Carbonic Anhydrase as CO₂ capturing agent: its Classes and Catalytic Mechanisms

Bashistha Kumar Kanth, Seung Pil Pack*
Department of Biotechnology and Bioinformatics
Korea University
Sejong, 339-700 Korea
E-mail: spack@korea.ac.kr

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Abstract- Carbonic anhydrase (CA; EC 4.2.4.1), metallo-enzyme, can catalyze reversible hydration of CO₂ (CO₂ + H₂O ↔ H⁺ + HCO₃⁻) with high efficiency (kcat ~10⁶ s⁻¹) and plays fundamental roles in many biological processes like photosynthesis, respiration, pH homeostasis and ion transport. Recently, CA has been considered as an important biocatalyst for CO₂ sequestration technology because the accumulation of CO₂ is the main cause for global climate change and it is critical to develop technologies that can reduce atmospheric CO₂ level. This review deals with the classes and mechanisms of several CAs as CO₂ capture agents.

Keywords— Carbonic Anhydrase; Classes, Mechanism

I. INTRODUCTION

Since 20th century, industrialization and civilization have developed extensively. Anthropogenic interventions have caused a rise in concentration of greenhouse gases, especially, carbon dioxide (CO₂) which has led to several undesirable consequences such as global warming and related changes [1]. The most easily addressable source of CO₂ is from the coal-fired power plants, which currently account for almost the half of the electricity generation. Due to industrial progress, concentration of CO₂ in the atmosphere is still increasing and it is unlikely that there will be a dramatic change in this situation in near futures. The outcome of this increase has already witnessed a profound effect on global environment. The climate changes and increasing environmental awareness have been driving global policy maker to find a solution for global warming [2, 3]. Thus, actions are being taken to alleviate the greenhouse gas emissions, especially in the case of large point source emissions, through various mechanisms and protocols.

In technology, there are various methods of reducing CO₂ concentration in the atmosphere. These methods are normally classified as (1) reduction of formation of CO₂ and (2) reduction of emission of CO₂. In case of reducing CO₂ emissions, one promising approach is CO₂ capture and storage (CCS)- CO₂ is captured at a power plant and sequestered for long-term storage in any of a variety of suitable geologic forms [4]. CO₂ capture from coal-fired power generation can be achieved by various approaches: post-combustion capture, pre-combustion capture, and oxy-combustion. A wide variety of separation techniques is being pursued, including gas phase separation, absorption into a liquid, and adsorption on a solid, as well as hybrid processes, such as adsorption / membrane systems. Although these approaches may be prominent, it is still too costly, so the efficiency of capture/sequestration should be increased. Much effort should be made not only for improving the state-of-the-art technologies but also for developing several innovative concepts, such as metal organic frameworks, ionic liquids, and enzyme-based systems.

Biologically based CO₂ capture systems are one of innovative approaches to improve the capture efficiency. These systems are based upon highly-efficient biological CO₂ mechanisms, which are naturally-occurring in living organisms. One of the most prominent possibilities is the use of the enzymes involved in such biological CO₂ reactions; the respiratory system in mammalian cells or photosynthetic systems in plant cells. On the basis of CO₂-catalyzing enzymes, we could make “bio-mimic” CO₂ capture systems, which can show high efficiency or performance in capture and release of CO₂ comparable to biomechanisms.

Carbonic anhydrase (CA, EC 4.2.1.1) is one of the enzymes, which can be employed for CO₂ sequestration technology development. CA is metallo-enzyme (mostly, a zinc-containing) and ubiquitous in nature, that is, found in animals and plants and even in the microbes. They exist in different forms, with different structures and molecular weights, and their activities vary from one to another [5]. CA catalyzes a reversible hydration of carbon dioxide: CO₂ + H₂O ↔ H⁺ + HCO₃⁻ (1.1) including varieties of other reactions. This enzyme is involved in basic cell processes, such as photosynthesis, respiration, transport of inorganic carbon (Cᵢ) and ions, calcification, and regulation of acid–base balance [6]. Interestingly, CA has the ability to catalyze the hydration of 600,000 molecules of carbon dioxide per molecule of CA per second comparable to a theoretical maximum rate of 1,400,000 [5, 6]. They are the fastest enzymes known e.g. it is reported that one molecule of hCA II from the human body can catalyze 1.4 ×10⁶ molecules of CO₂ per sec. CA can fix large quantities of CO₂ into CaCO₃ in presence of suitable cations at modest pH values in vitro.

Since CA has such unique CO₂-catalyzing properties as described above, CAs has been attended as prominent biocatalysts for CO₂ sequestration technology development. In this review, several classes CA are presented. This review will
be helpful for design of CA-based CO₂ sequestration systems.

