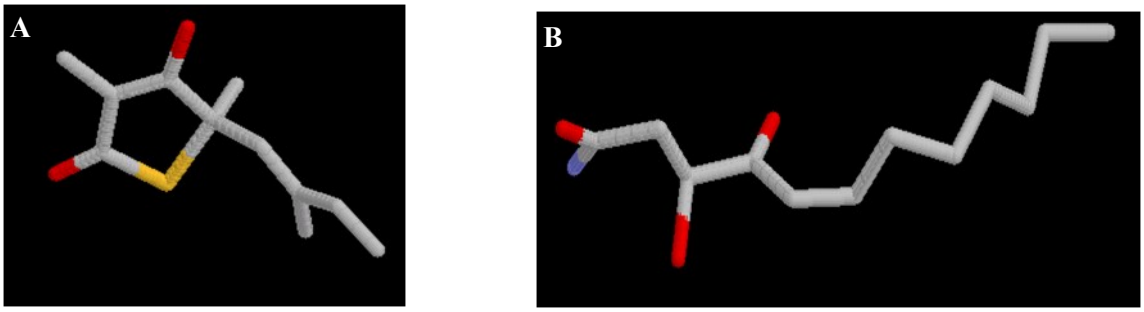

Figure 2. The structures of thiolactomycin (A) and cerulenin (B).

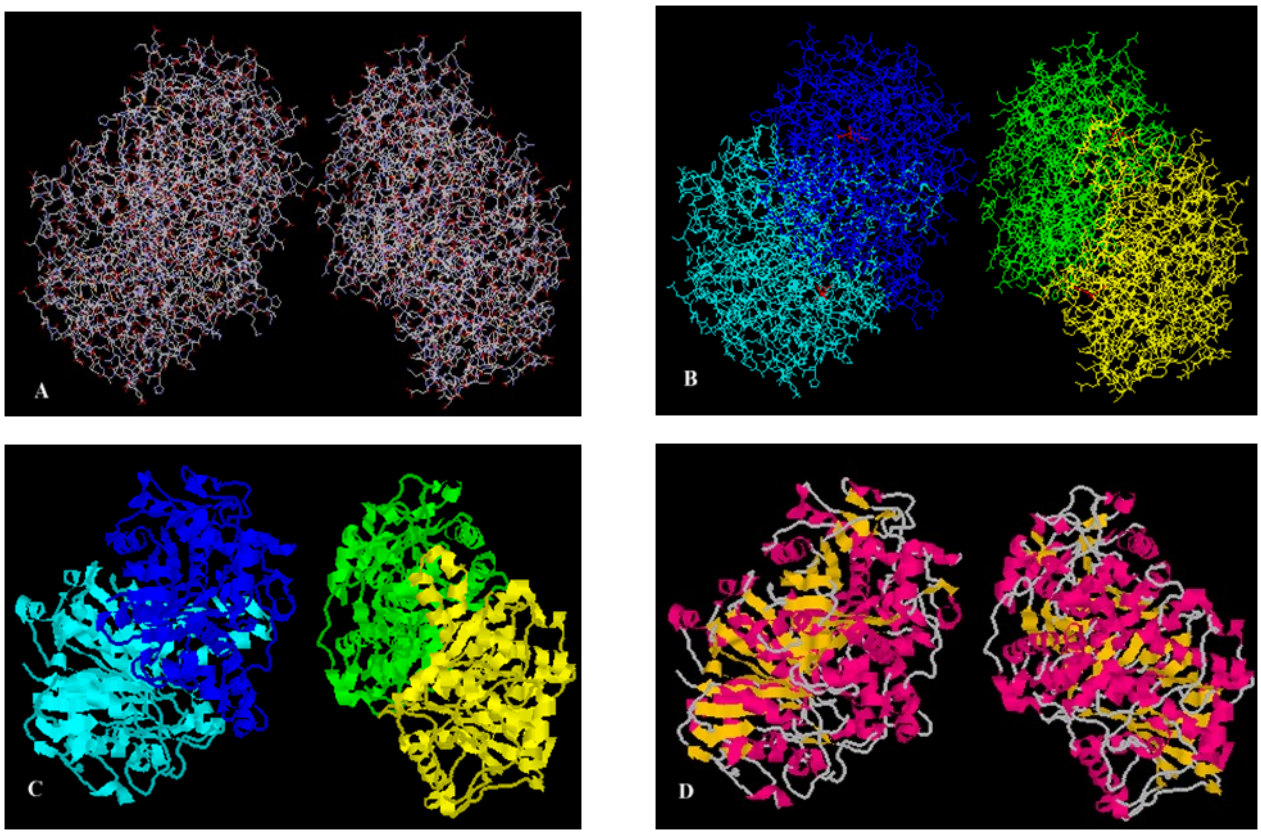

Figure 3. The structure of β-ketoacyl-[acyl-carrier-protein] synthase I, coloured in CPK (A), by chain (B), by chain in cartoon display with the protein's motifs visible (C) and by motif in cartoon mode (D). A-helices are in pink colour and β-sheets are in orange.

