A SINGLE AMINO ACID CHANGE IS RESPONSIBLE FOR EVOLUTION OF ACYLTRANSFERASE SPECIFICITY IN BACTERIAL METHIONINE BIOSYNTHESIS*

Chloe Zubieta, Kiani A.J. Arkus, Rebecca E. Cahoon, Joseph M. Jez

From the Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132 USA

Running title: Switching Acyltransferase Specificity

Address correspondence to: Joseph M. Jez, Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132 USA; Tele./Fax.: 314-587-1450/-1550; E-mail: jjez@danforthcenter.org.

Bacteria and yeast rely on either homoserine transsuccinylase (HTS, metA) or homoserine transacetylase (HTA; met2) for the biosynthesis of methionine. Although HTS and HTA catalyze similar chemical reactions, these proteins are typically unrelated in both sequence and three-dimensional structure. Here we present the 2.0 Å resolution x-ray crystal structure of the B. cereus metA protein in complex with homoserine, which provides the first view of a ligand bound to either HTA or HTS. Surprisingly, functional analysis of the B. cereus metA protein reveals that it does not use succinyl-CoA as a substrate. Instead, the protein catalyzes the transacetylation of homoserine using acetyl-CoA. Therefore, the B. cereus metA protein functions as an HTA, despite greater than 50% sequence identity with bona fide HTS proteins. This result emphasizes the need for functional confirmation of annotations of enzyme function based on either sequence or structural comparisons. Kinetic analysis of site-directed mutants reveals that the B. cereus metA protein and the E. coli HTS share a common catalytic mechanism. Structural and functional examination of the B. cereus metA protein reveals that a single amino acid in the active site determines acetyl-CoA (Glu111) versus succinyl-CoA (Gly111) specificity in the metA-like of acyltransferases. Switching of this residue provides a mechanism for evolving substrate specificity in bacterial methionine biosynthesis. Within this enzyme family, HTS and HTA activity likely arises from divergent evolution in a common structural scaffold with conserved catalytic machinery and homoserine binding sites.

Bacteria, fungi, and plants synthesize methionine from aspartate, which is also a precursor of lysine, threonine, and isoleucine (1-2). In addition to its role in protein synthesis, methionine is a key component for production of S-adenosylmethionine, a biologically important methyl group donor. S-adenosylmethionine, in turn, may be a metabolite in the biosynthesis of acylhomoserine lactones, which act as quorum sensing signals in pathogenesis and biofilm formation (3-4). Because the methionine biosynthetic pathway is essential for microbial and fungal growth, the enzymes of this pathway are potential targets for the development of small molecule inhibitors (5-9).

Homoserine is the last common metabolite in the biosynthesis of methionine, threonine, and isoleucine (2). Four enzyme-catalyzed reactions convert homoserine to methionine. In the first reaction, acylation of the homoserine hydroxyl group occurs (Fig. 1). In Escherichia coli, Salmonella typhimurium, and other Gram-negative bacteria, homoserine transsuccinylase (HTS); EC 2.3.1.46) catalyzes this reaction to yield O-succinylhomoserine (10-13). Alternatively, yeast (Saccharomyces cerevisiae and Schizosaccharomyces pombe) and many bacteria, including Bacillus subtilis, B. polymyxa, Pseudomonas aeruginosa, Haemophilus influenzae, and Mycobacterium tuberculosis, use homoserine transacetylase (HTA, EC 2.3.1.31) and acetyl-CoA for the acylation reaction (13-16). Either acylated substrate may be condensed with cysteine by cystathionine-γ-synthase (Fig. 1). Subsequent hydrolysis of cystathionine by cystathionine-β-lyase yields homocysteine, which is next methylated to form methionine.

Although HTS and HTA are encoded by genes (metA and met2, respectively) that share no sequence homology, these enzymes catalyze...
reactions using similar substrates. Both HTS and HTA use a ping-pong kinetic mechanism in which the acyl group of either succinyl- or acetyl-CoA is first transferred to an active site residue before subsequent transfer to the hydroxyl moiety of homoserine (12, 15-16). Mechanistic studies of the S. pombe HTA and E. coli HTS demonstrate the role of an active site serine (HTA) or cysteine (HTS) as a nucleophile (17-19). Structurally, HTS and HTA adopt distinct three-dimensional folds. The crystal structure of the H. influenzae HTA showed that the enzyme is a member of the a/b-hydrolase superfamily and contains a Rossmann-fold motif (20). Likewise, the structure of a putative HTS (metA) from B. cereus also contains a Rossmann-fold motif, but the overall topology of this protein differs dramatically from that of H. influenzae HTA (met2) (21).

Based on sequence similarity to the canonical HTS from E. coli, the B. cereus metA protein was annotated as an HTS (22). The B. cereus metA protein shares 51% amino acid sequence identity with E. coli HTS and less than 10% identity with the met2-type of HTA from yeast or bacteria (21). Biochemical studies of E. coli HTS (12, 18-19) and structural comparison with the H. influenzae HTA (20) suggest that the B. cereus metA protein contains a catalytic triad consisting of Cys142, His235, and Glu237, which is located at the bottom a surface groove between two α-helices (21).

