Chapter 5
Prokaryotic Carbonic Anhydrases of Earth's Environment

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Abstract Carbonic anhydrase is a metalloenzyme catalyzing the reversible hydration of carbon dioxide to bicarbonate. Five independently evolved classes have been described for which one or more are found in nearly every cell type underscoring the general importance of this ubiquitous enzyme in Nature. The bulk of research to date has centered on the enzymes from mammals and plants with less emphasis on prokaryotes. Prokaryotic carbonic anhydrases play important roles in the ecology of Earth's biosphere including acquisition of CO_2 for photosynthesis and the physiology of aerobic and anaerobic prokaryotes decomposing the photosynthate back to CO_2 thereby closing the global carbon cycle. This review focuses on the physiology and biochemistry of carbonic anhydrases from prokaryotes belonging to the domains *Bacteria* and *Archaea* that play key roles in the ecology of Earth's biosphere.

Keywords Anaerobe • Aerobe • Food chains • Biosphere • Decomposition • Prokaryotes • Carbon cycle

1 Introduction

There are five independently-evolved classes ($\alpha, \beta, \gamma, \delta, \zeta$) of carbonic anhydrases (CAs), with no structural or sequence similarity, that catalyze the reversible hydration of CO_2 ($CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$). The α class is restricted

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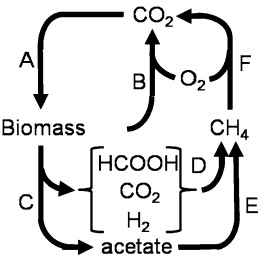

Fig. 5.1 The global carbon cycle

mostly to mammals and pathogenic prokaryotes, although a few have been described from non-pathogenic species [1]. The δ [2, 3] and ζ [3, 4] classes are found in marine diatoms [4]. A search of the Comprehensive Microbial Resource [5] of 656 completed prokaryotic genomes with the query "carbonic anhydrase" retrieved 1,300 sequences underscoring the widespread occurrence of this enzyme in diverse prokaryotes inhabiting Earth's biosphere. The great majority of prokaryotes harbor the β and γ class enzymes that play important roles in the global carbon cycle (Fig. 5.1). In the cycle, plants, algae and photosynthetic prokaryotes fix CO_2 into biomass (step A) that is decomposed back to CO_2 by O_2-requiring prokaryotes in aerobic environments (step B). Significant amounts of biomass enter diverse O_2-free environments where strictly anaerobic microbes decompose the biomass to methane and CO_2 (steps C,D and E). The remainder diffuses into aerobic environments where O_2-requiring prokaryotes oxidize it to CO_2 (Step F) closing the carbon cycle. CAs are a focus of investigation for developing technologies that capture and sequester CO_2 at point sources for reducing emissions that contribute to the greenhouse effect and global warming [6]. This review focuses on recent advances in understanding the physiology and biochemistry of CAs from prokaryotic microbes important to the ecology of Earth's biosphere.

2 Aerobic Species

Aerobes are prokaryotes that require O_2 to metabolize growth substrates. Cyanobacteria are aerobes and the most abundant photosynthetic organisms on Earth [11] that, together with plants, are responsible for the great majority of global CO_2 fixation into biomass (Step A, Fig. 5.1). Cyanobacteria utilize β and γ class CAs associated with an organelle called a carboxysome to enhance photosynthesis via a mechanism that concentrates and supplies CO_2 to ribulose-1,5-bisphosphate carboxylase [7]. Given that all cyanobacteria contain carboxysomes, and that cyanobacteria are considered the most abundant photosynthetic organisms on Earth [8], a substantial portion of global CO_2 fixation is dependent on CAs. The γ class CA (CcmM) from the aerobic cyanobacterium *Thermosynechococcus elongatus* strain BP-1 has been characterized [9]. The crystal structure closely resembles the archetype of the γ class (Cam) from *Methanosarcina thermophila* of the domain *Archaea* [10]. Cam is

