Structure of homoserine *O*-acetyltransferase from *Staphylococcus aureus*: the first Gram-positive ortholog structure

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Homoserine *O*-acetyltransferase (HTA) catalyzes the formation of L-*O*-acetyl-homoserine from L-homoserine through the transfer of an acetyl group from acetyl-CoA. This is the first committed step required for the biosynthesis of methionine in many fungi, Gram-positive bacteria and some Gram-negative bacteria. The structure of HTA from *Staphylococcus aureus* (SaHTA) has been determined to a resolution of 2.45 Å. The structure belongs to the α/β -hydrolase superfamily, consisting of two distinct domains: a core α/β -domain containing the catalytic site and a lid domain assembled into a helical bundle. The active site consists of a classical catalytic triad located at the end of a deep tunnel. Structure analysis revealed some important differences for SaHTA compared with the few known structures of HTA.

1. Introduction

Homoserine *O*-acetyltransferase (HTA; EC 2.3.1.31) lies at a branch point in the aspartate metabolic pathway (Viola *et al.*, 2011; Umbarger, 1978) which leads to the biosynthesis of the essential amino acids methionine and lysine in most plants, bacteria and fungi. The branch leading to the synthesis of methionine, as well as the essential methyl-transfer cofactor *S*-adenosylmethionine (AdoMet), starts with the acetylation of homoserine to form *O*-acetyl-homoserine (OAH) catalyzed by HTA. AdoMet is also the substrate for a group of enzymes that synthesize acylhomoserine lactones (AHLs), which are virulence signaling molecules in Gram-negative bacteria (Hanzelka & Greenberg, 1996; Schaefer *et al.*, 1996). The reaction catalyzed by HTA involves the acetylation of the γ -hydroxyl of homoserine through an acetyl-CoA-dependent acetylation *via* a double-displacement mechanism (Born & Blanchard, 1999) facilitated by a classic Ser–His–Asp catalytic triad which is located at the bottom of a narrow tunnel (Mirza *et al.*, 2005). This reaction represents a critical control point for cell growth and viability. Genetic studies in bacteria and yeast have shown that deletion of the *met2* gene that encodes HTA is lethal in minimal medium, and methionine supplementation is required for cell viability (Pascon *et al.*, 2004; Nazi *et al.*, 2007). Additionally, the *met2* gene has been established to be essential for virulence in human pathogens (Nazi *et al.*, 2007). Since the aspartate pathway is absent in mammals but is necessary for bacterial survival, the enzymes in this pathway are potential targets for the development of new antibiotics (Pavlovsky *et al.*, 2012).

In the present study, we report the structure of HTA from *Staphylococcus aureus* (SaHTA). *S. aureus* is a Gram-positive bacterium and is the most common species of *Staphylococcus* to cause staph infections. MRSA is a widespread strain of *S. aureus* that has evolved resistance to β -lactam antibiotics, increasing the urgency for the development of new drugs against this human pathogen. Because HTA catalyzes an important step in an essential metabolic pathway in *S. aureus*, this enzyme can potentially serve as a novel target (De Pascale *et al.*, 2011) for the development of *S. aureus*-selective drugs. This paper reports the structure of SaHTA determined to 2.45 Å resolution with an overall conserved secondary structure of the α/β -hydrolase superfamily. This study also shows differences in the structure from previously reported structures of *Haemophilus influenza* HTA (HiHTA; Mirza *et al.*, 2005) and *Leptospira interrogans* HTA (LiHTA; Wang *et al.*, 2007).

