



Industrial CO₂ Removal Using Carbonic Anhydrase: Potential, Promise and Challenges

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Atmospheric concentrations of greenhouse gases (GHG) such as carbon dioxide (CO₂), chlorofluorocarbons, methane, and nitrous oxide have been rising considerably due to human-induced processes [1]. One of the most abundant of the GHG is CO₂, and a main contributor to a rise in global temperatures [2]. The burning of fossil fuels has sharply increased the concentration of atmospheric CO₂ and has been correlated with increased global temperatures over the past century [3,4]. This presents a global threat that has recently been addressed by world leaders in the *Paris Climate Talks* [5] promoting an extensive effort to limit CO₂ production in industrial processes and to slow the rate of climate change. Despite the recognition of these issues, implementation of large scale CO₂ removal from the burning fossil-fuels has been limited [6]. Most of this is due to the use of harsh chemical processes and extreme temperatures to remove CO₂, which translates to an energy and cost inefficient process [4,7]. Therefore, more efficient CO₂ removal processes must be implemented. One such potential avenue is the utilization of enzymatic CO₂ sequestration [8]. Specifically, the use of the enzyme, carbonic anhydrase (CA) for CO₂ removal (CDR) has shown promise for its catalytic efficiency and its ability to be produced in large quantities from recombinant technology [7,9-11].

However, for a successful CA-mediated CDR, the enzyme must maintain its catalytic efficiency in extreme conditions, such as high temperature (up to 80°C), pressure, extreme pH levels (between pH 3 – 11), and more recently, resistance to anionic inhibition [10,12-16]. Furthermore, a mechanism to feasibly incorporate a CA-mediated CDR step in the fossil-fuel combustion process needs to be developed. To date, several possibilities have come in the form of CO₂ absorbers containing immobilized CA resins, or bioreactors containing algae that over express CA, all of which have been extensively reviewed by Frost and McKenna et al. [17]. A model depicting a CA-mediated CO₂ absorber is depicted in Figure 1 with favorable biochemical and biophysical characteristics of the enzyme highlighted.

Our group and others, have made efforts to characterize CAs from organisms that thrive in extreme environments [15,18] and utilize these biochemical and biophysical characteristics to engineer thermal and pH stable CA variants [11,19-21], to address the need for a suitable bio-catalytic CDR agent. Previously it has been shown: that truncating surface loops, the presence of an intramolecular disulfide bond, and dimerization allows CA to maintain its catalytic activity at 70°C, and a range of pH (from pH 5-9) [15,20]. In addition, it has been shown that the presence of charged residues in the catalytic site of CA can contribute to the reduction in anionic inhibition (common anions found in fossil-fuel by-products and their CA inhibition constants are shown in Table 1). Although these parameters still fall short of the ideal characteristics of a CA-mediated CDR agent, they provide us with avenues which we can further exploit to engineer a useful candidate to reduce fossil-fuel produced CO₂ emissions. Future studies will include implementing an enzymatic design of an oligomeric and compact CA, that exhibits resistance to anionic inhibition, and retains its activity in a range of pH from 3-11 and temperatures up to 80°C (Figure 1) [13-16,18]. These results can further be combined with current designs to

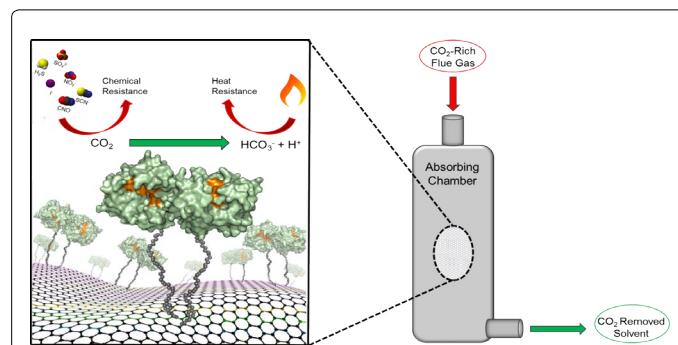


Figure 1: Model of a proposed CA-mediated CO₂ absorber to be used in industrial CDR processes. Highlighted are specific biochemical and biophysical characteristics that would be ideal for a bio-catalytic CA (green) immobilized on a resin (shown as mesh) within a CO₂ absorber. These would include: a compact and oligomeric structure to have thermal stability, exhibit enzyme activity in a broad pH range, and resistance to anionic inhibition (shown are common anions found in fossil-fuel combustion by-products). Figure was made in part using PyMol [22]. CA structure shown is that of the α-CA from *Thiomicrospira crunogena* (PDB: 4XZ5) [15].

Anion	TcrUCA	hCA II	SspCA
	K _i (mM) ^a		
Hg ²⁺	8.40	0.85	0.77
HSO ₃ ⁻	0.97	89	21.1
SO ₃ ²⁻	7.6	7.5	2.3
HS ⁻	0.70	0.04	0.58

TcrUCA: α-CA isolated from *Thiomicrospira crunogena* XL2 [15]; hCA II: α-CA from humans (isoform II); SspCA: α-CA isolated from *Sulfurihydrogenibium yellowstonense* YO3AOP1 [18]; ^aInhibition constants adapted from Mahon et al. [14]

Table 1: Selected anion inhibition constants of CAs suggested as CDR-agents.

implement a CA-mediated CDR system and provide an energy and cost efficient process to limit atmospheric CO₂. With the current global dependency on fossil-fuels for energy production, CA may provide a means to reduce human induced climate change.

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