II. HISTORICAL VIEW

In 1933, Carbonic anhydrase was independently discovered by Meldrum and Roughton (Meldrum and Roughton, 1933) and Stadie and O’Brien. CA was first characterized while searching a catalytic factor necessary for fast transportation of HCO₃⁻ from the erythrocyte to pulmonary capillary. Meldrum and Roughton purified the erythrocyte carbonic anhydrase. Keilin and Martin presented role for Zn in catalysis by finding that activity was directly proportional to the Zn content; thus, carbonic anhydrase was the first Zn metalloenzyme identified. The enzyme has been found to exist in all animals after examining for its presence. Neish reported its existence in 1939 in plants but the existence was confirmed by sulfhydryl protectants when used to preserve its activity during purification. CAs from plants differ with that from animals by size and decreased sensitivity to the sulfonamide family of inhibitors.

In 1963, Veitch and Blankenship became first to report CA from a prokaryote. They found in the nasal exudates of patients suffering from respiratory infections. Several microbes like Lactobacillus, nine strains of Neisseria, and Streptococcus salivarius were examined for its origin. The first CA enzyme was reported from Neisseria sicca strain and was found to share many of the same properties as the human CA. The first sequence of CA from E. coli was reported in 1992. The CA from Methanosarcina barkeri was first detected in the Archaea domain and an enzyme was found closely related to CA from M. thermophila in 1994. Roberts et al. (1997) reported CA from Thalassiosira weissflogii [7].

III. CLASSES AND MECHANISMS OF CA

Carbonic anhydrases are ubiquitous enzymes, present in prokaryotes and eukaryotes. There are five distinct CA families (α, β, γ, δ and ζ). These families have no significant similarity in amino acid sequence i.e. evolutionarily unrelated and in most cases are believed to be an example of convergent evolution [8-11].

Alpha-class carbonic anhydrase (α-CA)

Alpha-class CAs (α-CAs) are present in vertebrates, bacteria, algae and cytoplasm of green plants [6, 12-17]. Those CA enzymes found in mammals have different sub-cellular localization and tissue distribution. They are of 16 different and divided into four broad subgroups; cytosolic CAs (CA-I, CA-II, CA-III, CA-VII and CA-XIII), mitochondrial CAs (CA-VA and CA-VB), secretory CAs (CA-VI) and membrane-associated CAs (CA-IV, CA-IX, CA-XII, CA-XIV and CA-XV). There are three additional "acatalytic" CA isoforms (CA-VIII, CA-X, and CA-XI), whose functions remain unclear.

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+ \quad (1.1)
\]

\[
\text{CO.NH} + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{NCOOH} \quad (1.2)
\]

\[
\text{NH.C.NH} + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{NCONH}_2 \quad (1.3)
\]

\[
\text{RCHO} + \text{H}_2\text{O} \leftrightarrow \text{RCH(OH)}_2 \quad (1.4)
\]

\[
\text{RCOOAr} + \text{H}_2\text{O} \leftrightarrow \text{RCOOH} + \text{ArOH} \quad (1.5)
\]

\[
\text{RSO}_2\text{Ar} + \text{H}_2\text{O} \leftrightarrow \text{RSO}_2\text{H} + \text{ArOH} \quad (1.6)
\]

\[
\text{ArF} + \text{H}_2\text{O} \leftrightarrow \text{HF} + \text{ArOH} \quad (\text{Ar: 2,4 - dinitrophenyl}) \quad (1.7)
\]

\[
\text{PhCH}_2\text{OCOCl} + \text{H}_2\text{O} \leftrightarrow \text{PhCH}_2\text{OH} + \text{CO}_2 + \text{HCl} \quad (1.8)
\]

\[
\text{RSO}_2\text{Cl} + \text{H}_2\text{O} \leftrightarrow \text{RSO}_2\text{H} + \text{HCl} \quad (\text{R: Me, Ph}) \quad (1.9)
\]

Besides physiological reactions, the reversible hydration of CO₂ to bicarbonate [reaction (1.1)], α-CAs catalyze a variety of other reactions, such as hydration of cyanate to carbamic acid, or of cyanamide to urea [reactions (1.2) and (1.3)]; the aldehyde hydration to gem-diols [reaction (1.4)]; the hydrolysis of carboxylic, or sulfonic [reactions (1.5), (1.6)], as well as other less investigated hydrolytic processes, such as those described by reactions (1.7) - (1.9).