mammals (Sato *et al*. 2005, Sharma *et al.* 2007). It has also been found that Cerulenin has anti-neoplastic activities, from experiments in mice. Thiolactomycin, on the other hand, is a molecule that can reversibly inhibit type II fatty acid synthases, even though it is not active against type I (Ramya *et al*. 2007, Roberts *et al*. 2003). Thiolactomycin shows activity against Gram-negative anaerobes, from the periodontal disease, and from its ability to inhibit mycolic acid. It is also an antimycobacterial agent. Finally, this drug can be used as an agent against malaria and trypanosomes, which makes it an ideal antimicrobial research tool.

Results & Discussion

The Ligand-Protein Contacts (LPC) analysis was performed on both 1FJ4 and 1FJ8, both of which correspond to the KAS I protein, with thiolactomycin and cerulenin docked, respectively (Vlachakis *et al*. 2009). The active sites were studied in terms of their distance from the ligand, their exposed contact accessible area and the type of bond that they establish (Palaiomylitou *et al*. 2008). Four groups of interaction types were generated by the LPC analysis: hydrophilic-hydrophilic (hydrogen bond), aromatic-aromatic, hydrophobic-hydrophobic and hydrophobic-hydrophilic contact (Vangelatos *et al.* 2009).

As shown in Table 1, the most variable interaction type among the subunits is the hydrophobic-hydrophilic contact. Those bonds are not as strong as the h-bonds but are still very important in defining the affinity of the ligand's binding to the active site of the protein. Hydrophobic interactions are the major driving force between non-polar groups and water that force the non-polar groups to aggregate together in order to minimise their surface area and contact with the latter. It should be noted that hydrophobic/hydrophilic interactions belong to the dominant forces determining protein structure (Sellis *et al.* 2009). On the other hand, hydrogen bonds are intermolecular forces between strongly charged dipoles located on different molecules or different parts of a single large molecule. Although stronger than most other intermolecular forces, hydrogen bonds are much weaker than both ionic and covalent bonds. The hydrogen bond has only ~5% the strength of a covalent bond. However, when many hydrogen bonds are formed between two molecules (or parts of the same molecule), the resulting union can be sufficiently strong so as to be quite stable.

Table 1. LPC analysis on the characteristics and the interactions that take place between the KAS I protein and thiolactomycin (pdb entry: 1FJ4) or Cerulenin (pdb entry: 1FJ8).

Thiolactomycin																								
Residue	Dist				Surf				HB				Arom				Phob				DC			
163 CYS	3.2	3.2	3.2	3.3	13.0	12.4	13.0	12.5	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
206 ALA	5.5	5.6	5.4	5.6	1.8	1.8	2.0	1.8	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
229 PHE	3.4	3.4	3.5	3.5	40.2	39.7	39.7	37.9	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
265 ASP	5.2	5.2	5.1	4.4	0.4	0.2	0.4	0.9	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
268 ASP	3.8	3.8	3.7	3.7	5.2	6.1	6.7	5.2	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
270 VAL	3.5	3.4	3.5	3.6	13.0	12.8	13.7	12.3	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+
271 ALA	3.3	3.2	3.2	3.3	41.3	42.2	41.3	42.6	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
272 PRO	3.9	3.9	3.8	3.8	30.3	30.3	40.1	40.0	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
298 HIS	2.8	2.9	3.0	2.9	21.7	23.6	21.6	23.0	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
300 THR	3.7	3.7	3.8	3.7	20.9	20.0	19.3	20.6	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
302 THR	4.0	3.9	4.0	3.9	14.4	16.6	14.9	16.6	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
305 GLY	4.0	3.9	4.0	4.0	27.0	26.6	17.3	17.4	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+
333 HIS	2.6	2.7	2.7	2.7	31.2	30.6	30.9	30.4	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-
390 PHE	3.3	3.3	3.3	3.2	46.4	44.4	43.5	49.1	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-
391 GLY	3.5	3.6	3.6	3.5	18.8	19.3	19.1	18.2	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
392 PHE	3.7	3.8	3.6	3.9	30.1	29.7	31.0	28.7	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
393 GLY	3.8	3.8	3.9	3.9	4.5	5.4	3.8	4.5	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
394 GLY	3.3	3.4	3.3	3.3	35.2	33.0	34.1	34.1	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
396 ASN	5.0	5.0	5.0	4.8	5.6	5.6	5.6	6.1	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+