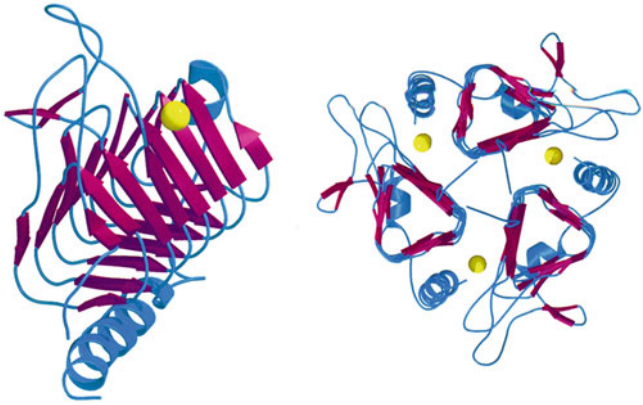

Fig. 5.2 Ribbon diagram of Cam, the archetype γ class CA. *Left panel*, a monomer. *Right panel*, view of the homotrimer along the axis of symmetry. The *yellow sphere* indicates the active site metal coordinated by two histidine residues from one of the three subunits and a third histidine ligand contributed by an adjacent subunit

a homotrimer with an overall left-handed β-helical fold (Fig. 5.2) [11]. However, CcmM differs from Cam by the addition of residues 198–207 that form a third α-helix stabilized by an essential cysteine 194-cysteine 200 disulfide bond. Deletion of this domain yields an inactive protein, the structure of which shows disordering of the N- and C-termini, and reorganization of the trimeric interface and active site. Under reducing conditions, CcmM is similarly partially disordered and inactive which underscores the importance of the disulfide bond. The crystal structure of the β class CA from the carboxysome of the aerobic species *Halothiobacillus neapolitanus* (CsoSCA) identifies it as one of three structural subclasses of the β class [12]. The active sites of β class enzymes from *Escherichia coli* [13], *Pisum sativum* [14], and the strict anaerobe *Methanothermobacter thermautotrophicus* [15] are organized as identical pairs through homodimerization. The enzyme from *Porphyridium purpureum*, a red alga, evolved an intermediate degree of symmetry wherein two domains are fused and organized with 2-fold pseudo-symmetry. The C-terminal domain of CsoSCA from *H. neapolitanus* diverged such that it lost the ligands for the catalytic zinc, including a loop on which one of the cysteine ligands would normally reside. Thus, the two domains in CsoSCA evolved such that only one in the pair retains a viable active site. It is proposed that this structure evolved to supply a new function specific to the carboxysome [12]. Nonetheless, the structural similarity among active site regions of the β class subclasses suggests a common catalytic mechanism.

A few non-photosynthetic chemolithotrophic aerobes contribute to CO_2 fixation such as the thermophiles *Sulfurihydrogenibium yellowstonense* and *Sulfurihydrogenibium azorense* [16]. These species, isolated from terrestrial hot springs, grow chemolithotrophically with CO_2 as the carbon source and H_2 as the energy source suggesting a potential role for CA in acquisition of CO_2 for

cell carbon [17]. Characterization of the α class CAs from these aerobes has revealed the fastest CA known with a k_{cat} of 4.40×10^6 s^{-1} and a k_{cat}/K_m of 3.5×10^8 M^{-1} s^{-1} [1].

The decomposition of complex biomass in Earth's aerobic biosphere is mediated by prokaryotes called heterotrophs (Step B). Carbon dioxide and bicarbonate are essential growth factors for environmentally important heterotrophs. Although sufficient CO_2 is produced during catabolism, levels below atmospheric lead to growth inhibition or possibly death [18–20]. For example, deletion of a gene from *Ralstonia eutropha* encoding a β class CA resulted in a requirement for heterotrophic growth of the mutant at CO_2 concentrations elevated compared to wild-type [21]. The requirement for CO_2 is attributed to fixation in anaplerotic or other biosynthetic reactions. A recent bioinformatics analysis indicates that CA-deficient prokaryotes evolved to only occupy high-CO_2 niches [22]. Comparisons between CA-retaining and CA-deficient genomes indicated that CA was lost in some species during the course of evolution as a consequence of dispensability. On the other hand, pathogenic prokaryotes seem to be adapted to high CO_2 concentrations in their host environment, as they usually require 5–10 % (vol/vol) CO_2 for growth (9, 45, 60). Although genomes of aerobic heterotrophs isolated from the environment include CAs [23], none have been biochemically and structurally characterized in detail.

Characterized CAs from heterotrophic aerobes are not limited to the upper regions of Earth's surface. *Geobacillus kaustophilus* strain HTA426 is a heterotrophic thermophile isolated from deep sea sediment at 10,897 m [24]. The organism contains a γ class CA for which the crystal structure and documentation of CA activity was recently published albeit with no further characterization [25]. Characterization of CAs from methane-oxidizing prokaryotes that close the carbon cycle (Fig. 5.1, Step F) have not been reported although the genomic sequences of methylotrophic species have annotations for several CAs [23].