Here we describe the determination of the three-dimensional structure of the B. cereus metA protein in complex with homoserine. This structure provides the first view of a ligand bound to either HTA or HTS and clearly defines the active site in the B. cereus metA protein. Surprisingly, functional analysis of the B. cereus metA protein reveals that it does not use succinyl-CoA as a substrate; instead, it catalyzes the transacylation of homoserine using acetyl-CoA. Therefore, the B. cereus metA protein functions as an HTA, and not an HTS despite its similarity with this protein (21-22). Kinetic analysis of site-directed mutants reveals that the B. cereus metA protein and the E. coli HTS share a common catalytic mechanism, and that a single amino acid in the active site determines acetyl- versus succinyl-CoA specificity in the metA-type acyltransferases involved in bacterial methionine biosynthesis.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis, Expression, and Purification.** Site-directed mutants of the B. cereus metA protein were generated using oligonucleotides (Integrated DNA Technologies) encoding the desired mutation and the QuikChange PCR method (Stratagene). Automated nucleotide sequencing confirmed the fidelity of the mutant gene sequences (Washington University Sequencing Facility, St. Louis, MO). Wild-type and mutant B. cereus metA were expressed in E. coli strain HK100 GeneHogs (Invitrogen) and E. coli BL21(DE3) (Novagen), respectively. Transformed E. coli were grown at 37 °C in Terrific broth containing 50 µg mL⁻¹ kanamycin until A 600nm~0.8. After induction with 0.15% (v/v) arabinose, cultures were grown at 25 °C for 4-8 h. Cells were pelleted by centrifugation and resuspended in 50 mM Tris (pH 8.0), 500 mM NaCl, 20 mM imidazole, 10% (v/v) glycerol, and 1% (v/v) Tween-20. After sonication and centrifugation, the supernatant was passed over a Ni²⁺-nitritotriacetic acid (NTA) agarose column. The column was washed with wash buffer (lysis buffer minus Tween-20). His-tagged protein was eluted with elution buffer (wash buffer with 250 mM imidazole), and the eluant incubated with His-tagged tobacco etch virus (TEV) protease (1 mg TEV protease per 10 mg eluted protein) during overnight dialysis at 4 °C against wash buffer. Dialyzed protein was reloaded on an Ni²⁺-NTA column equilibrated with wash buffer to deplete the sample of uncleaved B. cereus metA protein and TEV protease. The flow-through of this step was dialyzed overnight at 4 °C against 30% (v/v) glycerol, 25 mM Hepes (pH 7.5), 100 mM NaCl, and 1 mM dithiothreitol, then loaded onto a Superdex-200 size-exclusion FPLC column equilibrated in the same buffer without glycerol. Fractions containing purified protein were concentrated to 15 mg mL⁻¹ and stored at -80 °C.

**X-ray Crystallography.** The B. cereus metA protein (15 mg mL⁻¹) was crystallized using the hanging drop vapor diffusion technique. Protein (1 µL) was added to a 1 µL drop of
crystallization solution containing 1.4 M (NH₄)₂SO₄ and 0.1 M Tris (pH 8.0) at 4 °C. Crystals were harvested and soaked overnight in 1.8 M (NH₄)₂SO₄, 0.1 M Tris (pH 8.0), 15% (w/v) glycerol, and 10 mM homoserine. For data collection, crystals were flash frozen in liquid nitrogen. Data collection was performed at the Stanford Synchrotron Radiation Facility (SSRL) on monochromatic beamline 9-1. Crystals were indexed as space group P4₁2₁2₁. The data was integrated and reduced using XDS (23) and scaled with XSCALE (23). Molecular replacement using the apoenzyme structure (21) was performed with PHASER (24). Model building was performed in COOT (25) and all refinements were performed with REFMAC (26). Data collection, refinement, and model statistics are summarized in Table 1. Because ice rings were observed during data collection, reflections between 3.93-3.87, 3.70-3.64, 3.47-3.41, and 2.70-2.64 Å were omitted from the final data set. In the final model, 96.6% of residues were in the most favored region of the Ramachandran plot, and 3.4% in the additionally allowed region. The atomic coordinates (2VDJ) and structure factors (R2VDJSF) for the B. cereus metA•homoserine complex have been deposited.

Kinetic Analysis. Initial velocities were determined at 25 °C by monitoring the change in absorbance at 232 nm that results from hydrolysis of the thioester bond of acetyl-CoA (ε = 4,500 M⁻¹ cm⁻¹). Standard assay conditions are 50 mM potassium phosphate buffer (pH 7.5), 2 mM L-homoserine, and 0.5 mM acyl-CoA in a final volume of 500 μL. Kinetic parameters were determined by varying the concentration of one substrate at different fixed concentrations of the second substrate. Initial velocity kinetic data was fit to the equation for a two-substrate reaction, v = (V[A][B])/(Kₐ[B] + Kₖ[A] + [A][B]), where V is the observed rate and Kₐ and Kₖ are the Kₘ values for each substrate, in Sigmaplot (Systat Software, Inc.).