![Fig. 1. Mechanism of Alpha-type CA](image)

The metal ion Zn(II) in all α-CAs is essential for catalysis. In case of α-CA, human carbonic anhydrase II, hCAII (pdb: 2VVB) therefore was exemplified to explain structure-based mechanism of α-CA in detail. The metal ion, Zn(II), is situated at the bottom of a 15 Å deep active site cleft, being coordinated by three histidine residues (His94, His96 and His119) (Fig. 1(A)), with hydroxide bound to Zn(II) (Fig. 1(A)). This strong nucleophile attacks the CO₂ molecule bound in a hydrophobic pocket in its neighbourhood (substrate-binding site comprised residues Val1121, Val1143 and Leu198 in hCAII) (Fig.1(B)), leading to the formation of bicarbonate coordinated to Zn(II) (Fig. 1(C)). The bicarbonate ion is then displaced by a water molecule and liberated into solution, leading to the acid form of
The reaction (1.11) i.e., the proton transfer that regenerates the zinc-hydroxide species of the enzyme is the rate limiting step [18]. In the catalytically-active isozymes, such as CA II, CA IV, CA VII and CA IX, the process is assisted by a histidine residue placed at the entrance of the active site (His64 in hCAII), as well as by a cluster of histidines, which protrudes from the rim of the active site to the surface of the enzyme, assuring thus a very efficient proton transfer process for the most efficient CA isozyme, CA-II [18].

Two main classes of CA inhibitor are known: the metal complexing anions, and the unsubstituted sulfonamides, which bind to the Zn(II) ion of the enzyme either by substituting the non-protein zinc ligand or add to the metal coordination sphere generating trigonal-bipyramidal species.

**Beta-class carbonic anhydrase (β-CA)**

Beta-class CAs (β-CAs) are present predominantly in bacteria, algae and chloroplasts of both mono- as well as dicotyledons. β-CA have been obtained from a red alga (P. purpureum), a plant chloroplast (P. sativum), bacterium (E. coli), an archaea (M. thermoautotrophicum), two enzymes from pathogenic bacteria (M. tuberculosis), a carboxysome (H. neapolitanus), gram negative bacteria (Haemophilus influenzae) and so on.

All β-CAs share a unique α/β fold not found in any other proteins. Although β-CA can adopt a variety of oligomeric states with molecular masses ranging from 45 to 200 kDa, the fundamental structural unit appears to be a dimer or its structural equivalent. β-CAs are generally formed of 2-6 monomers of molecular weight 25-30 kDa. Some well-known β-CAs are PPCA (pdb code: 1DDZ) available from Porphyridium purpureum, PSCA (1EKJ) from Pisum sativum, ECCA (116P) from Escherichia coli and MTCA (1G5C) from Methanobacterium thermoautotrophicum.

The Porphyridium purpureum CA (PPCA) is a pseudo-tetramer composed of two pseudo-dimers. Its monomer is composed of two internally repeating structures, being folded as a pair of fundamentally equivalent motifs of an α/β domain and three projecting α-helices. The motif is very distinct from the motif of either α- or γ-CAs. This homodimeric CA looks like a tetramer [19]. The Zn(II) ion is essential for catalysis in both α- or β-CA families, but its coordination is different and rather variable for the β-CAs. Depending upon organization of active site region (ligation state of active site zinc ion, and the orientation & arrangement of nearby residues) in uncomplexed state, β-CAs have two structural classes- type I & type II (Rowlett, 2010). Thus, in the prokaryotic β-CAs, the Zn(II) ion is coordinated by two cysteinate residues, an imidazole from a His residue and a carboxylate belonging to an Asp residue (Fig. 2B(I), whereas the chloroplast CA has the Zn(II) ion coordinated by the two cysteines, the imidazole belonging to a His residue, and a water molecule (Fig. 2B(II)) (Kimber and Pai 2000; Kisker et al., 1996; Mitsuhashi et al., 2000).

![Fig. 2. Mechanism of Beta-type CA](image-url)

As arranged above, the proposed catalytic mechanism of β-CA is explained here by exemplifying PPCA. Since there are two homologous repeats in PPCA, two Zn(II) ions are coordinated by the four amino acids. In this case these pairs are Cys149/Cys403, His205/His459, Cys208/Cys462, and Asp151/Asp405 [19]. A water molecule is also present in the neighborhood of each metal ion, but it is not directly coordinated to it, forming a hydrogen bond with oxygen belonging to the zinc ligand Asp151 / Asp405 [Fig. 2(A)]. A proton transfer reaction may occur from this water molecule to the coordinated carboxylate moiety of the aspartate residue, with generation of a hydroxide ion which may be then coordinated to Zn(II) which acquires a trigonal bipyramidal geometry [Fig. 2(B)]. Thus, the strong nucleophile which may attack CO2 bound within a hydrophobic pocket of the enzyme is formed [Fig. 2(C)], with generation of bicarbonate bound to Zn(II) [Fig. 2(D)]. In the intermediate, the aspartic acid residue originally coordinated to zinc is proposed to participate to a hydrogen bond with the coordinated bicarbonate [Fig. 2(D)]. In the last step, the coordinated bicarbonate is released in solution, together with a proton, the aspartate generated re-coordinates the Zn(II) ion, and the accompanying water molecule forms a hydrogen bond with it. The enzyme is thus ready for another cycle of catalysis.