3 Anaerobic Species

Anaerobes are microbes that metabolize growth compounds in the absence of O_2. They inhabit environments such as freshwater and marine sediments, rice paddy soils, and the hind gut of termites to name a few. The metabolism of complex biomass is mediated by a diversity of anaerobes which include fermentatives, syntrophs, sulfate reducers and nitrate reducers.

3.1 Anaerobes Metabolizing Complex Biomass

Fermentative plus syntrophic species metabolize complex biomass to products (Fig. 5.1, Step C) that are substrates for methane-producing anaerobes

(Fig. 5.1, Steps D and E). CAs have been documented in several anaerobes producing acetate as the only product of their energy metabolism [26, 27]. These species reduce CO_2 to the methyl level for incorporation into the methyl group of acetate which places a high demand on acquisition of CO_2 from the environment; thus, a postulated role for CA is to facilitate a bicarbonate/acetate antiporter that transports acetate out of the cell and bicarbonate in. A similar physiological role has been postulated for the γ class CA encoded in the genome of *Pelobacter carbinolicus* whereby the enzyme functions in conversion of cytosolic bicarbonate to CO_2 for reduction to formate that is excreted by exchange with extracellular bicarbonate [28]. The Type I β class CA from *Clostridium perfringens* strain 13 is the only β class enzyme characterized from a strictly anaerobic species of the domain *Bacteria* [29]. Analyses of a deletion mutant showed the CA is strictly required for growth when cultured in semi-defined medium and an atmosphere without CO_2 suggesting a role in anaplerotic CO_2 fixation reactions by supplying bicarbonate to carboxylases.

Although the genomes of anaerobic prokaryotes are richly annotated with CAs [23], few have been characterized. The sequence and crystal structure of YrdA from *Escherichia coli* [30], a facultative anaerobe of the domain *Bacteria*, identifies YrdA as a homolog of the archetype γ class CA (Cam) from *Methanosarcina thermophila* belonging to the domain *Archaea* [10]. However, YrdA has no significant CA activity and does not contain residues corresponding to Glu62, Glu84 and Asn202 important for CA activity of Cam [31]. Interestingly, an acidic loop around the putative catalytic site of YrdA shows alternative conformations leading the authors to suggest that the enzyme accommodates substrates larger than carbonate. The only other characterized Cam homolog from an anaerobe is from *Pyrococcus horikoshii* which also belongs to the domain *Archaea* [32]. The crystal structure of the enzyme over-produced in *E. coli* and purified in the presence of air shows the overall fold similar to Cam with zinc in the active site, although activity was not reported and the enzyme was not further characterized. Interestingly, calcium was also shown to be present in the *P. horikoshii* structure.

3.2 Anaerobes Producing Methane

There are two major pathways (Fig. 5.1, steps D and E) producing methane from the products of fermentative and syntrophic species (Fig. 5.1, step C) which constitutes an anaerobic microbial food chain converting complex biomass to methane. Both methanogenic pathways involve CO_2 as either a reactant or product suggesting a role for CA in either CO_2 acquisition or removal. The CO_2 reduction pathway involves the reduction of CO_2 to methane with electrons derived from either H_2 or formate (Fig. 5.1 step D), whereas the aceticlastic pathway produces equimolar amounts of methane and CO_2 (Fig. 5.1, step E). Clearly there is a high demand for CO_2 in the CO_2 reduction pathway where CA could play a role in acquisition and retention of CO_2 by conversion to membrane impermeable bicarbonate inside the

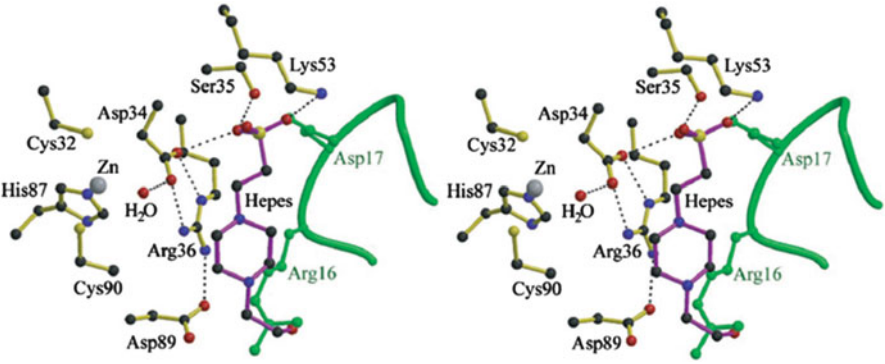

Fig. 5.3 Stereo view of the active-site of Cab from *Methanothermobacter thermautotrophicus* ΔH

