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The γ Class of Carbonic Anhydrases

James G. Ferry

Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania, 16801 USA

Abstract

Homologs of the γ class of carbonic anhydrases, one of five independently evolved classes, are found in the genomic sequences of diverse species from all three domains of life. The archetype (Cam) from the *Archaea* domain is a homotrimer of which the crystal structure reveals monomers with a distinctive left-handed parallel β -helix fold. Histidines from adjacent monomers ligate the three active site metals surrounded by residues in a hydrogen bond network essential for activity. Cam is most active with iron, the physiologically relevant metal. Although the active site residues bear little resemblance to the other classes, kinetic analyses indicate a two-step mechanism analogous to all carbonic anhydrases investigated. Phylogenetic analyses of Cam homologs derived from the databases show that Cam is representative of a minor subclass with the great majority belonging to a subclass (CamH) with significant differences in active site residues and apparent mechanism from Cam. A physiological function for any of the Cam and CamH homologs is unknown, although roles in transport of carbon dioxide and bicarbonate across membranes has been proposed.

Keywords

carbonic anhydrase; gamma class; iron; mechanism; *Archaea*; methane

Carbonic anhydrases are metalloenzymes catalyzing the reversible hydration of carbon dioxide to bicarbonate ($\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+$). The enzymes are widely distributed among metabolically diverse species from all three domains of life (*Eucarya*, *Bacteria* and *Archaea*) reflecting the importance of this enzyme in biology [1,2]. Five independently evolved classes (α , β , γ , δ , and ζ) of carbonic anhydrases have been identified that have no significant sequence or structural identity, save the active site. Thus, Nature has repeatedly invented carbonic anhydrase which further underscores the broad physiological importance of this enzyme. Although diverse and widespread, the current understanding of the biochemistry and biological roles of carbonic anhydrases are based largely on several α and β class enzymes from mammals and plants. Only one each from the δ and ζ classes have been characterized, both isolated from a marine diatom of the *Eucarya* domain [3]. Carbonic anhydrases are widely distributed among diverse prokaryotes [4], yet few from the α and β classes have been characterized from the *Bacteria* domain of life. Remarkably, only two carbonic anhydrases have been biochemically characterized from the *Archaea* domain, one each from the β class (Cab) [5-8] and the γ class (Cam). Cam is the γ class archetype isolated from *Methanosarcina thermophila*, an anaerobic methane-producing species from the *Archaea* domain [9]. The γ class is widely distributed in diverse species from all three domains of life [2,10]; however, only Cam has been characterized

Tel.: 814 863 5721; fax: 814 863 6217 jgf3@psu.edu.

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biochemically and shown to have carbonic anhydrase activity. This review focuses on the current status of the γ class highlighting the phylogeny, biochemistry and physiology.

1. Phylogeny

Although Cam is the only γ class carbonic anhydrase characterized biochemically and reported to have activity, homologs have been identified. Structural modeling and sequence analysis of homologs from the plant species *Arabidopsis thaliana* show conservation with Cam of the overall fold, active site metal ligands (Cam His 81, His 117, His 122) and catalytically important residues (Cam Gln75, Gln73) [11]. Notably, Cam residues Glu62, Glu84 and Asn202 that are essential or important for catalysis are not conserved in the *A. thaliana* homologs and carbonic anhydrase activity was not detectable in the proteins overproduced in *Escherichia coli*. Furthermore, absent in the *A. thaliana* homologs is the acidic loop in Cam (Fig. 1) on which Glu84 resides [12]. A database search in 2004 queried with one of the *A. thaliana* homologs retrieved sequences of putative γ class homologs from cyanobacteria, α and γ proteobacteria, plants and green algae [11]. Analysis of all the sequences showed conservation with Cam residues essential for metal binding and catalysis except Glu62 and the acidic loop residues that include Glu84. The only other Cam homolog investigated from the *Archaea* domain is from *Pyrococcus horikoshii*, an anaerobic species that does not produce methane and grows optimally at 98°C utilizing amino acids. The crystal structure of the enzyme overproduced in *E. coli* [13] shows the overall fold similar to Cam [14] with zinc in the active site (Fig. 2). However, the acidic loop including Glu84 of Cam and catalytic residues Glu62 and Asn202 are not conserved in the *P. horikoshii* enzyme (Fig. 3) and carbonic anhydrase activity was not reported. Thus, all Cam homologs reported in the literature are missing Glu62 and the acidic loop containing the proton shuttle residue Glu84 consistent with an earlier evolutionary analysis of γ class Cam homologs in 2000 [15]. These results suggest that the γ class is largely populated with homologs of a subclass in which the acidic loop of Cam and catalytically important residues Glu62 and Glu84 are missing. Indeed, a BLAST search of databases performed with Mt-Cam from *M. thermophila* as the query (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed the first 100 sequences with an expect cut-off of 6e-13 with at least 47% identity to Mt-Cam of which 84 conserved the Mt-Cam residues Glu62, Asn73, Gln75, Asn202, His81, His117 and His122 important for catalysis and metal ligation (Figure 1). However, of the 100 sequences retrieved, 66 were missing PSR Glu84 and all or part of the acidic loop of Mt-Cam consistent with previous database searches and typical of the homolog from *P. horikoshii* (Fig. 3). The subclass without the acidic loop and catalytic residues in Cam is hereafter referred to in this review as the CamH subclass based on the CamH designation for the homolog in *Methanosarcina acetivorans* [16]. The Blast search retrieved CamH subclass homologs from diverse species in all three domains of life.