Cerulenin																								
Residue	Dist				Surf				HB				Arom				Phob				DC			
106 GLY	3.2	3.4	3.1	3.3	29.2	28.5	31.9	30.1	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
107 GLY	3.5	3.7	3.6	3.6	9.0	7.6	7.2	7.6	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
110 PRO	4.3	4.5	4.3	4.4	9.2	7.9	7.6	7.6	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
162 ALA	3.3	3.3	3.3	3.3	17.9	17.3	15.9	16.6	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
163 CYS	1.9	1.9	1.9	1.9	65.1	61.4	58.4	58.6	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+
191 GLU	4.7	4.8	4.9	4.8	0.9	0.2	0.2	0.7	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
197 MET	3.4	3.4	3.3	3.4	31.6	30.1	33.2	38.8	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	+
200 GLU	2.8	2.7	2.9	2.8	30.3	30.3	28.9	34.5	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
201 PHE	3.0	2.9	2.7	2.8	45.3	44.8	49.8	49.9	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
229 PHE	5.0	5.0	4.8	4.9	7.2	7.0	7.7	6.3	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
298 HIS	3.0	2.7	2.8	2.8	18.3	28.8	20.4	20.2	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
300 THR	4.0	4.0	3.8	3.9	2.9	2.9	3.8	3.4	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+
333 HIS	2.8	2.8	2.6	2.7	25.7	17.5	29.4	28.5	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
335 LEU	4.2	4.2	4.3	4.2	18.2	17.3	17.9	17.9	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
390 PHE	3.7	3.6	3.8	3.7	7.4	7.6	5.9	6.7	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+
391 GLY	2.9	2.8	2.9	2.8	15.8	16.8	10.1	15.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
392 PHE	2.7	2.7	2.7	2.7	72.8	70.4	67.6	70.3	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
113 GLN	3.5	3.3	2.9	4.3	19.3	27.1	34.5	11.0	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-
133 VAL	X	4.6	X	X	X	0.7	X	X	X	-	X	X	X	-	X	X	X	-	X	X	X	+	X	X
134 VAL	3.3	3.5	3.4	3.5	18.1	25.0	19.2	15.9	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
137 ALA	3.3	3.4	3.3	3.4	27.6	16.6	11.7	24.7	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+
138 MET	3.4	3.6	3.4	3.6	34.8	34.3	35.7	32.1	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-

Legend

Colors: Red = subunit A, Green = subunit B, Blue = subunit C, Gray = subunit D.
Brown = subunit A, Light Blue = subunit B, Purple = subunit C, Orange = subunit D.
Dist: nearest distance (Å) between atoms of the ligand and the residue
Surf: contact surface area (Å^2) between the ligand and the residue
HB: hydrophilic-hydrophilic contact (hydrogen bond)
Arom: aromatic-aromatic contact
Phob: hydrophobic-hydrophobic contact
DC: hydrophobic-hydrophilic contact (destabilizing contact)
+/-: indicates presence/absence of a specific contacts
Underlined: indicates residues contacting ligand by their side chain (including CA atoms)