2. Materials and methods

2.1. Expression and purification of recombinant SaHTA

The sequence of the putative *met2*-encoded HTA gene from *S. aureus* has been reported. Two oligonucleotide primers, 5'-AGAAAGCTGGGTCCTTAGCTTAAAA-3' and 5'-AAAAAG-CAGGCTTCGAAGGAGATAGA-3', were designed and synthesized (IDT) to be complementary to the amino-terminal and carboxyl-terminal ends of the gene, respectively. These primers were used to amplify the *SaHTA* gene from *S. aureus* genomic DNA using standard PCR conditions. The gene was cloned into pDEST42 vector containing a carboxyl-terminal hexahistidine tag by Gateway Technology (Invitrogen). The BL21 (DE3) *Escherichia coli* cell line transformed with the plasmid DNA was used for the expression of recombinant native *SaHTA*. Transformed cells were grown at 310 K until the culture density reached an OD₆₀₀ of 0.7–0.8. Cells were then cooled to 289 K followed by the addition of IPTG to a final concentration of 1 mM and were allowed to grow for an additional

16 h at 289 K (De Pascale *et al.*, 2011). Cells were harvested by centrifugation at 17 000g for 10 min. The cell paste (5 g) was resuspended in 25 ml of a buffer consisting of 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 500 mM NaCl, 20 mM imidazole, 5 mM β -mercaptoethanol, 10% glycerol pH 7.5 and the cells were homogenized using a hand homogenizer. The cells were lysed by sonication for a total of 8 min at 30 s intervals with a sonic dismembrator and the cell debris was removed by centrifugation at 12 000g for 30 min. The supernatant was filtered through a 0.8 μ m membrane syringe filter and loaded onto an Ni-IMAC column for initial purification. The enzyme was further purified on a Source 30Q anion-exchange column using an ÄKTA chromatographic system (GE Biosciences). The fractions that showed catalytic activity and contained a single band on a Coomassie-stained polyacrylamide gel were pooled and dialyzed overnight in a buffer consisting of 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM dithiothreitol (DTT), 0.1 mM ethylenediaminetetraacetic acid (EDTA). The protein was then concentrated using 10 kDa cutoff ultracentrifugal concentrators

Figure 1

(a) Stereoview of *SaHTA* in a ribbon representation. The β -sheets colored yellow and the α -helices colored red compose the core domain, while the α -helices of the lid domain are colored blue. The catalytic triad residues Ser131, Asp267 and His296 are shown in stick representation. (b) Schematic diagram of the topology of *SaHTA* showing the two domains and the catalytic residues.

Table 1 Data-collection and refinement statistics for *Sa*HTA.

Values in parentheses are for the highest resolution shell.

Data collection	
Wavelength (Å)	1.033
Space group	$P6_122$
Unit-cell parameters (Å, °)	$a = 49.16, b = 49.16, c = 481.47,$ $\alpha = \beta = 90, \gamma = 120$
Resolution (Å)	50–2.45 (2.54–2.45)
Observed reflections	73534
Unique reflections	21299
Multiplicity	3.5 (2.8)
Completeness (%)	88.7 (54.9)
R_{merge} (%)	0.148 (0.370)
$\langle I/\sigma(I) \rangle$	7.3 (2.6)
Wilson B factor (Å ²)	39.9
Refinement	
R_{work}	0.24
R_{free}	0.29
No. of protein atoms	2660
No. of waters	31
R.m.s.d., bond lengths (Å)	0.008
R.m.s.d., bond angles (°)	1.359
Average B factor (Å ²)	59.8
Ramachandran plot statistics†, residues in (%)	
Favored region	93.5
Allowed region	5.6
Outlier region	0.9

† As reported by Coot (Emsley *et al.*, 2010).

BALBES was further refined by *Buccaneer* (Cowtan, 2006), an automated protein model-building program in the *CCP4* suite (Winn *et al.*, 2011). The program automatically located most of the protein residues and the model generated had an overall figure of merit (FOM) of 0.60 with $R_{\text{work}} = 0.30$ and $R_{\text{free}} = 0.35$. The model was further refined and diffraction phases to 2.45 Å resolution were calculated and improved using *Coot* (Cowtan, 2006; Emsley *et al.*, 2010) and *REFMAC5* (Murshudov *et al.*, 2011). Weighting of the X-ray and geometric components in the minimization function in *REFMAC5* was carried out by choosing a geometric weighting factor of 0.03. A series of cycles of manual building and iterative restrained refinement with the data resolution extended to 2.45 Å produced the final structure with $R_{\text{work}} = 0.24$ and $R_{\text{free}} = 0.29$. The refined model had an overall FOM of 0.73. The stereochemical quality of the final model was checked by *PROCHECK* (Laskowski *et al.*, 1993). The final structure was visualized and analyzed using *PyMOL* (DeLano, 2002). Atomic coordinates and structure factors have been deposited in the Protein Data Bank (Berman *et al.*, 2003) with accession code 4ql0. The statistics of the structure refinement are summarized in Table 1.