RESULTS

Overall Structure. Crystal structures of the unliganded forms of H. influenzae HTA and the B. cereus metA protein have been solved (20-21), but no details are available on how substrates or inhibitors bind to these proteins. The three-dimensional structure of the B. cereus metA protein in complex with homoserine was determined by x-ray crystallography at 2.0 Å resolution (Table 1 and Fig. 2A). The overall structure is a homodimer, and within the structure of each monomer, residues 1-16, 75-86, and 297-301 were disordered. The overall fold of the metA•homoserine complex is similar to that of the apoenzyme structure (21) with a 0.25 Å² r.m.s. deviation of Cα-atoms between the two structures. When bound with homoserine, the largest structural change occurs in a loop region preceding helix H1. Residues 43 to 46 of this loop shift up to 2.3 Å toward the active site; however, none of the residues in this loop contact the bound homoserine molecule and are 15 Å away from the proposed catalytic residues.

The position of bound homoserine clearly defines the location of the active site in the B. cereus metA protein. Unambiguous electron density in the initial 2Fₒ-Fₑ and Fₑ-Fₒ maps was visible for homoserine (Fig. 2B). Substrates access the putative catalytic residues (Cys142, His235, and Glu237) through a short tunnel leading from the enzyme surface (Fig. 2C). The proposed catalytic cysteine is positioned at the bottom of this pocket. Consistent with the ping-pong kinetic mechanism of the HTA and HTS from various bacteria (12, 15-16), in which only one substrate binds at each step of the reaction, homoserine binding in the entrance prevents access of other molecules (i.e., acyl-CoA) to the active site.

The Homoserine Binding Site. The three-dimensional structure of the B. cereus metA protein in complex with homoserine provides the first view of the molecular interactions involved in substrate binding at the active site of either HTA or HTS. Multiple side-chain, main-chain, and water-mediated contacts bind homoserine in the B. cereus metA active site (Figs. 3A and 3B). Binding of homoserine positions the homoserine hydroxyl group near Cys142 and within hydrogen bonding distance of His235 (Figs. 3A and 3B). Adjacent to Cys142, the Nᵦ of His235 is within hydrogen-bonding distance of the cysteine sulphydryl...
group. The Nδ of His235 is positioned to interact with the carboxylate of Glu237. These residues form the proposed catalytic triad of the enzyme. In addition, the homoserine hydroxyl group is within hydrogen bonding distance of an active site water molecule, which in turn interacts with another water molecule bound by the main-chain nitrogens of Ala108 and Trp143. This water molecule’s location between Ala108 and Trp143 likely approximates the position of an oxyanion hole that stabilizes the tetrahedral reaction intermediates of the chemical mechanism. Additional interactions between the substrate and the protein are formed. The carboxylate of homoserine forms a bidentate charge-charge interaction with Arg249 and a charge-charge interaction with Lys163. An active site water molecule and the backbone carbonyl and side-chain hydroxyl groups of Ser192 provide hydrogen bonds with the amino moiety of the substrate. Amino acid sequence comparison between \textit{E. coli} HTS and \textit{B. cereus} metA shows that the three catalytic residues (Cys142, His235, and Glu237) and the residues forming the homoserine-binding site (Ala108, Trp143, Lys163, Ser192, Arg249, and Glu250) are invariant between the two proteins.

**Functional Analysis.** The \textit{B. cereus} metA gene was annotated as an HTS based on sequence similarity with bona fide HTS proteins and genes (22). To verify the activity of the protein, \textit{B. cereus} metA was assayed with different acyl-CoAs and homoserine. Surprisingly, no activity was observed when succinyl-CoA and homoserine were tested as potential substrates; however, the \textit{B. cereus} metA protein was active with acetyl-CoA and homoserine (Table 2). The catalytic efficiency ($k_{\text{cat}}/K_m$) of the \textit{B. cereus} metA protein for homoserine is comparable to that of the \textit{S. pombe} HTA ($8,830 \text{ M}^{-1} \text{ s}^{-1}$), although the $k_{\text{cat}}/K_m$ of the \textit{Bacillus} protein for acetyl-CoA is 98-fold lower than the fungal enzyme (17). Compared to the \textit{H. influenzae} HTA (16), the $K_m$ values of the \textit{B. cereus} metA protein for either substrate are less than 2-fold different, but the turnover numbers are roughly 100-fold lower. Nonetheless, the metA protein from \textit{B. cereus} is not an HTS, as annotated by genome comparisons and structural genomics (21-22).

**Mutagenesis of Proposed Catalytic Residues.** To examine the role of Cys142, His235, and Glu237 in the transacetylation reaction catalyzed by the \textit{B. cereus} metA protein, a series of point mutants were generated for kinetic analysis. In addition, mutations of Lys47 were generated to test the role of this residue, which was previously suggested to have a catalytic role in the bacterial HTS (27). Expression and purification of the mutants used the same methods as wild-type enzyme. All proteins were obtained in yields and homogeneity comparable with wild-type enzyme, and all were isolated as homodimers, based on size-exclusion chromatography.