**Gamma-class carbonic anhydrase (γ-CA)**

Gamma-class CAs (γ-CAs) are mainly found in Archaea and some in bacteria. The γ-CA (Cam) has been isolated from the methanogenic archaean Methanosarcina thermophile growing...
in hot springs [20]. The protein fold is composed of a left-handed β-helix motif interrupted by three protruding loops and followed by short and long α-helices. The Cam monomer self-associates in a homo-trimer with the approximate molecular weight of 70 kDa [20]. The Zn(II) ion remain within the active site and is coordinated by three histidine residues, like in α-CAs, the active site of this γ-CA contains additional metal-bound water ligands, so that the overall coordination geometry is trigonal bipyramidal for the zinc-containing Cam. Two of the His residues coordinating the metal ion belong to one monomer whereas the third one is from the adjacent monomer. Thus, the three active sites are located at the interface between pairs of monomers [20]. The catalytic mechanism of γ-CAs has been proposed to be similar with α-class enzymes. It is matter of controversy whether Zn(II) is not tetra-coordinated as originally reported or penta-coordinated [20] with two water molecules bound to the metal ion. At this moment, the zinc hydroxide mechanism is accepted as being valid for γ-CAs, as it is probable that equilibrium exists between the trigonal bipyramidal and the tetrahedral species of the metal ion from the active site of the enzyme.

Ligands at the active site make contacts with the side chain of Glu62 suggesting that this side chain is probably protonated. In the uncomplexed zinc-containing Cam, the side chains of Glu62 and Glu84 appear to share a proton; additionally, Glu84 exhibits multiple conformations and Glu84 may act as a proton shuttle because histidine as an active site residue generally plays this function, usually His64. Anions and sulfonamides were shown to bind to Cam [21].

**Delta-class carbonic anhydrase (δ-CA)**

Delta-class CA (δ-CA) has been found in diatoms. In 1997, Morel et al. reported the purified CA (represented by TWCA1) from the marine diatom Thalassiosira weissflogii. The active site of TWCA1 is similar to that of mammalian α-CAs. The diatom carbonic anhydrase do not show significant sequence similarity with other carbonic anhydrases and represent convergent evolution at the molecular level [7, 22, 23]. The zinc has three histidine ligands and a single water molecule, being quite different from the β-CAs of higher plants in which zinc is coordinated by two cysteine thiolates, one histidine, and a water molecule. TWCA1 enzyme is upregulated by low pCO2 [13, 23] and under Zn-limited conditions, the zinc ion at the active site can be substituted by Co(II) in vivo.

**Zeta-class carbonic anhydrase (ζ-CA)**

Zeta-class CA (ζ-CA) is found in the same species Thalassiosira weissflogii. The marine diatom, further growing under low concentration of Zn(II) ions (like in the sea water) and in the presence of Cd2+ or low CO2 pressure, produces this zeta-class CA (represented by CDCA1) [7,13, 23]. ζ-CA occurs exclusively in bacteria, in particular a few chemolithotrophs, marine cyanobacteria containing cso-carboxysomes (So et al., 2004) and diatoms. ζ-CA naturally uses Cd(II) as its catalytic metal ion [22, 23]. CDCA1 consists of three tandem CA repeats (R1-R3), which share 85% identity in their primary sequences [23].

Three-dimensional structural analysis suggests that CDCA1 bears some structural resemblance to β-CA, particularly near the metal ion site. Thus, the two forms may be distantly related, though amino acid sequence has diverged considerably. CDCA1 plays crucial role in CO2 fixation in marine diatom. This CA is involved in the use of cadmium as an algal nutrient in the sea water though cadmium is biologically toxic. Another structural analysis of CDCA1 shows its unique ability to exchange Cd(II) with Zn(II) keeping for both metal ions high catalytic efficiency, while the other classes CAs are severely inhibited by the Cd(II) ions [24].

**IV. REMARKS**

Carbonic anhydrase (CA) is a zinc-containing metallo-enzyme present in virtually every tissues, cell type, subcellular organelles, and in organisms ranging from unicellular microbes to mammals. Presently, CAs are divided to five classes (α, β, γ, δ, ζ), which have no sequence similarity each other and are supposed to be evolutionally independent. CA catalyze a rapid inter-conversion of CO2 and water to HCO3- and protons. The high efficiency of CA is fundamental to many biological processes like photosynthesis, respiration, pH homeostasis, ion transport, water and electrolyte balance, etc. Recently, CA has been considered as an important biocatalyst for CO2 sequestration technology because the accumulation of CO2 is the main cause for global climate change and it is critical to develop technologies that can reduce atmospheric CO2 level [25-27]. This review deals with the classes and mechanisms of several CAs as CO2 capture agents.

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