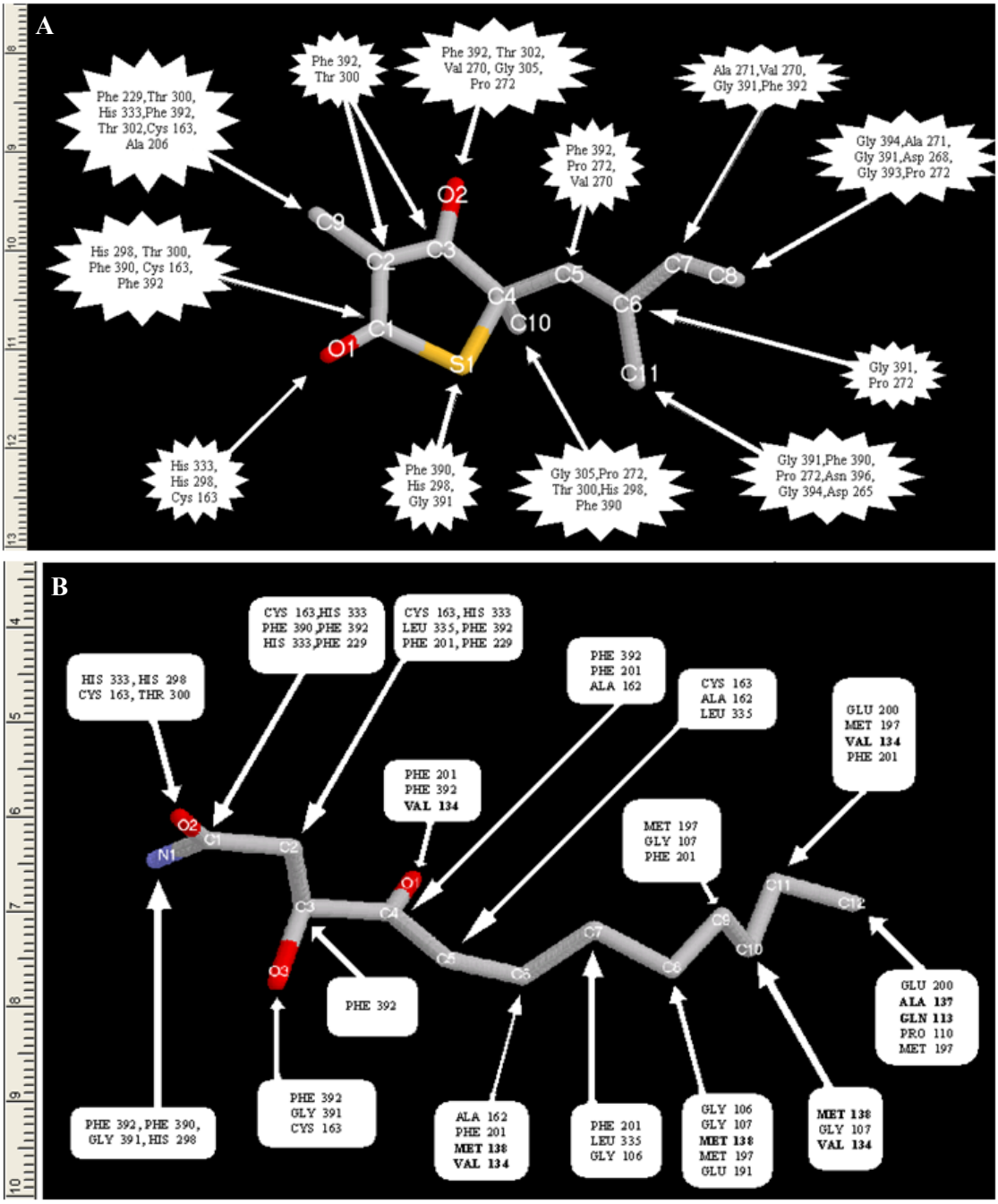

Figure 4. Interactions of the KAS I enzyme. The interactions that thiolactomycin (**A)** and cerulenin (**B**) establish with the active site of the KAS I enzyme (data from LPC analysis of the 1FJ4 and 1FJ8 pdb files, respectively). Note that cerulenin only interacts with Val134 (in bold) in subunit B.

The differences in the interaction between the four subunits of the KAS enzyme when thiolactomycin and cerulenin bind are shown in Figure 4. In the case of thiolactomycin, there are only differences in the hydrophobic-hydrophilic contacts between the protein and the ligand. Because those differences though are not many and aren't particularly strong, one could even ignore them; even though, as it has already been mentioned, the hydrophobic-hydrophilic contacts are key contacts, which determine the protein structure.

On the other hand, when cerulenin binds to the KAS enzyme, there are more significant differences among the four subunits of the protein. More specifically, subunits A and B both establish a hydrogen bond between the residue Cys163 and the ligand, whereas subunits C and D don't. All the other interactions between the ligand and the active site of the protein are exactly the same, as far as Cys163 is concerned. Another case with varying hydrogen bond interactions is Thr300. This residue establishes a hydrogen bond with