Substitution of either alanine or serine for Cys142 yielded inactive enzyme, which is consistent with the proposed role of this residue as the catalytic nucleophile. Likewise, mutation of His235 to alanine, glutamine, or asparagine resulted in inactivation of the enzyme. Mutants of Glu237 retained catalytic activity albeit with 6- to 65-fold decreases in $k_{\text{cat}}$ (Table 2). Overall, these results are similar to those reported for changes in the catalytic triad of the HTA from yeast and the HTS from bacteria (17-19), suggesting a conserved reaction mechanism between these enzymes of different three-dimensional structures.

Mutation of Lys47 affects the reaction catalyzed by the enzyme (Table 2). The K47R mutant primarily affects the $K_m$ value for acetyl-CoA. Similar results were observed with the K47M mutant, which reduces the turnover number by 14-fold and the $K_m$ for acetyl-CoA by 17-fold. Given the location of this residue in the structure (i.e., 15 Å from the active site cysteine), this residue appears to function in acyl-CoA binding, most likely by interaction with CoA and not the acyl group as previously suggested (19,27), and is not directly involved in catalysis or homoserine binding.

**Mutagenesis of Homoserine Binding Residues.** The contribution to catalysis of residues with side-chains that form interactions with homoserine was probed using site-directed mutagenesis. The K163M, S192A, R249M, and E250A mutants were expressed, purified, and assayed using acetyl-CoA and homoserine as
substrates (Table 2). Each mutant retained activity and showed less than a 3-fold change in $K_m$ for acetyl-CoA. Replacement of Arg249 with methionine resulted in the largest changes in kinetic parameters compared to the other mutations. The R249M mutant displayed a 10-fold reduction in $k_{cat}$ and a 64-fold higher $K_m$ for homoserine compared to the wild-type enzyme. The R249M mutant also showed a 13- to 14-fold increase in the homoserine $K_m$ values. A 5-fold increase in the $K_m$ for homoserine was observed with the S192A mutant. These results indicate that each residue forming the binding site plays a role in homoserine binding, but contribute little to acyl-CoA interaction.

**DISCUSSION**

The three-dimensional structure of the *B. cereus* metA protein in complex with homoserine provides insight on the chemical reaction mechanism and how acyltransferase specificity is determined in the bacterial metA-type HTA and HTS. The location of a catalytic triad (Cys143, His235, and Glu237) at the base of a short tunnel from the enzyme surface allows access of only one substrate (i.e., either acetyl-CoA or homoserine) at a time. Within the active site, the pattern of hydrogen bond interactions between the sulfhydryl group of Cys142 and N\textsubscript{ε} of His235 and between the carboxylate of Glu237 and N\textsubscript{δ} of His235 is identical to that observed in the crystal structure of the fungal HTA, except that a cysteine replaces the serine found in this enzyme (20). Although the structures of the metA-type of HTS/HTA (21) and the met2-type of HTA (20) are different, these scaffolds have convergently evolved nearly identical active site constellations for a shared chemical reaction. This arrangement of catalytic amino acids readily allows for activation of the nucleophilic cysteine residue in the *B. cereus* metA protein.

**The Aeryltransferase Substrate Specificity Switch.** Modeling of the acetyl-enzyme intermediate in the homoserine complex structure (Fig. 4A) shows that the *B. cereus* metA active site readily accommodates this reaction intermediate. Examination of the model showed that Glu111 is oriented towards the reactive cysteine residue, and sterically occludes fitting of a succinyl-enzyme intermediate in the active site. Comparison with *E. coli* HTS showed that the corresponding residue is a glycine, suggesting that the identity of this amino acid may control acyl-CoA substrate specificity. Modeling of the glycine substitution in the active site (Fig. 4B) indicates that this mutation removes a negative charge-charge interaction and enlarges the space available to accommodate a succinyl group.

To test this hypothesis, the *B. cereus* metA protein E111G mutant was generated, expressed, and purified. The purified mutant protein showed no detectable activity with acetyl-CoA, but catalyzed an acyltransferase reaction using succinyl-CoA and homoserine. The steady-state kinetic parameters of the E111G mutant ($k_{cat} = 48.2 \pm 6.4$ min\(^{-1}\), $K_m$ succinyl-CoA = 273 \pm 22.1 \mu M; $K_m$ homoserine = 200 \pm 37 \mu M) were determined. The catalytic efficiency of the mutant with homoserine (4,020 M\(^{-1}\) s\(^{-1}\)) is comparable to wild-type enzyme and the $k_{cat}/K_m$ for succinyl-CoA (2,940 M\(^{-1}\) s\(^{-1}\)) is less than 2-fold different from that of the metA protein for acetyl-CoA (Fig. 4).
hydroxyl group, which then acts as a nucleophile to attack the thioester of the acyl-enzyme intermediate (Fig. 5). Subsequent collapse of the second transition state yields O-acetylhomoserine as the product.

