

Review Article

Carbonic Anhydrase: An Efficient Enzyme with Possible Global Implications

Christopher D. Boone, Sonika Gill, Andrew Habibzadegan, and Robert McKenna

Department of Biochemistry & Molecular Biology, University of Florida, P.O. Box 100245, Gainesville, FL 32610, USA

Correspondence should be addressed to Robert McKenna; rmckenna@ufl.edu

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As the global atmospheric emissions of carbon dioxide (CO₂) and other greenhouse gases continue to grow to record-setting levels, so do the demands for an efficient and inexpensive carbon sequestration system. Concurrently, the first-world dependence on crude oil and natural gas provokes concerns for long-term availability and emphasizes the need for alternative fuel sources. At the forefront of both of these research areas are a family of enzymes known as the carbonic anhydrases (CAs), which reversibly catalyze the hydration of CO₂ into bicarbonate. CAs are among the fastest enzymes known, which have a maximum catalytic efficiency approaching the diffusion limit of 10⁸ M⁻¹s⁻¹. As such, CAs are being utilized in various industrial and research settings to help lower CO₂ atmospheric emissions and promote biofuel production. This review will highlight some of the recent accomplishments in these areas along with a discussion on their current limitations.

1. Introduction

The atmospheric concentrations of greenhouse gases such as carbon dioxide (CO₂), methane, chlorofluorocarbons, and nitrous oxides have been increasing accordingly due to human-induced activities [1]. CO₂ is the most abundant greenhouse gas, being produced primarily by the burning of fossil fuels such as coal, oil, and natural gas. The atmospheric concentration of CO₂ has increased since preindustrial era from ~280 ppm [2] to 400 ppm in 2013 [3]. According to Antarctic ice core extractions, these levels are significantly higher than those at any time during the past 800,000 years [4–7]. Less direct geological evidence, based on boron-isotope ratios in ancient planktonic foraminifer shells, suggests that such high CO₂ atmospheric concentrations were last seen about 20 million years ago [8].

Since 1896, a trend has been associated with elevated CO₂ levels in the atmosphere and an increase in the average global temperature [9]. In ~100 years (1906–2005), the average global temperature increased by 0.7 ± 0.2°C, compared to a relatively constant average over the previous two thousand years [10]. This rise in CO₂ levels is linked not only to surface temperature increases (with rising sea levels, melting of

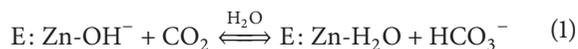
the glacier, and polar ice caps) but also to increased ocean acidity [11–13]. Fossil fuel burning accounts for 75% of the elevated atmospheric CO₂ levels from human activity over the past 20 years, with the remainder being associated primarily with deforestation [14].

2. Carbonic Anhydrase

The need for development of an efficient and inexpensive carbon sequestration system and the drive for an alternative fuel supply have both been led by a group of enzymes called the carbonic anhydrases (CAs; EC 4.2.1.1). The CAs are ubiquitously found in all kingdoms of life and are mostly zinc metalloenzymes that catalyze the interconversion between carbon dioxide and bicarbonate [15–17]. CAs exist in three structurally distinct and evolutionarily unrelated classes: α , β , and γ . The α -class is found throughout the animal kingdom and in the periplasmic function of *Chlamydomonas reinhardtii*, which is a type of green alga [18]. In addition, α -CAs can be found in *Neisseria gonorrhoeae* and other *Neisseria* species [19–21]. The β -CAs are found in plants with C3 and C4 metabolism, as well as monocotyledons and dicotyledons, arthropods, and bacteria [17, 22–27]. The γ -CA

was first isolated and characterized in the *Methanosarcina thermophila*, a methanogenic archaebacterium [28]. Homologous sequences of γ -CAs have been found in eubacteria; however, it is not known whether these sequences represent functional CAs [17].

Despite their structural differences, all CAs utilize the same catalytic mechanism: a two-step ping-pong mechanism that catalyzes the reversible hydration/dehydration of CO_2 into bicarbonate and a proton [17] as



In the first reaction, the zinc-bound hydroxide (ZnOH) nucleophilically attacks the CO_2 to form bicarbonate. Then, a molecule of water coordinates directly to the zinc ion, which promotes diffusion of the bicarbonate out of the active site (Reaction (1)). The zinc-bound water molecule (ZnH_2O) is deprotonated in the next reaction to regenerate the ZnOH (Reaction (2)). The proton is transferred from the ZnH_2O to the bulk solvent (B) via a highly ordered array of water molecules in the active site [29–32]. The amino acid residue that acts as the proton shuttle residue (i.e., the link between the buried ZnH_2O in the active site and bulk solvent) is His64 in the case of human CA isoform II (HCA II) [33] and several other HCAs [15]. HCA II is a highly efficient catalyst with a turnover rate (k_{cat}) on the order of one per microsecond and an overall catalytic efficiency (k_{cat}/K_M) approaching the diffusion rate limit of $10^8 \text{ M}^{-1} \text{ s}^{-1}$ [15, 29, 34–37]. Due to this highly efficient CO_2 hydration, there is considerable interest in using CAs in carbon sequestration systems, as well as in biofuel and calcite production (Figure 1 and Table 1). This review will outline the current status, advantages, and limits of these applications.