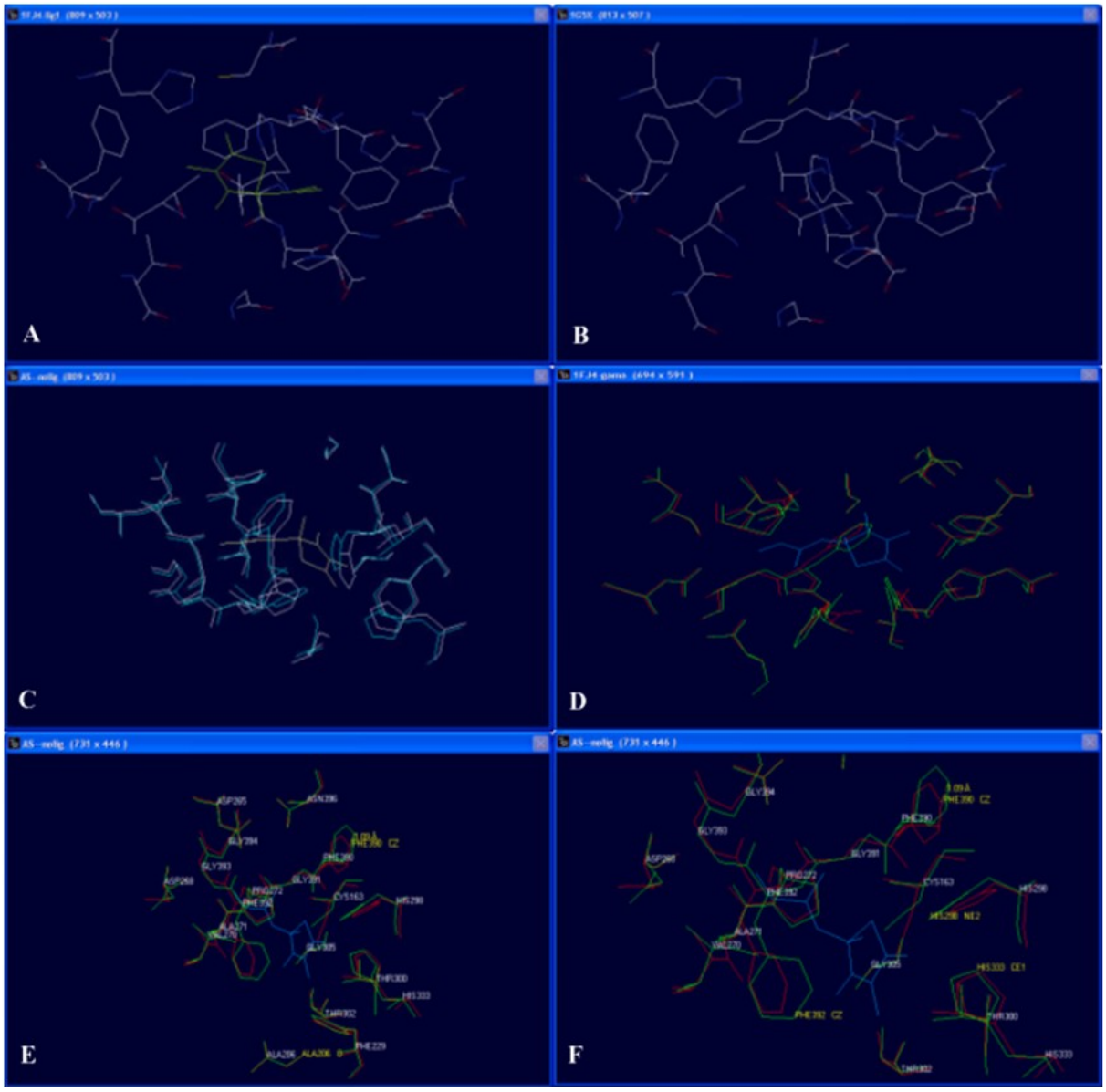

Figure 5. The active site of the KAS I enzyme.

the ligand only in subunit C. The greatest difference among the active sites of the four subunits is the Val133 residue, which is only found in the B subunit. The existence of Val133 in the active site of the B subunit has an effect on the properties of this particular site as it establishes a DC interaction with the ligand. Another difference between the active sites involves the non-establishment of DC bonds between the ligand and Gln113 in subunit D, the establishment of a DC bond between the ligand and Met138 in subunit C, the establishment of a DC bond between the ligand and Ala137 in subunit D and finally, the establishment of a DC bond between cerulenin and Met197, only in the B and D subunits of the protein.