Kinetic analysis of *B. cereus* metA proteins with mutations in the catalytic triad is consistent with the proposed reaction mechanism (Table 2). Replacement of either Cys142 or His235 yields inactive protein, and substitutions of Glu237 impair activity. Removal of the nucleophilic cysteine prevents catalysis. Similarly, substitution of His235 likely affects the activation of the catalytic sulfhydryl group and removes the general base required for the second half reaction. Mutations of Glu237 may alter positioning and/or reactivity of the imidazole side-chain of His235, but would still permit the cysteine-histidine pair to retain a degree of catalytic function. Moreover, the results of detailed kinetic studies of *E. coli* HTS (18-19) also strongly support the use of a common catalytic mechanism in the related metA-type of HTA and HTS.

In addition to conservation of the catalytic machinery, the amino acids forming the homoserine-binding site of the *B. cereus* metA protein and *E. coli* HTS are invariant. The structure presented here provides the first experimental representation of a substrate bound to either an HTA or HTS. Site-directed mutagenesis of Lys163, Ser192, Arg249, and Glu250 indicate that each residue significantly contributes to homoserine binding (Table 2). Based on the steady-state kinetic parameters of the R249M mutant compared to wild-type enzyme, the interaction between Arg249 and the carboxylate of homoserine is a dominant interaction. Substitution of Glu250 with an alanine may change the orientation of Arg249 and its contact with the substrate. The K163M and S192A mutants remove side-chain interactions with homoserine, and the kinetic parameters of these proteins reflect these changes.

The original annotation of the *B. cereus* metA gene as encoding an HTS was based on the greater than 50% sequence identity with bacterial metA genes encoding bona fide HTS (10-13) versus the 10% sequence identity with known bacterial and fungal met2 HTA genes (13-16, 28-29); however, functional analysis of the *B. cereus* metA protein clearly demonstrates it as an HTA, not an HTS (30). Similarly, a gene from *Thermota maritima* with 50% sequence identity to the *E. coli* HTS and no discernible homology with met2-type of HTA was recently shown to encode a functional HTA, instead of an HTS (31).

Structural and functional analysis of wild-type and mutant *B. cereus* metA demonstrates that changing Glu111 to a glycine switches substrate preference from acetyl-CoA to succinyl-CoA (Fig. 4). Further examination of the metA-like proteins in the NCBI non-redundant sequence databases shows that these proteins can be separated into two families based on the identity of residue 111, i.e., glycine versus glutamate (See Supplemental Data Table 3). Functionally characterized metA-like proteins that function as HTS have a glycine at this position in the active site. In contrast, this residue is a glutamate in the metA-like proteins that prefer acetyl-CoA as a substrate. Within enzyme superfamilies that share a common three-dimensional scaffold and related amino acid sequence, the divergence of substrate specificity with retention of core active site residues and chemistry provides an efficient means of evolving new enzyme activity by modifying substrate specificity (32-33). Diversification of acyl-CoA specificity in HTA and HTS involved in methionine biosynthesis appears to have occurred in different bacterial species through a single amino acid difference in the active site.

The details of the methionine biosynthetic pathway in the *Bacillus* genus are poorly understood (34), but in the context of the pathway, HTA and HTS are functionally equivalent (Fig. 1). Examination of the genome of *B. cereus* shows that the metA gene is in a cluster of metabolic genes that includes genes for O-acetylserine sulfhydrylase (*cysD*), a cysteine biosynthesis enzyme, and homoserine dehydrogenase (*hom*), another methionine biosynthetic enzyme (21). As noted here, the *B. cereus* genome contains a gene, located near no other amino acid biosynthesis genes, that is annotated as an HTA. This putative HTA gene...
shares less than 30% sequence identity with authentic bacterial HTA genes and encodes an uncharacterized protein of unknown function. Interestingly, both *B. subtilis* and *B. polymyxa* use O-acetylhomoserine, not O-succinylhomoserine, for methionine synthesis (15, 34). In addition, *B. subtilis* cystathionine-γ-synthase, the next enzyme in the methionine biosynthesis pathway, specifically accepts O-acetylhomoserine as a substrate, and is inactive with the succinylated molecule (34). Although the *B. cereus* metA protein is catalytically less efficient than other bacterial HTA and HTS homologs, it may provide sufficient activity for methionine synthesis in *B. cereus*.

Analysis of the *B. cereus* metA protein reinforces the need for functional confirmation of annotations of enzyme function based on either sequence or structural comparisons. Annotation of gene and protein function based solely on structural comparisons is insufficient in many cases because it neglects the biological framework. The *B. cereus* metA provides an example of the need for scrutiny when using structural and sequence results alone to annotate function. Often, biochemical confirmation of protein function is needed to overcome the limitations inherent to high throughput structural initiatives in determining protein function.