3. Carbon Sequestration

The typical industrial flue gas contains 10%–20% CO_2 , which by current processes is cost-inefficient and requires harsh chemical environments at elevated temperatures [60]. Selectively capturing CO_2 out of a mixture of waste gas that may also include nitrogen, sulfur, and other compounds can be technically challenging and expensive [61]. Current industrial protocol separates postcombustion CO_2 via amine scrubbing, mineral carbonation, pressure storage, or absorption into solids or into liquids. An attractive alternative to these methods includes the use of an environmentally benign, renewable, selective, and inexpensive biomimetic CO_2 sequestering agent.

The CAs are the leading candidate for this application as they are fairly inexpensive to be produced, are reusable, and can work at ambient temperatures and under mild conditions [38]. Adequate bovine CA can be purified from simple chloroform extraction or ammonium sulfate precipitation of slaughterhouse blood samples [62]. Human (and other mammalian) CAs are easily overexpressed in bacteria [63] and are commercially available for purchase. While both human and bovine CAs have been used in the industrial

setting, there is a growing need for improved CAs with higher stability and/or catalytic rates (to improve reusability and cost-efficiency, resp.) [38, 64]. A technique currently employed in the industrial setting to improve the stability of CAs is via immobilization onto a variety of inorganic [39–43] and biopolymer surfaces [44, 45], including enriched microorganisms [21, 46], as well as onto matrices containing acrylamide, alginate, and chitosan-alginate [47, 48]. Other techniques include site-directed mutagenesis to rationally design faster [65] and/or more stable [64, 66] variants of HCA II. Research on stable CAs isolated from halo- and thermotolerant microorganisms could provide further CO_2 sequestration candidates in the industrial setting.

Once CO_2 has been scrubbed from the flue gas, it can be chemically converted into stable compounds such as various carbonates or it can be pressurized to a liquid state for mass transport for geosequestration (storage either underground or in the ocean) [67, 68]. However, there are concerns over the cost, stability, and the long-term biological impacts (e.g., the release of CO_2 upon contact with acidic rain) associated with geosequestration [68]. Chemical conversion of CO_2 into ecologically friendly products, such as calcite (CaCO_3), has gained recent interest. Calcite is the main constituent of shells in marine organisms [69, 70] and is readily prepared by reacting CaCl_2 with bicarbonate, the product of CA catalysis. As such, calcite is routinely used in the making of cement and other building materials, but it can also be used as a pigment for paint formulation or as an acid neutralizer. Sequestered CO_2 can also be converted into other useful products such as polycarbonates, acrylates, methane, carbonate storage polymers, and other construction materials [56–58].

4. Biofuel Production

The potential long-term global environmental effects and the limited availability of oil and natural gas sources have prompted many countries, including the US, to initiate methods to find an environment friendly alternate fuel source [59]. There are an estimated 60 billion gallons of diesel and 120 billion gallons of gasoline used in the US per year [71]. This equates ~140 billion gallons of biodiesel needed for total transportation fuel in the US each year. Biodiesel is preferred over conventional diesel as it does not contribute to CO_2 or sulfate levels in the atmosphere, emits less gaseous pollutants, and is nontoxic [49]. Soybean oil accounts for over half of the source of US biodiesel production [72]. However, only ~15% of the biodiesel demand could be met if all the arable land in the US were used to grow soybean for oil production [59]. Additionally, the current production of biofuels displaces croplands, previously used for food, and has been associated with increased consumer prices [73, 74].

An alternative to the soybean-derived biodiesel is algae-based systems. Algae are attractive candidates as they have higher oil production and carbon fixation rates compared to terrestrial plants [75, 76] and do not compete with traditional agriculture as they can be cultivated in ponds or in closed photobioreactors located on nonarable land [59]. Continuous