The active site of 1FJ4, which was isolated using the LPC analysis software, is depicted in Figure 4. 1FJ4 is a complex of the KAS protein with thiolactomycin. The pdb file was edited and only Subunit A was retained. Then, based on the results of the LPC analysis, only the residues of the active site and the ligand were retained. Following the same procedures

Residues	Surface (Å^2)	Distance (Å)
Phe390	46.4	1.09
Phe392	30.1	1.21
His298	21.7	1.68
Ala206	1.8	0.13
Asp265	0.4	0.21

Table 2. Contact surface area (Å^2) and movement distance (Å) of some residues in the KAS I protein's active site.

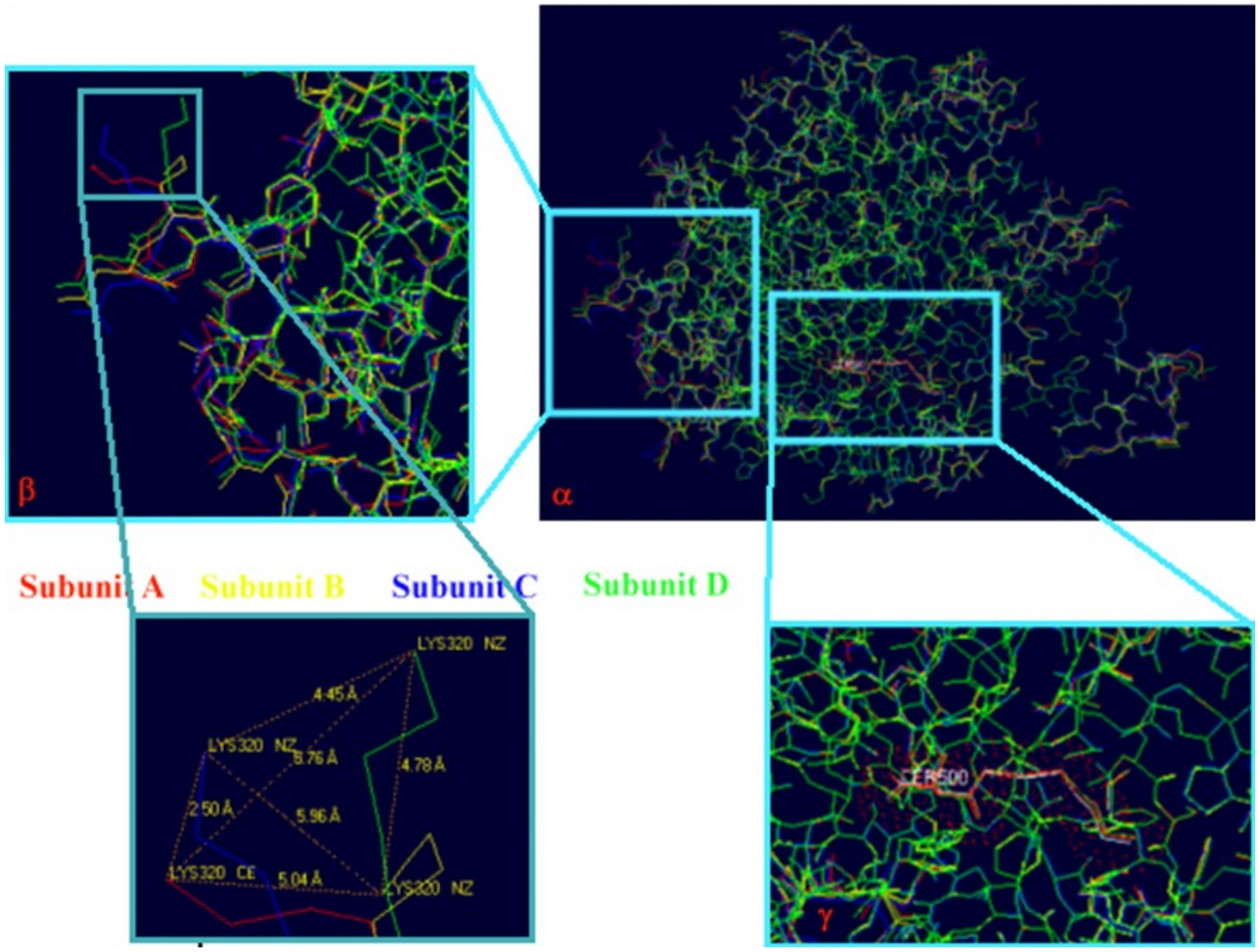

Figure 6. Different views of the superimposition of the four subunits of the KAS I enzyme with cerulenin docked (pdb entry: 1FJ8).

the active site of KAS I without any ligand docked was also isolated (pdb entry: 1G5X).