**ACKNOWLEDGEMENTS**

We thank the JCSG for providing the *B. cereus* metA expression construct.
REFERENCES
1. Umbarger, H.E. (1978) Annu. Rev. Biochem. 47, 532-606
2. Viola, R.E. (2001) Acc. Chem. Res. 34, 339-349
3. HanzeIka, B.L. and Greenberg, E.P. (1996) J. Bacteriol. 178, 5291-5294
4. Val, D.L. and Cronan, J.E. (1998) J. Bacteriol. 180, 2644-2651
5. Bareich, D.C., Nazi, I., and Wright, G.D. (2003) Chem. Biol. 10, 967-973
6. Ejim, L., Mirza, I.A., Capone, C., Nazi, I., Jenkins, S., Chee, G.L., Berghuis, A.M., and Wright, G.D. (2004) Bioorg. Med. Chem. 12, 3825-3830
7. Jacques, S.L., Mirza, I.A., Ejim, L., Koteva, K., Hughes, D.W., Green, K., Kinach, R., Honje, J.F., Lai, H.K., Berghuis, A.M., and Wright, G.D. (2003) Chem. Biol. 10, 989-995
8. Aguilar, A., Perez-Diaz, J.C., Baquero, F., and Asensio, C. (1982) Antimicrob. Agents Chemo. 21, 381-386
9. Rohl, F., Rabenhorst, J., and Zahner, H. (1987) Arch. Microbiol. 147, 315-320
10. Rowbury, R.J. and Woods, D.D. (1964) J. Gen. Microbiol. 36, 341-358
11. Kaplan, M.M. and Flavin, M. (1966) J. Biol. Chem. 241, 4463-4471
12. Born, T.L. and Blanchard, J.S. (1999) Biochemistry 38, 14416-14423
13. Brush, A. and Paulus, H. (1971) Biochem. Biophys. Res. Comm. 45, 735-741
14. Datko, A.H., Giovanelli, J., and Mudd, S.H. (1974) J. Biol. Chem. 249, 1139-1155
15. Wyman, A. and Paulus, H. (1975) J. Biol. Chem. 250, 3897-3903
16. Born, T.L., Franklin, M., and Blanchard, J.S. (2000) Biochemistry 39, 8556-8564
17. Nazi, I. and Wright, G.D. (2005) Biochemistry 44, 13560-13566
18. Ziegler, K., Noble, S.M., Mutumanje, E., Bishop, B., Huddler, D.P., and Born, T.L. (2007) Biochemistry 46, 2674-2683
19. Coe, D.M. and Viola, R.E. (2007) Arch. Biochem. Biophys. 461, 211-218
20. Mirza, I.A., Nazi, I., Korczynska, M., Wright, G.D., and Berghuis, A.M. (2005) Biochemistry 44, 15768-15773
21. Zubieta, C., Krishna, S.S., McMullan, D., Miller, M.D., Abdubek, P., Agarwalla, S., Ambing, E., Astakhova, T., Axelrod, H.L., Carlton, D., et al. (2007) Proteins 68, 999-1005
22. Ivanova, N., Sorokin, A., Anderson, I., Galleron, N., Candelon, B., Kapralav, V., Bhattacharyya, A., Reznik, G., Mikhailova, N., Lapidus, A., et al. (2003) Nature 423, 87-91
23. Kabsch, W. (1993) J. Appl. Cryst. 26, 795-800
24. McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C., and Read, R.J. (2007) J. Appl. Cryst. 40, 658-674
25. Emsley, P. and Cowtan, K. (2004) Acta Cryst. D60, 2126-2132
26. Murshudov, G.N., Vagin, A.A., and Dodson, E.J. (1997) Acta Cryst. D53, 240-255
27. Rosen, R., Becher, D., Buttner, K., Biran, D., Hecker, M., and Ron, E. (2004) FEBS Letters 577, 386-392
28. Bourhy, P., Martel, A., Margarita, D., Saint Girons, I., and Belfaiza, J. (1997) J. Bacteriol. 179, 4396-4398
29. Park, S.D., Lee, J.Y., Kim, Y., Kim, J.H., and Lee, H.S. (1998) Mol. Cells 30, 286-294
30. Ziegler, K., Yusupov, M., Bishop, B., and Born, T.L. (2007) Biochem. Biophys. Res. Comm. 361, 510-515.
31. Goudarzii, M. and Born, T.L. (2006) Extremophiles 10, 469-478
32. Jensen, T.A. (1976) Annu. Rev. Microbiol. 30, 409-425
33. Penning, T.M. and Jez, J.M. (2001) Chem. Rev. 101, 3027-3046
34. Auger, S., Yuen, W.H., Danchin, A., and Martin-Verstraete, I. (2002) Microbiol. 148, 507-518
FOOTNOTES

* This work was supported by funds from the Donald Danforth Plant Science Center to J.M.J.

1 Present address: Cell and Molecular Biology Program, Duke University.

2 To whom correspondence should be addressed: Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132 USA; Tele./Fax.: 314-587-1450/-1550; E-mail: jjez@danforthcenter.org.