3. Results and discussion

3.1. Structure overview

The full-length *Sa*HTA enzyme consists of 322 amino acids, while the structural model accounts for residues 3–322 as well as the additional seven amino acids (DPAFLYK) which were derived from the vector. The structure of *Sa*HTA is a member of the α/β -hydrolase fold family (Ollis *et al.*, 1992; Heikinheimo *et al.*, 1999; Nardini & Dijkstra, 1999), sharing the same overall structural features as *Hi*HTA and *Li*HTA. *Sa*HTA is organized into two distinct domains: a core α/β -domain (residues 3–161 and 239–322) and a helical bundle lid domain (residues 162–238) forming a canopy over the core domain (Fig. 1*a*). The core domain consists of an eight-stranded, predominantly parallel β -sheet ($\beta 1, \beta 4$ – $\beta 10$), with the connectivity of the β -sheet achieved by five α -helices (αA – αE) on one side and one (αF) on the opposite side (Fig. 1*b*). Two short antiparallel β -strands,

$\beta 2$ and $\beta 3$, provide the connection between $\beta 1$ and $\beta 4$. The lid domain is organized as five α -helices ($\alpha L1$ – $\alpha L5$). This domain is connected to the core domain *via* links between $\alpha L1$ and $\beta 8$ and between $\alpha L5$ and αD (Fig. 1*b*), with the intervening residues lacking defined secondary structure. The electron density for residues 197–208 is significantly weaker and similar observations were made for the *Li*HTA structure (Wang *et al.*, 2007), in which the electron density for residues 252–268 was also quite poor. This is the same region in both

Figure 3

Ribbon diagram showing (a) superimposition of *Sa*HTA (cyan) and *Hi*HTA (green) and (b) *Sa*HTA (cyan) and *Li*HTA (magenta).

structures, constituting the α L4 of the lid domain. The previously proposed flexibility in this region that would allow structural changes in response to substrate binding is supported by our current structure.

Similar to the *Hi*HTA (PDB entry 2b61; Mirza *et al.*, 2005) and *Li*HTA (PDB entry 2pl5; Wang *et al.*, 2007) structures, *Sa*HTA crystallizes with only one HTA molecule in the asymmetric unit. However, both *Hi*HTA and *Li*HTA have been shown by gel-filtration and sedimentation-velocity analysis to exist as dimers in solution (Born *et al.*, 2000; Wang *et al.*, 2007). Helices L1 and L2 from each subunit form a classical antiparallel four-helix bundle with the hydrophobic core, while helices L3 and L4 are involved in dimer stabilization flanking the four-helix bundle. In *Sa*HTA the four-helix bundle is found to be left-handed, similar to that seen in *Hi*HTA, while in the *Li*HTA structure this bundle is right-handed.

3.2. Active-site residues

The catalytic triad consisting of a serine, a histidine and an aspartic acid is the most conserved feature of the α/β -hydrolase family. In the

*Sa*HTA structure, the catalytic triad is composed of the Ser131 nucleophile, the His296 base and the Asp267 acidic residue. Ser131 is located on a loop between β 7 and α C, Asp267 is on a loop between β 9 and α E, and His296 is on a loop between β 9 and α E. This structural arrangement is conserved among all members of the HTA family.