cell. Indeed, a β class CA (Cab) has been characterized from *Methanothermobacter thermoautotrophicus* (f. *Methanobacterium thermoautotrophicum* strain ΔH) [33] which reduces CO_2 to methane with H_2 and synthesizes all cell carbon starting with CO_2 which places an additional demand on acquisition and retention of CO_2. Enzymes of biosynthetic pathways require either CO_2 or bicarbonate suggesting additional roles for CAs in supplying the required form of inorganic carbon.

Cab is the only β class CA characterized, both biochemically and structurally, from a methane-producing species. Although the overall fold is similar to that of other β-class CAs that are tetrameric and octameric, Cab is a dimer in the crystal structure [15]. The crystal structure shows the active site metal at the interface of the two monomers, although the two cysteines and one histidine coordinating the metal originate from the same monomer. The catalytic water is the fourth ligand (Fig. 5.3) which hydrogen bonds to an aspartate residue conserved in all β class CAs. The aspartate is in turn hydrogen bonded to an arginine also conserved in the active sites of β class CAs. Further, the crystal structure of Cab shows the active-site cavity open to bulk solvent such that a HEPES buffer molecule is present 8 Å from the catalytic metal (Fig. 5.3) distinct from the *P. sativum* enzyme. The kinetic analysis of Cab is consistent with a two-step metal-hydroxide mechanism proposed for all CAs. The conserved aspartate residue in the active site of Cab when replaced with alanine significantly reduces the k_{cat}/K_m value [34]. When the arginine interacting with aspartate is replaced with alanine, a still larger decrease in k_{cat}/K_m is observed. When the interaction between aspartate and arginine is disrupted, the aspartate may swing toward the active site and ligate the catalytic metal in a dead-end inactive conformation. Imidazole rescues the activity of both the replacement variants which suggests a role for the conserved residues in the proton transfer step. Thus, an exchange of ligands to aspartate during catalysis may be necessary if the pK_a of aspartate were decreased when interacting with arginine such that the aspartate residue is unable to abstract a proton from the zinc-bound water. In the aceticlastic pathway, the methyl group of acetate is reduced to methane with electrons derived from oxidation of the carbonyl group of acetate to CO_2.

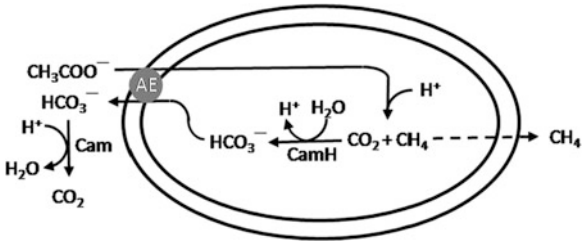

Fig. 5.4 Postulated roles for the γ class CAs Cam and CamH in the pathway of acetate conversion to methane and carbon dioxide in *Methanosarcina* species. The *double line* represents the cell membrane. *AE* anion exchanger

Methanosarcina thermophila contains two γ class CAs, Cam and CamH, postulated to facilitate transport of acetate into the cell and CO_2 out via an acetate/bicarbonate anion exchanger (Fig. 5.4) in analogy to the mammalian chloride/bicarbonate exchange system [35]. Cam was first investigated with the enzyme over produced in *Escherichia coli*. When purified from *E. coli* in the presence of air, Cam contains zinc in the active-site. However, when the zinc is exchanged with ferrous iron in vitro, the catalytic efficiency increases 3-fold over the zinc form [36]. Ferrous iron is present in the active-site when purified from *E. coli* in an atmosphere void of O_2. When exposed to air, ferrous is oxidized to ferric with rapid loss of CA activity. Cam from *M. thermophila* over-produced in the close relative *Methanosarcina acetivorans* contains stoichiometric amounts of ferrous iron when purified in the absence of O_2 establishing ferrous iron as the physiologically relevant metal in vivo [37]. When purified from *E. coli* in the presence of air, ferric iron is exchanged with contaminating amounts of zinc present in untreated buffers. A crystal structure is unavailable for Fe-Cam, although it is proposed to be similar to in vitro cobalt-substituted Cam with a coordination sphere containing three waters in addition to the metal ligands [11].