Figures 5A and 5B show the active site of the KAS I enzyme, with and without the ligand docked, respectively (in 5A thiolactomycin is in yellow). In Figures 5C and 5D, the two active sites have been superimposed. In Figure 5C the active site without the ligand is in blue and in 5D in red. It is obvious that the conformation of the active site changes significantly with the ligand binding. The RMSd value of the two active site superimposition was found to be 0.67 A, with 19 residues of each one of the two active sites involved in the calculation. Movement of the residues in the active site when the ligand binds can also be seen in Figures 5E and 5F, were the labels of the residues have been added as well.

From the LPC analysis it was found that the contact surface area (calculated in $Å^2$) between the ligand and the residue is directly proportional to the degree of the residue's movement when the ligand binds to the active site. In Table 2 the contact surface area ($Å^2$) and the movement distance (Å) of some residues have been summarized.

The LPC analysis revealed a several differences between the four active sites of the KAS I enzyme with cerulenin bound to it. In order to investigate the conformational differences between the four subunits of the KAS I protein (with cerulenin in the active site), they were superimposed. Figure 6α shows the four superimposed subunits together with a subunit-colour reference below. Then in 6β, a part of the superimposition near the outer area of the four subunits is magnified, whereas 6γ focuses on the superimposed area around the active sites of the subunits. It is very clear that the superimposition in the active-site area of the subunits has been done smoothly, as there are no big movements and conformational changes. On the other hand, a lot differences between the four subunits can be observed on the outside of the protein. In Figure 6δ, a part of 6β has been further magnified and the distances between the same residues on different subunits have been measured. These are huge differences as they can be as large as 5.76 A. One could dismiss these

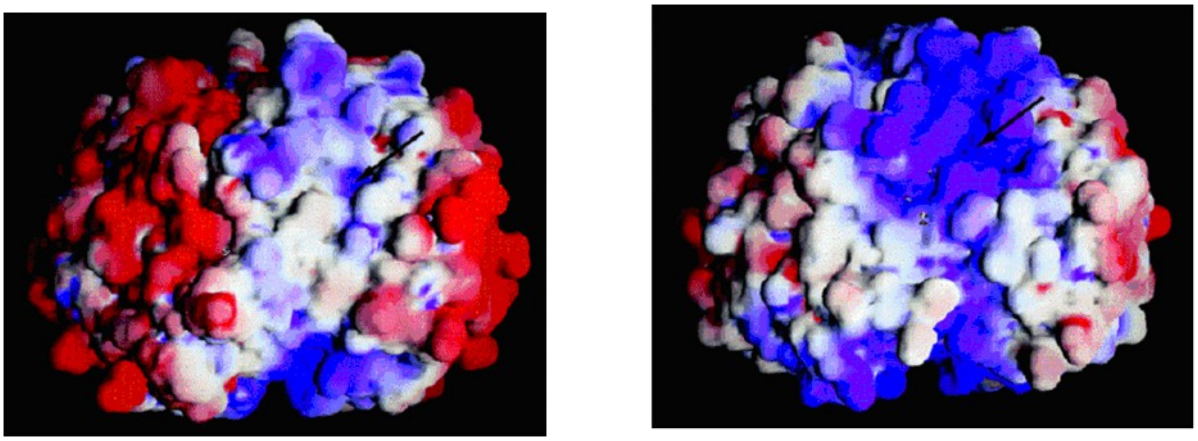

Figure 7. Accessible surface map representation of electrostatic potential distribution on the KAS I and KAS II dimer, respectively. Positive and negative electrostatic potentials are colored blue and red, respectively. Notice that the overall distribution of charges is the same: Positive potential around the active site flanked by strong negative potential. An arrow points to the active site entrances.

differences as insignificant to the function of the protein, as they are far away from the active site. However, according to the work by Olsen *et al.* (2001) it has been found that there is a path the ligand has to follow to reach the active site of the KAS I protein. As a result, the conformation of the outside of the protein might have an effect on the easiness or difficulty that the ligand will have to cope with, in order to reach the active site. Thus, the conformation on the outside of the subunit could affect its affinity for binding the ligand as a whole. Figure 7 shows part of the work done by Olsen *et al.* (2001) emphasising the entrance of the active site of the KAS I subunit. It is assumed that conformational changes between KAS I and KAS II proteins are responsible for ligand selectivity and binding affinity.