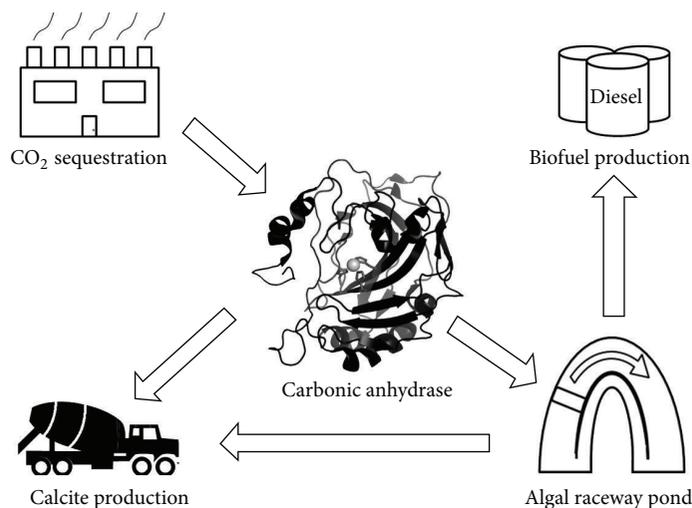


FIGURE 1: Schematic of the centralized role for CA in converting CO_2 into beneficial products. The catalytic conversion of CO_2 produced during the combustion of fossil fuels into bicarbonate (HCO_3^-) via CA yields a valuable source of inorganic carbon for algal cultures grown in raceway ponds. The lipids and oils from the algae cultures are excellent sources for biofuels, whereas the “waste” product yields additional beneficial proteins, vitamins, minerals, and dietary supplements. The algal CA can also serve as a great source for calcite (CaCO_3) production, being critical in many construction, agricultural, and industrial materials.

TABLE 1: CA usage in industrial settings.

Technique	Principles	CA utilization	References
Carbon sequestration	Capture of atmospheric CO_2 produced during the burning of fossil fuels	Immobilized onto a variety of surfaces including enriched microorganisms, alginates, and inorganic material	[38–48]
Biofuel production	Mass algal growth and harvesting as an alternative fuel source	Provides inorganic carbon in the soluble form of bicarbonate to Rubisco, the rate-limiting step in biomass production	[49–55]
Calcite production	Chemical conversion of bicarbonate to calcite, used in construction and agricultural materials	Provides bicarbonate at a rapid rate via the catalytic hydration of CO_2 that is captured as a result of carbon sequestration and biofuel production	[56–59]

cultivation of algae would also yield beneficial medicinal agents such as proteins, fatty acids, vitamin A, minerals, pigments, dietary supplements, and other biomolecules [77].

The CAs play an important role in the carbon fixation pathways in photosynthetic organisms (plants, algae, and cyanobacteria). The rate-limiting step of biomass production in these organisms is the uptake of CO_2 into cells as bicarbonate. A carbon-concentrating mechanism that includes CA delivery of inorganic carbon to RuBisCo was evolved to counterattack this limitation [59, 78]. The CO_2 is fixated into phosphoglyceric acid, enters the Calvin cycle and ultimately results in sugar. Numerous research efforts are being conducted to improve the efficiency of carbon fixation pathways aiming to improve food crop cultivation and biomass production [79].

Endogenous algal and cyanobacteria CA can also be used indirectly in calcite deposition, as evidenced by the enhanced CO_2 capture and sequestration in the presence of the algal species *Chlorella* and *Spirulina* [50–53]. These algal species also provide the extra benefit of producing calcite during cell with *Chlorella* produced the greatest yield of lipid biomass in photobioreactors [50]. Other studies in simulated

raceway ponds showed similar results with the additional observation of decreased CO_2 capture with increasing levels of acetazolamide, a commonly used CA inhibitor [50, 80]. The role of CA in this calcite precipitation is proposed to be a vital one, as it provides inorganic carbon in a soluble form as bicarbonate, the preferred source for a variety of algal strains (Figure 1 and Table 1) [54, 55]. However, much work is needed in determining the right conditions (pH, substrate/nutrient availability, aeration, etc.) to optimize the simultaneous production of biofuels and calcite with algal species.

5. Conclusion

Man’s dependence on fossil fuels and other natural gas products has brought forth an era when CO_2 atmospheric levels are higher than those at any other time in recorded history. The long-term environmental consequences of this pandemic caused by humanities need for energy are bleak but also unpredictable. The global reduction of greenhouse gas emissions is the first critical step in the reversing process

as an alternative source of fuel is found. The need for a solution has prompted much research into studying the viability of utilizing CAs for both of these challenges. Ideally, an optimized system would include a cyclic production of biofuels via algal and/or microalgal cultures that would substitute in fossil fuel combustion. The flue gas could then be scrubbed for CO₂ by the CAs in the same algal cultures, which would also promote the formation of bicarbonate, inducing further biomass production and increasing the rate of calcite precipitation (Figure 1). As such, this system would provide the benefits of reduced CO₂ emissions while also providing an essentially self-enclosed fuel and calcite generator, provided other essential ingredients and nutrients are available. Much research is also needed, however, in designing a highly active and stable variant of CA that can be easily overexpressed in such system.

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