Cam is the most extensively investigated of the few γ class CAs characterized. The kinetic mechanism for a the reversible hydration of CO_2 by Cam is a two-step ping pong mechanism similar to all characterized CAs as shown in the following equations where E represents enzyme residues, M is metal and B is buffer.

$$\text{E-M}^{2+}\text{-OH}^- + \text{CO}_2 = \text{E-M}^{2+}\text{-HCO}_3^- \tag{5.1a}$$

$$\text{E-M}^{2+}\text{-HCO}_3^- + \text{H}_2\text{O} = \text{E-M}^{2+}\text{-H}_2\text{O} + \text{HCO}_3^- \tag{5.1b}$$

$$\text{E-M}^{2+}\text{-H}_2\text{O} = \text{H}^+\text{-E-M}^{2+}\text{-OH}^- \tag{5.2a}$$

$$\text{H}^+\text{-E-M}^{2+}\text{-OH}^- + \text{B} = \text{E-M}^{2+}\text{-OH}^- + \text{BH}^+ \tag{5.2b}$$

In step 5.1a, a lone pair of electrons on the metal-bound hydroxide attack carbon dioxide producing metal-bound bicarbonate that is displaced by water in step 5.1b. In step 5.2a, a proton is extracted from the metal-bound water and then transferred to buffer in step 5.2b. Cam exhibits a $k_{cat} > 10^4\ s^{-1}$ which is faster than the fastest

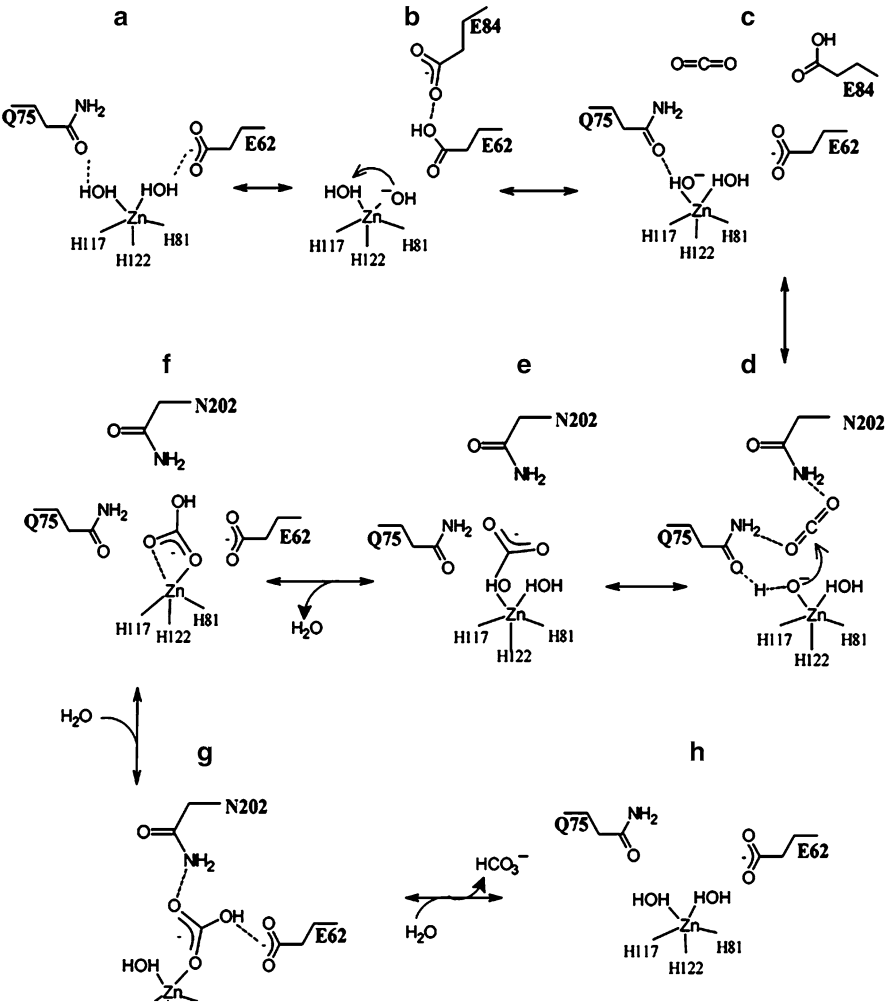

Fig. 5.5 The reaction mechanism proposed for the archetype γ class CA from *Methanosarcina thermophila*