The apparent absence of carbonic anhydrase activity in the two purified proteins from the CamH subclass [13,17] calls into question the catalytic potential, metal content, and other properties of this subclass. However, recent biochemical characterization of the CamH homolog from *Methanosarcina thermophila* reveals carbonic anhydrase activity (unpublished results). Nonetheless, with only two representatives validated with carbonic anhydrase activity and biochemically characterized, an overall understanding of the γ class is incomplete.

2. Structure and function

2.1. Crystal structures

The only two γ class crystal structures reported are the γ class archetype Cam from *Methanosarcina thermophila* [18] and a CamH homolog from *Pyrococcus horikoshii* [13], both from the *Archaea* domain (Fig. 2). The homotrimeric structures reveal the monomer with a distinctive left-handed parallel β -helix fold predicted by a unique sequence motif shared with

the superfamily of proteins comprised mainly of acyltransferases [15,18]. Particularly distinct are the left-handed crossover connections between parallel β -strands. Arg59 is important for stability of the Cam trimer that in turn is essential for activity [19]; thus, as expected, this residue is conserved in the *P. horikoshii* CamH homolog (Fig. 3) and CamH homologs retrieved from the Blast database search. The three active site metals of both the *Methanosarcina thermophila* Cam and *P. horikoshii* CamH structures are located at the interface between monomers wherein one monomer contributes one histidine ligand and the adjacent monomer contributes the remaining two histidine ligands (Figs. 2, 5 and 6). A major distinction between the Cam and CamH structures, aside from the acidic loop, is the presence of Ca^{2+} ions located at the interfaces between monomers of the CamH structure. Comparison of the two structures suggests that Ca^{2+} binding at the same site of Cam is unlikely due to the presence of loop structures not present in the CamH structure (Fig. 2).

The active sites of Cam from *Methanosarcina thermophila* and CamH from *P. horikoshii* (Figs. 5 and 6) have in common an extensive hydrogen bond network that is characteristic of the α , β and ζ classes for which crystal structures have been determined [8,20-22]. Remarkably, although the sequence and overall fold of Cam and CamH have no resemblance to any of the other four independently evolved classes, the topography of the histidine ligands superimpose nearly perfectly on the three histidine ligands of the α class suggesting convergent evolution constrained by the chemical requirements for catalysis of the same reaction [18]. However, active site residues of Cam *versus* CamH bear little resemblance. Although Cam was initially overproduced in *E. coli* and purified aerobically with Zn^{+2} in the active site (Zn-Cam), the metal can be replaced with Co^{+2} (Co-Cam) by unfolding and refolding in the presence of Co^{+2} [14] revealing different coordination geometries (Fig. 5). The coordination of Zn-Cam exhibits distorted trigonal bipyramidal geometry whereas the coordination of Co-Cam exhibits distorted octahedral geometry.