Conclusions

The KAS protein consists of four identical subunits with an active site on each one. It was found that the way the ligand binds is different among the four active sites of the protein. Thiolactomycin binds by establishing different sets of interactions among the active sites of the protein. The residues of the active site though are the same in all four active sites of the protein. On the other hand, cerulenin not only binds by establishing different interactions among the active sites, but it also uses one more residue in subunit B. As a result, it is not only the type of interactions that varies from active site to active site, but it is also the number of residues too. Moreover, superimpositions of the empty active site over the same active site with the ligand present, reveal significant active-site residue movement when the ligand binds. This occurs for both thiolactomycin and cerulenin. It was found that the distance that the active site's residues move when the ligand binds, depends on the exposed contact surface of each residue. Residues with a large contact surface area moved a lot, whereas residues with little exposed contact surface area either moved little or not at all. The key residues are the ones that make up the active site of the protein (especially the two histidines, which establish H-bonds) and those that are generally conserved (evolutionary). This preliminary work will pave the way towards a holistic future docking, *de novo* drug-design and lead optimization of potent anti-plasmodium agents.

References

Klein EY 2013 Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread. *Int J Antimicrob Agents* **41** 311-317

Komaki-Yasuda K, Okuwaki M, Nagata K, Kawazu S & Kano S 2013 Identification of a Novel and Unique Transcription Factor in the Intraerythrocytic Stage of Plasmodium falciparum. *PLoS One* **8** e74701

Ménard D, Ariey F & Mercereau-Puijalon O 2013 Plasmodium falciparum susceptibility to antimalarial drugs: global data issued from the Pasteur Institutes international network. *Med Sci (Paris)* **29** 647-655

Olsen JG, Kadziola A, von Wettstein-Knowles P, Siggaard-Andersen M & Larsen S 2001 Structures of beta -ketoacyl-acyl carrier protein synthase I complexed with fatty acids elucidate its catalytic machinery. *Structure* **9** 233-243

Olupot-Olupot P & Maitland K 2013 Management of severe malaria: results from recent trials. *Adv Exp Med Biol* **764** 241-250

Palaiomylitou M, Tartas A, Vlachakis D, Tzamarias D & Vlassi M 2008 Investigating the structural stability

of the Tup1-interaction domain of Ssn6: Evidence for a conformational change on the complex. *Proteins* **70** 72-82

Ramya TN, Mishra S, Karmodiya K, Surolia N & Surolia A 2007 Inhibitors of nonhousekeeping functions of the apicoplast defy delayed death in Plasmodiumfalciparum. *Antimicrob Agents Chemother* **51** 307-316

Roberts CW, McLeod R, Rice DW, Ginger M, Chance ML & Goad LJ 2003 Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. *Mol Biochem Parasitol* **126** 129-142

Sato S & Wilson RJ 2005 The plastid of Plasmodium spp.: a target for inhibitors. *Curr Top Microbiol Immunol* **295** 251-273

Sellis D, Drosou V, Vlachakis D, Voukkalis N, Giannakouros T & Vlassi M 2012 Phosphorylation of the arginine/serine repeats of lamin B receptor by SRPK1-Insights from molecular dynamics simulations. Biochim Biophys Acta **1820** 44-55

Sharma S, Sharma SK, Modak R, Karmodiya K, Surolia N & Surolia A 2007 Mass spectrometry-based systems approach for identification of inhibitors of Plasmodium falciparumfatty acid synthase. *Antimicrob Agents Chemother* **51** 2552-2558

Vangelatos I, Vlachakis D, Sophianopoulou V & Diallinas G 2009 Modelling and mutational evidence identify the substrate binding site and functional elements in APC amino acid transporters. *Mol Membrane Biol* **26** 356-370

Vlachakis D 2009 Theoretical study of the Usutu virus helicase 3D structure, by means of computer-aided homology modeling. *Theor Biol Med Model* **6** 9

Waller RF, Keeling PJ, Donald RG, Striepen B, Handman E, Lang-Unnasch N, Cowman AF, Besra GS, Roos DS & McFadden GI 1998 Nuclear-encoded proteins target to the plastid in Toxoplasma gondii and Plasmodium falciparum. *Proc Natl Acad Sci U S A* **95** 12352-12357

Zhang YM, Rao MS, Heath RJ, Price AC, Olson AJ, Rock CO & White SW 2001 Identification and analysis of the acyl carrier protein (ACP) docking site on beta-ketoacyl-ACP synthase III. *J Biol Chem* **276** 8231 -8238