rate at which protons can transfer from the zinc-bound water with a pK_a of 7 to water. Thus, Cam with $k_{cat} > 10^4$ s^{-1} must transfer the proton from the zinc-bound water to an intermediate proton shuttle residue (H^+-E in steps 5.2a and 5.2b) and then to the external buffer molecule. Replacement of active-site residues via site-directed mutagenesis, and kinetic analyses of the variant proteins, has identified several residues either essential or important for catalysis leading to the proposed mechanism for catalysis shown in Fig. 5.5 [31]. In the mechanism, glutamate 62 extracts a proton from either of two metal-bound waters (A) producing a hydroxyl group which extracts a proton from the adjacent water producing the metal-bound

hydroxyl Zn-OH^- that attacks the carbon of CO_2 producing metal-bound HCO_3^- (B-F). Glutamine 75 and asparagine 73 participate in a hydrogen bond network to orient and increase the nucleophilicity of the metal-bound hydroxide. The bound HCO_3^- undergoes a bidentate transition state wherein the proton either rotates or transfers to the nonmetal-bound oxygen of HCO_3^- (G). Hydrogen bonding with glutamate 62 destablizes the HCO_3^- which is further destabilized by an incoming water that replaces one of the metal-bound oxygens (H). A second incoming water displaces HCO_3^- with product removal and regeneration of the water-bound active-site metal. Not shown in Fig. 5.5, tryptophan 19 and tyrosine 200 are second shell residues distant from the catalytic metal that fine tune catalysis. Tryptophan 19 is important for both CO_2/bicarbonate interconversion and the proton transfer step [38].

Inhibitor studies of Cab and Cam show differences in inhibition response to sulfonamides and metal-complexing anions, when compared to α class CAs [39–42]. In addition, inhibition between Cab and Cam differ. These inhibition patterns are consistent with the idea that although, α-, β-, and γ-class CAs participate in the same two-step mechanism, diverse active site architecture among these classes predicts variations on the catalytic mechanism.

CamH of *M. thermophila* when over produced in *E. coli* contains 0.15 iron per monomer and only a trace amount of zinc when purified in the absence of O_2 [43]. The enzyme losses CA activity when exposed to air which suggests ferrous iron has a role in the active site. Databases queried with the Cam sequence reveal the great majority of homologs comprising a subclass homologous to CamH for which there is conservation of most residues essential for the archetype Cam except an acidic loop often missing the proton shuttle residue glutamate 84 of Cam [43]. Interestingly, the γ class CAs isolated from *P. horikoshii* and *E. coli* belong to the CamH subclass for which neither is reported to have CA activity. Clearly, more research is warranted on CAs from this subclass.

References

1. Luca VD, Vullo D, Scozzafava A, Carginale V, Rossi M, Supuran CT, Capasso C (2012) An alpha-carbonic anhydrase from the thermophilic bacterium *Sulphurihydrogenibium azorense* is the fastest enzyme known for the CO_2 hydration reaction. Bioorg Med Chem 21:1465–1469
2. Cox EH, McLendon GL, Morel FM, Lane TW, Prince RC, Pickering IJ, George GN (2000) The active site structure of *Thalassiosira weissflogii* carbonic anhydrase 1. Biochemistry 39:12128–12130
3. Tripp BC, Smith K, Ferry JG (2001) Carbonic anhydrase: new insights for an ancient enzyme. J Biol Chem 276:48615–48618
4. Lane TW, Saito MA, George GN, Pickering IJ, Prince RC, Morel FM (2005) Biochemistry: a cadmium enzyme from a marine diatom. Nature 435:42
5. Anonymous (2013) The comprehensive microbial resource. J Craig Venter Inst. http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi
6. Savile CK, Lalonde JJ (2011) Biotechnology for the acceleration of carbon dioxide capture and sequestration. Curr Opin Biotechnol 22(6):818–823