Cam from *Methanosarcina thermophila* contains zinc in the active site when overproduced in *E. coli* and purified aerobically [9,23]. However, Cam has 3-fold greater carbonic anhydrase activity and contains Fe^{2+} in the active site (Fe-Cam) when purified anaerobically from *E. coli* or overproduced in the closely related species *M. acetivorans* and purified anaerobically establishing Fe^{2+} as the physiologically relevant metal [24,25]. Carbonic anhydrase activity is rapidly lost on exposure of Fe-Cam to air, the result of oxidation to Fe^{3+} and loss of the metal from the active site that is subject to replacement with Zn^{2+} during aerobic purification. The Irving-Williams series predicts that the stability of complexed Zn^{2+} is much greater than that for Fe^{2+} ligated with the nitrogen atoms of histidine residues coordinating the active-site metal in Cam. Thus, aerobic purification results in the loss of Fe^{3+} and substitution with Zn^{2+} that contaminates buffers not treated with chelating agents. Soluble Fe^{2+} is abundant in oxygen-free environments and available to anaerobic microbes that utilize this metal in a host of enzymes. Thus, it is paramount that the purification and characterization of carbonic anhydrases from anaerobes is performed under oxygen-free conditions. In this context, it is interesting to note that activity was not reported for the CamH homolog from the strict anaerobe *P. horikoshii* overproduced in *E. coli* and purified aerobically [13]. Interestingly, the activity of the α class carbonic anhydrase from duck erythrocytes is higher in the presence of iron when compared to that induced by similar concentrations of zinc consistent with a role for iron in the active site [26].

2.2. Catalytic mechanism

Kinetic analyses of the γ class archetype Cam from *Methanosarcina thermophila* suggest a two-step ping pong mechanism for the reversible hydration of carbon dioxide to bicarbonate similar to that for the α class [27,28] as is shown in the following equations where E represents enzyme residues, M is metal and B is buffer.

In step 1a, a lone pair of electrons on the metal-bound hydroxide attack carbon dioxide producing metal-bound bicarbonate that is displaced by water in step 1b. In step 2a, a proton is extracted from the metal-bound water and then transferred to buffer in step 2b. Cam exhibits a $k_{cat} > 10^4 \text{ s}^{-1}$ which is faster than the fastest rate at which protons can transfer from the zinc-bound water with a pK_a of 7 to water. Thus, Cam with $k_{cat} > 10^4 \text{ s}^{-1}$ must transfer the proton from the zinc-bound water to an intermediate proton shuttle residue (H^{+} -E in steps 2a and 2b) and then to the external buffer molecule.

In the α class enzymes, a hydrogen bond network is of fundamental importance to the catalytic mechanism. In the network, Thr199 hydrogen bonds with the zinc-bound hydroxide *via* the $O\gamma$ atom thereby lowering the pK_a to physiological levels near neutrality and positioning the lone pair of electrons for nucleophilic attack on the carbon atom of carbon dioxide [20,29,30]. The backbone amide of Thr199 tethers and polarizes the carbon dioxide substrate for nucleophilic attack and also serves to bind bicarbonate substrate. An analogous hydrogen bond network has been revealed for Cam through replacement of active site residues *via* site-directed mutagenesis and kinetic analyses of the variant enzymes [20,27]. Based on the results, a catalytic mechanism has been proposed as shown in Figure 6 [27]. Although the mechanism features Zn-Cam, it is also generally applicable to Co-Cam that contains an extra metal-coordinated water. In panel A, Gln75 and Glu62 hydrogen bond with separate waters as revealed in crystal structures [14]. Glu62 coordinates the non-catalytic metal-bound water of which a proton is shared in a hydrogen bond (not shown) with the previously documented [12,31] proton shuttle residue Glu84 residing on the acidic loop exposed to solvent (Fig. 1). As shown in panel B, the metal-bound hydroxide extracts a proton from the adjacent metal-bound water hydrogen bonded by Gln75 (not shown). Next, the catalytic hydroxide hydrogen bonded to Gln75 is primed for nucleophilic attack on the incoming carbon dioxide while the proton is relayed to Glu84 and ultimately shuttled out to buffer (Panel C). The incoming carbon dioxide is tethered by hydrogen bonds contributed by the functional group amides of Gln75 and Asn202 (Panel D). The polarized carbon dioxide is attacked by the lone pair of electrons of the metal-bound hydroxide producing metal-bound bicarbonate as shown in panel E. As shown in panel F, the bicarbonate swings down, binding in a bidentate fashion to the metal, displacing the coordinated water previously engaged in a hydrogen bond with Glu62. As revealed in structures of both Zn-Cam and Co-Cam, the bidentate bound bicarbonate displaces the same coordinating water [14]. The proton of the hydroxyl of the bound bicarbonate either shifts to the oxygen tethered by Asn202 or the hydroxyl rotates *via* the carbon bond as described for the α class carbonic anhydrases. An incoming water displaces an oxygen producing monodentate-bound bicarbonate *via* hydrogen bonds with Asn202 and Glu62 as shown in panel G. Finally, a second water displaces bicarbonate from the active site as shown in panel H.