3 The abbreviations used are: HTA, homoserine transacetylase (EC 2.3.1.31); HTS, homoserine transsuccinylase (EC 2.3.1.46); NTA, nitrilotriacetic acid; TEV, tobacco etch virus.
FIGURE LEGENDS

FIGURE 1. Overview of methionine biosynthesis steps involving homoserine transacetylase and homoserine transsuccinylase.

FIGURE 2. Structure of the *B. cereus* metA•homoserine complex. (A) Overall structure of the *B. cereus* metA homodimer showing homoserine bound in each active site. One monomer is shown as a surface rendering and the other as a ribbon diagram. The N- (residue 17) and C-termini (residue 296) of the ribbon diagram monomer are indicated. The location of the active site in each monomer is indicated by an arrow. In each monomer, homoserine is shown as a cpk model. In the ribbon diagram, key active site residues are labeled and shown as stick models. (B) The electron density of homoserine is shown as a SIGMAA-weighted Fo-Fc omit map (2.0 σ). (C) Stereo-view surface rendering of the active site entrance. Homoserine is shown as a stick model. Surfaces corresponding to Cys142 (yellow) and His235 (blue) are colored.

FIGURE 3. Homoserine binding in the active site. (A) Protein-ligand interactions. The stereo-view shows homoserine binding in the *B. cereus* metA active site. Water molecules are shown as red spheres. Dotted lines represent hydrogen bonds. (B) Schematic of distances (Å) in the active site.

FIGURE 4. Structural models of acyl-enzyme intermediates in the reaction catalyzed by *B. cereus* metA. (A) Model of homoserine addition to the acetyl-enzyme intermediate. (B) Model of homoserine addition to the succinyl-enzyme intermediate of the E111G mutant. Positions of amino acids and homoserine are as crystallographically observed. Acyl-enzyme intermediates were manually built into the structure using the position of Cys142 and the oxyanion hole formed by Ala108 and Trp143 as constraints. The catalytic efficiencies of wild-type (A) and E111G (B) *B. cereus* metA protein for acetyl- and succinyl-CoA are shown.

FIGURE 5. Proposed reaction mechanism of *B. cereus* metA.
**TABLE ONE: Crystallographic statistics**

**Crystal**
- Space group: P 4₁2₂
- Cell dimensions: \( a = b = 96.39 \, \text{Å}, \ c = 75.58 \, \text{Å}; \ \alpha = \beta = \gamma = 90.0^\circ \)

**Data Collection**
- Wavelength (Å): 0.9790
- Resolution range (Å): 20.0 - 2.0
- Reflections (total/unique): 157,362 / 22,875
- Completeness (highest shell): 92.7% (98.5%)\(^a\)
- \( <I/\sigma > \) (highest shell): 17.1 (2.50)\(^a\)
- \( R_{\text{sym}} \) (highest shell): 11.4 (51.5)\(^a\)

**Model and Refinement**
- Resolution range (Å): 19.47 - 2.0
- Reflections (total/test): 21,729 (1,144)
- \( R_{\text{cryst}} / R_{\text{free}} \)\(^d\): 19.9% / 24.6%
- No. of protein atoms: 2,226
- No. of water molecules: 212
- No. of ligand atoms: 8 homoserine, 5 sulfate
- R.m.s. deviation, bond lengths: 0.007 Å
- R.m.s. deviation, bond angles: 0.987°
- Average isotropic B-factor (Å\(^2\)): 26.9
- ESU\(^e\) based on \( R_{\text{free}} \): 0.166

\(^a\)Highest resolution shell (2.11 - 2.00 Å) in parenthesis.  \(^b\)\( R_{\text{sym}} = \Sigma |I_h| - <|I_h|>/\Sigma |I_h|, \) where \(<|I_h|>\) is the average intensity over symmetry.  \(^c\)\( R_{\text{cryst}} = \Sigma |F_o| - <|F_o|>/\Sigma |F_o|, \) where summation is over the data used for refinement.  \(^d\)\( R_{\text{free}} \) is defined the same as \( R_{\text{cryst}} \), but was calculated using 5% of data excluded from refinement.  \(^e\)ESU = estimated overall coordinate error.
|     | $k_{\text{cat}}$ (min$^{-1}$) | $K_m$ (µM) | $k_{\text{cat}}/K_m$ (M$^{-1}$ s$^{-1}$) | $K_m$ (µM) | $k_{\text{cat}}/K_m$ (M$^{-1}$ s$^{-1}$) |
|-----|-------------------------------|------------|-----------------------------------------|------------|-----------------------------------------|
| Acetyl-CoA | Homoserine |
| WT   | 58.6 ± 5.7         | 185 ± 28  | 5280                                   | 214 ± 20  | 4560                                   |
| E237D| 9.5 ± 1.3          | 174 ± 26  | 910                                    | 405 ± 43  | 391                                    |
| E237Q| 3.1 ± 1.0          | 143 ± 29  | 361                                    | 287 ± 39  | 180                                    |
| E237A| 0.9 ± 0.1          | 108 ± 17  | 139                                    | 850 ± 94  | 17.6                                   |
| K47R | 39.3 ± 8.8         | 1350 ± 390| 485                                    | 946 ± 103 | 692                                    |
| K47M | 4.2 ± 2.9          | 3070 ± 535| 22.8                                   | 1380 ± 217| 50.7                                   |
| K163M| 19.7 ± 2.6         | 266 ± 39  | 1230                                   | 2780 ± 179| 118                                    |
| S192A| 32.6 ± 2.9         | 249 ± 38  | 2180                                   | 1020 ± 127| 533                                    |
| R249M| 6.1 ± 1.5          | 199 ± 27  | 511                                    | 13800 ± 2210| 7.4                                  |
| E250A| 26.1 ± 3.7         | 165 ± 36  | 2640                                   | 2950 ± 305| 147                                    |