7. Kinney JN, Salmeen A, Cai F, Kerfeld CA (2012) Elucidating essential role of conserved carboxysomal protein CcmN reveals common feature of bacterial microcompartment assembly. J Biol Chem 287:17729–17736
8. Partensky F, Hess WR, Vaulot D (1999) Prochlorococcus, a marine photosynthetic prokaryote of global significance. Microbiol Mol Biol Rev 63:106–127
9. Pena KL, Castel SE, de Araujo C, Espie GS, Kimber MS (2010) Structural basis of the oxidative activation of the carboxysomal γ-carbonic anhydrase, CcmM. Proc Natl Acad Sci U S A 107:2455–2460
10. Alber BE, Ferry JG (1994) A carbonic anhydrase from the archaeon *Methanosarcina thermophila*. Proc Natl Acad Sci U S A 91:6909–6913
11. Iverson TM, Alber BE, Kisker C, Ferry JG, Rees DC (2000) A closer look at the active site of γ-carbonic anhydrases: high resolution crystallographic studies of the carbonic anhydrase from *Methanosarcina thermophila*. Biochemistry 39:9222–9231
12. Sawaya MR, Cannon GC, Heinhorst S, Tanaka S, Williams EB, Yeates TO, Kerfeld CA (2006) The structure of β-carbonic anhydrase from the carboxysomal shell reveals a distinct subclass with one active site for the price of two. J Biol Chem 281:7546–7555
13. Cronk JD, Endrizzi JA, Cronk MR, O'Neill JW, Zhang KY (2001) Crystal structure of *E. coli* β-carbonic anhydrase, an enzyme with an unusual pH-dependent activity. Protein Sci 10:911–922
14. Kimber MS, Pai EF (2000) The active site architecture of *Pisum sativum* β-carbonic anhydrase is a mirror image of that of α-carbonic anhydrases. EMBO J 19:1407–1418
15. Strop P, Smith KS, Iverson TM, Ferry JG, Rees DC (2001) Crystal structure of the "cab"-type β-class carbonic anhydrase from the archaeon *Methanobacterium thermoautotrophicum*. J Biol Chem 276:10299–10305
16. Nakagawa S, Shtaih Z, Banta A, Beveridge TJ, Sako Y, Reysenbach AL (2005) *Sulfurihydrogenibium yellowstonense* sp. nov., an extremely thermophilic, facultatively heterotrophic, sulfur-oxidizing bacterium from Yellowstone National Park, and emended descriptions of the genus *Sulfurihydrogenibium*, *Sulfurihydrogenibium subterraneum* and *Sulfurihydrogenibium azorense*. Int J Syst Evol Microbiol 55:2263–2268
17. Aguiar P, Beveridge TJ, Reysenbach AL (2004) *Sulfurihydrogenibium azorense*, sp. nov., a thermophilic hydrogen-oxidizing microaerophile from terrestrial hot springs in the Azores. Int J Syst Evol Microbiol 54:33–39
18. Brown OR, Howitt HF (1969) Growth inhibition and death of *Escherichia coli* from CO2 deprivation. Microbios 3:241–246
19. Dehority BA (1971) Carbon dioxide requirement of various species of rumen bacteria. J Bacteriol 105:70–76
20. Gladstone GP, Fildes P, Richardson GM (1935) Carbon dioxide as an essential factor in the growth of bacteria. Br J Exp Pathol 16:335–348
21. Kusian B, Sultemeyer D, Bowien B (2002) Carbonic anhydrase is essential for growth of *Ralstonia eutropha* at ambient CO_2 concentrations. J Bacteriol 184:5018–5026
22. Ueda K, Nishida H, Beppu T (2012) Dispensabilities of carbonic anhydrase in proteobacteria. Int J Evol Biol 2012:324549
23. Anonomous (2013) The comprehensive microbial resource. J Craig Venter Inst. http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi
24. Takami H, Nishi S, Lu J, Shimamura S, Takaki Y (2004) Genomic characterization of thermophilic *Geobacillus* species isolated from the deepest sea mud of the Mariana Trench. Extremophiles 8:351–356
25. Ragunathan P, Raghunath G, Kuramitsu S, Yokoyama S, Kumarevel T, Ponnuraj K (2013) Crystallization, characterization and preliminary X-ray crystallographic analysis of GK2848, a putative carbonic anhydrase of *Geobacillus kaustophilus*. Acta Crystallogr Sect F Struct Biol Cryst Commun 69:162–164
26. Braus-Stromeyer SA, Schnappauf G, Braus GH, Gossner AS, Drake HL (1997) Carbonic anhydrase in *Acetobacterium woodii* and other acetogenic bacteria. J Bacteriol 179:7197–7200
27. Pitriuk AV, Pusheva MA, Kupriianova EV (2006) Carboanhydrase activity in halophilic anaerobes from soda lakes. Mikrobiologiia 75:18–21