The mechanism shown in Figure 6 indicates Glu62 playing a pivotal role by facilitating product removal, contributing to the carbon dioxide hydration step, and proton transfer. Structural and kinetic analysis [12,14] of the double E62A/E84A variant suggests these two residues act in an additive manner during proton transfer. Glu84 resides 8 Å from the metal-bound water [14], indicating that either an intervening residue or a water molecule is needed to relay the proton from the metal to buffer. Cam crystal structures fail to show conserved water(s) that could act as a relay; however, Glu62 is optimally positioned to shuttle protons from the adjacent metal-bound water to Glu84. Indeed, in structures of Cam not complexed with bicarbonate, Glu62 is shifted sharing a proton with Glu84 [14]. Furthermore, Glu84 assumes multiple conformations (Fig. 1) analogous to the α class proton shuttle residue His64 for which multiple conformations are important for proton transfer (see Chapter ?).

The active site architecture of the CamH homolog from *P. horikoshii* (Fig. 4) complexed with bicarbonate reveals an extensive hydrogen bond network [13]. Notably, two water molecules are ligated to zinc *versus* one water in Zn-Cam (Fig. 5). Substantial differences in the active sites of the Cam and CamH structures portend differences in the catalytic mechanisms. However, although a kinetic analysis has not been reported and roles for the residues have not been investigated biochemically, sequence alignments (Fig. 3) are consistent with roles for Asn57 and Gln59 analogous to Asn73 and Gln75 of Cam. Further, the authors have postulated roles for Tyr159 analogous to Cam Asn202 [13]. Residues that function analogous to Cam Glu62 and Glu84 are not readily apparent although an unspecified role in proton transfer was speculated for His68 located adjacent to the active site [13]. It is reported that bicarbonate can function as a proton donor to the metal-bound hydroxyl during dehydration of bicarbonate by Cam [32]; thus, for CamH homologs that lack Glu62 and Glu84, bicarbonate may be an obligate proton donor for the dehydration reaction in this subclass.

2.3. Activators and inhibitors of Cam

Activation of Cam from *Methanosarcina thermophila* with several natural and non-natural amino acids and aromatic/heterocyclic amines reveal a profile of activating efficacy with natural, L- and D-amino acids that is substantially less than from enzymes of the α class [33]. Compounds 2-pyridylmethylamine and 1-(2-aminoethyl)-piperazine were effective towards Zn-Cam with K_A 's of 10.1-11.4 μ M whereas serotonin, L-adrenaline and 2-pyridylmethylamine were the most effective towards the Co-Cam with K_A 's of 0.97-8.9 μ M. Most likely, the differences are the consequence of diversity in the active sites (Fig. 5).

Sulfonamides are weaker inhibitors of Zn-Cam and Co-Cam than of α class carbonic anhydrases [34], likely a consequence of the different active site architecture of Cam that could allow entry of bulkier compounds without significant rearrangement. Indeed, catalytic residues Gln75 and Asn202 may be better positioned to interact strongly with the oxygen atoms of R-NH₄SO₂, or other negative atoms of the inhibitor, that more effectively inhibit catalysis. Sulfamate (HOSO₂NH₂) is a more effective inhibitor of Zn-Cam *versus* Co-Cam [34]. Unexpectedly, sulfamide (H₂NSO₂NH₂) that has structural similarity to sulfamate is the least effective Zn-Cam inhibitor with a K_i of $\sim$ 70 μ M. In general, anions are stronger inhibitors of Cam than most α class enzymes [35,36] with the exception of halides that are weak inhibitors of both Zn-Cam and Co-Cam. The anions CNO⁻, SCN⁻, and CN⁻ that bind monodentately to metal inhibit Zn-Cam to a greater extent than Co-Cam [36]. However, HCO₃⁻, CO₃⁻, NO₃⁻, and HSO₃⁻ that bind bidentately are most effective towards Co-Cam [36]. These different inhibition patterns for Zn-Cam and Co-Cam are best explained by the different coordination geometries for Zn²⁺ and Co²⁺ with different preferences for binding monodentate *versus* bidentate anions (Fig. 5).