In the *Li*HTA apoenzyme structure the active-site histidine was found to exist in two conformations with similar occupancy (Wang *et al.*, 2007). In our *Sa*HTA structure His296 is somewhat disordered, with a relatively high temperature factor ($B = 72 \text{ \AA}^2$) for the imidazole ring relative to the other active-site functional groups, while in the *Hi*HTA structure this histidine has well defined density and exists in a single conformation. This observed conformational flexibility of this active-site histidine in HTAs is consistent with its proposed role in catalysis. The $k_{\text{cat}}/K_{\text{m}}$ values for acetyl-CoA and L-homoserine with *Hi*HTA were reported to be 6.5×10^5 and $7.1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively (Born *et al.*, 2000). For *Sa*HTA, the $k_{\text{cat}}/K_{\text{m}}$ values for acetyl-CoA and L-homoserine were found to be 4.0×10^5 and $3.3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. These values are each within a factor of two

(a)

Figure 4

(a) Cross section of *Sa*HTA highlighting the active-site tunnel. The catalytic triad residues Ser131, Asp267 and His296 are shown as green sticks. Comparison of the active-site tunnel between (b) *Hi*HTA, (c) *Li*HTA and (d) *Sa*HTA, with the active-site serine colored red.

of those reported for the Gram-negative ortholog, consistent with the highly conserved active site in this enzyme family.

3.3. Secondary- and tertiary-structure comparison among the HTAs

Despite the many similarities of *SaHTA* to *LiHTA* and *HiHTA*, there are some significant structural differences. *SaHTA* is a shorter protein (322 amino acids) compared with *LiHTA* (366 amino acids) and *HiHTA* (377 amino acids) (Fig. 2). These deletions are primarily located in the loop regions. The loop connecting $\beta 6$ and αB (amino acids 81–105) is shorter in *SaHTA*. Similarly, the 15-amino-acid loop connecting $\alpha L1$ and $\alpha L2$ has essentially been eliminated, as has the 15-amino-acid loop between $\alpha L3$ and $\alpha L4$, when compared with these regions in *LiHTA* and *HiHTA* (Fig. 2). Helix αE is also shorter in *SaHTA*. Overall, *SaHTA* can be viewed as a more compact version of *HiHTA*, with several truncated loops in this new structure but with no significant changes in the orientation of the secondary-structural elements (Fig. 3a). In contrast, there are several differences in the secondary-structural arrangement between *SaHTA* and *LiHTA*. For example, in *SaHTA* the loop connecting $\beta 6$ and $\beta 7$ is flanked by a short helix which is not seen in *LiHTA* (Fig. 3b).

3.4. Surface view and active-site tunnel of *SaHTA*

An important common structural feature of HTA is the presence of a tunnel formed at the junction of the two domains (Fig. 4a). This tunnel leads to the active-site nucleophile, Ser131, and appears to act as a channel for substrate access to the active site. The cross section of the tunnel reveals the size and shape of this channel leading to the active site. When compared with the tunnel present in *HiHTA* (Fig. 4b) and *LiHTA* (Fig. 4c), this feature in *SaHTA* is much narrower and more compact (Fig. 4d), suggesting the possibility of some specificity differences in the *S. aureus* enzyme.

The natural product ebelactone A inhibits *HiHTA* with a K_i value of $203 \pm 12 \mu M$ (De Pascale *et al.*, 2011). This compound was shown to form a covalent adduct with the active-site serine by accessing it through the catalytic tunnel. When this compound was tested with *SaHTA* no inhibition was observed at concentrations as high as 1 mM. This greater than 50-fold discrimination in binding between these two homologous enzymes is likely to be due to the narrower catalytic tunnel in *SaHTA* which restricts access to the active-site serine.

4. Conclusions

The HTA from *S. aureus* has a similar overall structure to the HTA orthologs from two Gram-negative bacteria with the same active-site catalytic triad. Flexibility in the linkages between the two domains would allow protein conformation changes in response to substrate binding. Differences have been found in the dimensions of the tunnel

that potential substrates must traverse to enter the active site. This unique characteristic tunnel in the *S. aureus* enzyme can serve to limit the size of compounds that can access the active site, allowing this enzyme form to potentially avoid inhibition from known inhibitors of other HTA orthologs (De Pascale *et al.*, 2011).

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