28. Aklujkar M, Haveman SA, Didonato R Jr, Chertkov O, Han CS, Land ML, Brown P, Lovley DR (2012) The genome of *Pelobacter carbinolicus* reveals surprising metabolic capabilities and physiological features. BMC Genomics 13:690
29. Kumar RS, Hendrick W, Correll JB, Patterson AD, Melville SB, Ferry JG (2013) Biochemistry and physiology of the β class carbonic anhydrase (Cpb) from *Clostridium perfringens* strain 13. J Bacteriol 195(10):2262–2269
30. Park HM, Park JH, Choi JW, Lee J, Kim BY, Jung CH, Kim JS (2012) Structures of the gamma-class carbonic anhydrase homologue YrdA suggest a possible allosteric switch. Acta Crystallogr D Biol Crystallogr 68:920–926
31. Zimmerman SA, Ferry JG (2006) Proposal for a hydrogen bond network in the active site of the prototypic γ-class carbonic anhydrase. Biochemistry 45:5149–5157
32. Jeyakanthan J, Rangarajan S, Mridula P, Kanaujia SP, Shiro Y, Kuramitsu S, Yokoyama S, Sekar K (2008) Observation of a calcium-binding site in the γ-class carbonic anhydrase from *Pyrococcus horikoshii*. Acta Crystallogr D Biol Crystallogr 64:1012–1019
33. Smith KS, Ferry JG (1999) A plant-type (beta-class) carbonic anhydrase in the thermophilic methanoarchaeon *Methanobacterium thermoautotrophicum*. J Bacteriol 181:6247–6253
34. Smith KS, Ingram-Smith C, Ferry JG (2002) Roles of the conserved aspartate and arginine in the catalytic mechanism of an archaeal β-class carbonic anhydrase. J Bacteriol 184:4240–4245
35. Sterling D, Alvarez BV, Casey JR (2002) The extracellular component of a transport metabolon. Extracellular loop 4 of the human AE1 Cl/HCO_3 exchanger binds carbonic anhydrase IV. J Biol Chem 277:25239–25246
36. Tripp BC, Bell CB, Cruz F, Krebs C, Ferry JG (2004) A role for iron in an ancient carbonic anhydrase. J Biol Chem 279:6683–6687
37. MacAuley SR, Zimmerman SA, Apolinario EE, Evilia C, Hou Y, Ferry JG, Sowers KR (2009) The archetype γ-class carbonic anhydrase (Cam) contains iron when synthesized in vivo. Biochemistry 48:817–819
38. Zimmerman S, Domsic JF, Tu C, Robbins AH, McKenna R, Silverman DN, Ferry JG (2013) Role of Trp19 and Tyr200 in catalysis by the gamma-class carbonic anhydrase from *Methanosarcina thermophila*. Arch Biochem Biophys 529:11–17
39. Zimmerman SA, Ferry JG, Supuran CT (2007) Inhibition of the archaeal β-class (Cab) and γ-class (Cam) carbonic anhydrases. Curr Top Med Chem 7:901–908
40. Zimmerman S, Innocenti A, Casini A, Ferry JG, Scozzafava A, Supuran CT (2004) Carbonic anhydrase inhibitors. Inhibition of the prokaryotic β and γ-class enzymes from Archaea with sulfonamides. Bioorg Med Chem Lett 14:6001–6006
41. Innocenti A, Zimmerman S, Ferry JG, Scozzafava A, Supuran CT (2004) Carbonic anhydrase inhibitors. Inhibition of the beta-class enzyme from the methanoarchaeon *Methanobacterium thermoautotrophicum* (Cab) with anions. Bioorg Med Chem Lett 14:4563–4567
42. Innocenti A, Zimmerman S, Ferry JG, Scozzafava A, Supuran CT (2004) Carbonic anhydrase inhibitors. Inhibition of the zinc and cobalt γ-class enzyme from the archaeon *Methanosarcina thermophila* with anions. Bioorg Med Chem Lett 14:3327–3331
43. Zimmerman SA, Tomb JF, Ferry JG (2010) Characterization of CamH from *Methanosarcina thermophila*, founding member of a subclass of the γ class of carbonic anhydrases. J Bacteriol 192:1353–1360