3. Physiological functions

3.1. Anaerobic prokaryotes

The archetype γ class carbonic anhydrase Cam was isolated from *Methanosarcina thermophila*, an anaerobic microbe from the *Archaea* domain, that obtains energy for growth by metabolizing acetate to methane and carbon dioxide. Methane-producing species are one of several metabolic groups of microbes that together transform complex organic matter to methane in diverse environments devoid of oxygen such as the sediment of freshwater lakes and wetlands, the rumen of cattle, the hindgut of termites, and the lower intestinal tract of humans. The process is a significant link in the global carbon dioxide cycle. In the cycle, carbon dioxide is fixed into plant material that is oxidized back to carbon dioxide by oxygen-requiring prokaryotes in aerobic environments. However, a significant portion of the plant material enters diverse anaerobic environments devoid of oxygen where anaerobic fermentative species, largely from the *Bacteria* domain, decomposes the plant material to acetate and hydrogen gas that are further metabolized by methane-producing species from the *Archaea* domain. The process is harnessed to convert organic wastes and renewable plant material to methane (natural gas) as an alternative to fossil fuels.

The pathway for conversion of acetate to methane and carbon dioxide by *Methanosarcina thermophila* is shown in Fig. 7. In the pathway acetate is transported into the cell and converted to acetyl-CoA that is cleaved at the C-C and C-O bonds liberating methyl and carbonyl groups. The methyl group is attached to the cofactor coenzyme M and the carbonyl group is oxidized to provide electrons for reduction of the methyl group to methane. The mechanism for transport of acetate into the cell is unknown, although a role for Cam and CamH can be envisioned (Fig. 8). Genes encoding Cam and CamH homologs are among the annotations for genomic sequences of all acetate-utilizing methane-producing *Methanosarcina* species [16]. Further, Cam is up-regulated in acetate-grown *Methanosarcina thermophila* versus cells grown on other substrates [9] as are Cam homologs in *Methanosarcina mazei* [37] and *M. acetivorans* [38].

The proposed roles for Cam and CamH shown in Figure 8 are analogous to the well-studied mammalian trans-membrane bicarbonate/anion exchange system involving cytosolic and extracellular carbonic anhydrases that catalyze production or consumption of bicarbonate on opposing sides of the membrane thereby facilitating anion exchange [39]. The proposed location of carbonic anhydrase on the outer aspect of the cytoplasmic membrane is supported by a 5' region of the *cam* gene of which the deduced sequence is absent in Cam purified from *Methanosarcina thermophila*, a result consistent with a N-terminal leader peptide required for transport [9]. Furthermore, the putative leader is conserved in the deduced sequences of *cam* homologs identified in the sequenced genomes of the acetate-utilizing methane-producing species *M. mazei*, *M. acetivorans* and *Methanosarcina barkeri* (Fig. 9). The N-terminal sequences share significant identity and reveal a common motif consistent with a protease binding site. A role for this N-terminal sequence in maturation of Cam homologs is ruled out by over-production of Cam from *Methanosarcina thermophila* in either *E. coli* or *M. acetivorans*, without the putative leader, that is trimeric and displays robust carbonic anhydrase activity [23,24]. Deduced sequences with identity to the putative leader are absent in the genes encoding CamH from all four of the above mentioned acetate-utilizing *Methanosarcina* species [16] consistent with a cytoplasmic location of CamH. Furthermore, experiments with classical inhibitors are consistent with a location for a carbonic anhydrase located in the soluble fraction of *M. barkeri* [40]. Diamox, which is only moderately diffusible across membranes, inhibits carbonic anhydrase activity of whole cells only after a lag whereas inhibition with freely diffusible cyanide is immediate. These results are consistent with the cytoplasmic location of a carbonic anhydrase in *M. barkeri*, possibly CamH. Although an acetate/bicarbonate exchange protein (AE, Fig. 8) has not been identified, one potential candidate is the homolog of a novel acetate transporter (Ady2) from *Saccharomyces cerevisiae* [41] reported present in all the