$^a$All reactions were performed as described in the Experimental Procedures. WT = wild-type. All $k_{\text{cat}}$ and $K_m$ values are expressed as a mean ± SE for an $n = 3$. 

TABLE TWO: Steady-state kinetic parameters


Figure 1

- homoserine transacetylase (HTA) or homoserine transsuccinylase (HTS)

- CoA

- acetyl-CoA or succinyl-CoA

- O-acetylhomoserine or O-succinylhomoserine

- cystathionine-\gamma\text{-synthase}

- cystathione

- acetate or succinate

- cysteine
Figure 2

A

B

C
Figure 4
Figure 5
**SUPPLEMENTAL DATA - TABLE THREE: Summary of species and acyltransferase types**

| MetA-fold HTS and HTA | bacteria with HTS-specific residue 111 (glycine) |
|-----------------------|-----------------------------------------------|
|                       | *Escherichia coli*, *Salmonella typhimurium*, *Shigella boydii*, *S. sonnei*, *Enterobacter sakazakii*, *En. flexneri*, *En. dysenteriae*, *Klebsella pneumoniae*, *Serratia proteamaculans*, *Erwinia carotovora*, *Citrobacter koseri*, *Yersinia pestis*, *Y. enterocolitica*, *Y. mollaretii*, *Y. bercovsenii*, *Y. frederiksenii*, *Photobacterium luminescens*, *Vibro cholerae*, *V. vulnificum*, *V. angustum*, *Photobacterium profundum*, *Aeromonas hydrophila*, *A. salmonicida* |

|                      | bacteria with HTA-specific residue 111 (glutamate) |
|----------------------|---------------------------------------------------|
|                      | *Bacillus cereus*, *Thermotoga maritima*, *B. thuringinesis*, *B. anthracis*, *B. clausis*, *B. halodurans*, *Clostridium thermocellum*, *Cl. beijerinckii*, *Cl. cellulolyticum*, *Cl. phytofermentans*, *Cl. leptum*, *Cl. acetobutylicum*, *Cl. novyi*, *Cl. kluveri*, *Geobacillus kaustophilus*, *Pertotoga mobilis*, *Rhodobacter sphaeroides*, *Bacteroides ovatus*, *B. thailandicus*, *Ba. vulgatus*, *Ba. uniform*, *Ba. capillosus*, *Ba. caccae*, *Ba. fragilis*, *Ruminococcus torques*, *Ru. obeam*, *Ru. gaense*, *Roseobacter denitriificans*, *Dorea longicatena*, *Parabacteroides merdae*, *Pa. distasonis*, *Alkaliphilus metallireddens*, *Dinoroseabacter shibae*, *Oceanobacillus iheyensis*, *Silicibacter pomeroyi*, *Faecalibacterium prausnitzi*, *Roseovarius nubinhibens*, *Rhizobacterium leguminosarum*, *Sagitrella stellata*, *Oceanicola batsensis*, *Agrobacterium tumefaciens*, *Streptococcus thermophilus*, *St. suis*, *Coprococcus eutactus* |

| Met2-fold HTA | yeast |
|---------------|-------|
|               | *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Pichia pastoris*, *Neurospora crassa*, *Aspergillus nidulans*, *Ustilago maydis* |

| Met2-fold HTA | bacteria |
|---------------|----------|
|               | *Haemophilus influenzae*, *Leptospira meyeri*, *Corynebacterium glutamicum*, *Mycobacterium tuberculosis*, *Pseudomonas aerogenosa*, *Comamonas testosteroni*, *Mesorhizobium loti*, *Bradyrhizobium japonicum*, *Corynebacterium diptheriae*, *Neisseria gonorrhoeae* |

Organisms with metA-like proteins sharing greater than 50% sequence identity, based on BLAST searching, with the *B. cereus* metA protein are shown. A representative sampling of Met2-like containing organisms are shown. Proteins from species with either verified HTA or HTS activity are noted in bold.
A single amino acid change is responsible for evolution of acyltransferase specificity in bacterial methionine biosynthesis

Chloe Zubieta, Kiani A. J. Arkus, Rebecca E. Cahoon and Joseph M. Jez

J. Biol. Chem. published online January 22, 2008

Access the most updated version of this article at doi: 10.1074/jbc.M709283200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2008/01/23/M709283200.DC1