sequenced genomes of acetate-utilizing *Methanosarcina* species [42] and *Methanosaeta thermophila* which is the only other acetate-utilizing methane-producing species described. Another potential candidate is a hypothetical transport protein identified adjacent to genes encoding Cam in the sequenced genomes of the acetate-utilizing species *M. mazei* and *M. acetivorans* [16].

The stoichiometry of the proposed bicarbonate/acetate exchange system (Fig. 8) is consistent with the stoichiometry of the metabolism of acetate to carbon dioxide (Fig. 7); thus, the acetate/bicarbonate exchange system also addresses removal of carbon dioxide from the cytoplasm. As previously discussed [9,40], removal of carbon dioxide is essential for growth since the energy available for conversion of acetate to carbon dioxide and methane is marginal under standard conditions ($\Delta G^{0'} = -36 \text{ kJ/mol acetate}$).

Curiously, the sequenced genome of *Methanosaeta thermophila* contains genes encoding two CamH subclass homologs and no Cam subclass homologs [42]. Neither of the CamH homologs have identity to the putative leader sequence of Cam homologs in *Methanosarcina* species. *Methanosaeta thermophila*, however, is unique in that it metabolizes acetate at much lower concentrations than *Methanosarcina* species [42,43]. Thus, *Methanosaeta thermophila* may have another system for active transport of acetate and removal of carbon dioxide from the cytoplasm for which roles of the CamH and Ady2 homologs remain obscure.

3.2. Eukarya

The proposed functions of carbonic anhydrases in higher plants are: (a) conversion of bicarbonate to carbon dioxide as a substrate for RuBisCO, (b) conversion of carbon dioxide to bicarbonate as a substrate for phosphoenolpyruvate carboxylase in C_4 plants, (c) to facilitate diffusion of carbon dioxide across the plasma membrane and chloroplast envelope and (d) to participate in the active transport of carbon dioxide across the plasma membrane by conversion to bicarbonate [44]. The β class carbonic anhydrases of higher plants are found in the cytoplasm and chloroplasts of leaves, and γ class homologs of the CamH subclass are found in Complex I of mitochondria where the proposed function is to play a role in the carbon transport system between mitochondria and chloroplasts to increase the efficiency of photosynthetic carbon dioxide fixation [45-47].

4. Conclusions

Although the γ class of carbonic anhydrases is widely distributed among all three domains of life, the biochemical and physiological understanding is primarily limited to two examples from the *Archaea* domain. Although the archetype Cam from *Methanosarcina thermophila* is the best understood, phylogenetic analyses indicate that the γ class is dominated by a subclass (CamH) that lacks an acidic loop structure and proton shuttle residues essential for Cam. Clearly, biochemical and structural analyses of the CamH subclass is paramount to understanding the γ class. Least understood of the γ class are the physiological functions that are almost certain to be as diverse as the species in which they reside. Among the diverse species are prokaryotes from the *Bacteria* and *Archaea* domains that are strict anaerobes proliferating in oxygen-free reduced environments where iron is freely available. The finding that iron is the physiologically relevant metal in Cam from an anaerobic species raises the possibility that iron could function in γ class enzymes, and possibly other classes, from diverse anaerobes.

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Figure 1. Active site of the γ class archetype Cam from *Methanosarcina thermophila* showing the acidic loop containing Glu84 in two conformations. Reproduced [12] with permission.

Figure 2.

Crystal structures of the γ class archetype Cam from *Methanosarcina thermophila* and CamH from *Pyrococcus horikoshii*. Panel A, the Cam trimer viewed along the three-fold axis. Panel B, side view of the Cam monomer. The β -strands are in purple and α -helices are in cyan. The active site metal is in yellow. Panel C, the CamH trimer viewed along the three-fold axis. The Ca²⁺ ion is in magenta and Zn²⁺ is in brick red. Panel D, the CamH monomer. Zn²⁺ and Ca²⁺ are shown in magenta and yellow spheres. Reproduced [13,18] by permission.

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#
Mt  QEITVDEFSNIRENFPVTPWNPEPSAPVIDPTAYIDPEASVIGEV TIGANVMVSPMASIRS 60
      P I P+A++D  A VIG+V +      V P A +R
Ph  MAIYEINGKKPRIHPSAFVDENAVVIGDVVLEEKTSVWPSAVLRG 45

# @      @ @#      %      @      %
Mt  DEGMP I FVGDRSNVQDGVVLHALETINEE G EPIEDNIVEVDGKEYAVYIGNNVSLAHQSQ 120
      D      I+VG  SNVQD V +H      + Y      IG  V++ H +
Ph  DIEQ-IYVGKYSNVQDNVSIH-----TSHGYPT EIGEYVTIGHNAM 85

%
Mt  VHGPAAVGDDTFIGMQAFVFK-SKVGNNCVLEPRSA AIGVTIPDGRYIPAGMVVTSQA EA 180
      VHG A VG+      IG+ + +      +K+G+++ +      G +P + IP +VL
Ph  VHG-AKVGNYVIIGISSVILDGAKIGDHVII-----GAGAVVPPNKEIPDYSVLGVPGK 140

@
Mt  DKLPEVTDDYAYSH TN-EAVVYNVHLAEGYKETS 213
      +      +      V      + G K
Ph  VRQLTEEEIEWTKNAETYVELAEKHIKGRKRI- 173

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Figure 3.

Sequence alignment of the γ class archetype Cam from *Methanosarcina thermophila* vs. CamH from *Pyrococcus horikoshii*. The Cam sequence is the sequence of the enzyme as purified from *M. thermophila* with the putative N-terminal leader sequence absent. Symbols: (Mt) *Methanosarcina thermophila*, (Ph) *Pyrococcus horikoshii*, (@) catalytically relevant residues in Cam, (%) metal ligands in Cam, (#) structurally relevant residues in Cam. Acidic loop residues are underlined which include the proton shuttle residue Glu84 in Cam. Active site residues in CamH are in bold. Aligned using the CLUSTAL 2.0.10 multiple sequence alignment program.

Figure 4. The active site coordination of CamH from *Pyrococcus horikoshii*. BCT, bicarbonate. Reproduced [13] by permission. Residues labeled A or B distinguish locations on adjacent monomers.

Figure 5.

Active site coordination of the γ class archetype Zn-Cam and Co-Cam from *Methanosarcina thermophila*. Panel A, Zn-Cam. Panel B, Co-Cam. Panel C, Zn-Cam complexed with bicarbonate. Panel D, Co-Cam complexed with bicarbonate. Reproduced [14] by permission. Residues labeled with or without “b” distinguish locations on adjacent monomers.

Figure 6.
Mechanism proposed for hydration of carbon dioxide by Zn-Cam from *Methanosarcina thermophila*. Reproduced [27] by permission.

Figure 7.
 Pathway for conversion of acetate to methane and carbon dioxide by *Methanosarcina* species.
 CoA, coenzyme A; CoM, coenzyme M.

Figure 8.
Proposed mechanism for exchange of acetate and bicarbonate in *Methanosarcina* species. AE, anion exchanger.

* * * * * *

MMFNKQIFTILILSLSLALAGSGCIS-SEGAED-NVAQ--EITVD-EFSNIR... M. thermophila
M-KINRIFLALLFSLALTLAGSGCVSQGEGAEDGESADT-E--VESEVSNIR... M. acetivorans
 MALLSLAITLAGSGCVSQGEGAE EGENIEAEEVEANVEESNIR... M. mazei
MRFNKQTFTILILSLSLALLGSGCISEGEGAE-GNVTQGITE---SEFSNIR... M. barkeri

Figure 9.

Comparison of the N-terminal sequence deduced from the gene encoding the γ class archetype Cam from *Methanosarcina thermophila* versus Cam homologs from other *Methanosarcina* species. Sequences were obtained from [9] and the Comprehensive Microbial Resource [16]. Asterisks signify conserved residues.