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Carbonic Anhydrase: Mechanism, Structure and Importance in Higher Plants

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ABSTRACT

Carbonic anhydrase, a zinc-containing metalloenzyme, is an essential enzyme for photosynthesis because of its property to convert CO_2 to HCO_3- reversibly. Besides its role in carboxylation, it plays an important part in stomatal closure in plant leaves. Carbonic anhydrase is the second most abundant enzyme in the chloroplast representing about 20.1% of total soluble protein and is one of the fastest acting enzymes in biological system. Carbon Dioxide being main Constituent of Global warming is directly related to Carbonic anhydrase activity in Plants This review attempts to present a picture of the physiological role of carbonic anhydrase in plants, in the fixation of greenhouse gas like CO_2 from the atmosphere and its role as a biochemical marker for carbon sequestration.

Keywords: Carbonic anhydrase, Photosynthesis, Metalloenzyme, Carbon sequestration, Enzyme

Abbreviations: CO_2: Carbon Dioxide; CAM: Crassulacean Acid Metabolism; CA: Carbonics Anhydrase; HCO_3-: Bicarbonate Ion; RuBisCO: Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase; CCM: CO_2 Concentrating Mechanism

INTRODUCTION

Carbonic anhydrase is a ubiquitous zinc-metalloenzyme important for photosynthesis because of its property to convert CO_2 to HCO_{3-} reversibly. The occurrence of carbonic anhydrase in plants was confirmed by Bradfield in 1947 [1]. The rate of photosynthesis and CO_2 fixation gets directly affected due to any change in the activity of carbonic anhydrase. It is the only carbon metabolism enzyme which shows fluctuations in the activity among different species with varying CO_2 concentration in the environment. Carbonic anhydrase is one of the fastest enzymes known for hydrating 106 molecules of CO_2 per second. The rate of reaction of this enzyme is typically limited by the rate of diffusion of its substrates.

In leaves of C3 plants the enzyme represents 1% to 2% concentration of the total soluble protein, second only to RuBisCO in concentration [2]. About 86% to 95% of the total carbonic anhydrase is found in chloroplasts [3], while the rest is completely restricted to the cytosol of mesophyll cells [4]. A correlation was found by Khan [5] between carbonic anhydrase activity and photosynthetic rate suggesting that the enzyme serves as a biochemical marker for productivity as this helps in carbon sequestration. The activity of Carbonic anhydrase is mainly keeping up by light, Zn and CO_2 concentration [6]. This enzyme provides the supply of CO_2 to the phosphoenolpyruvate carboxylase in C4 and Crassulacean Acid Metabolism (CAM) plants and RuBisCO in Calvin-Benson cycle (C3 plants) (Figure 1). Carbonic anhydrase is concerned in a mixture of physiological processes [7]. The present review, however, lays emphasis on recent aspects of carbonic anhydrase in higher plants.

Mechanism of action of carbonic anhydrase

The carbonic anhydrase active sites contain a zinc ion and all share a similar catalytic activity. In the carbonic anhydrase enzyme, a zinc prosthetic group is coordinated in three positions by histidine side-chains. The active site of the enzyme "E" contains a specific pocket for CO_2 which brings it closer to the hydroxide group attached to the zinc. Therefore, this causes the electron-rich hydroxide ion to attack the CO_2 thereby creating a bicarbonate molecule.

Structure

Carbonic Anhydrase structures are unique and are evolutionary characterized into dissimilar classes, such as alpha

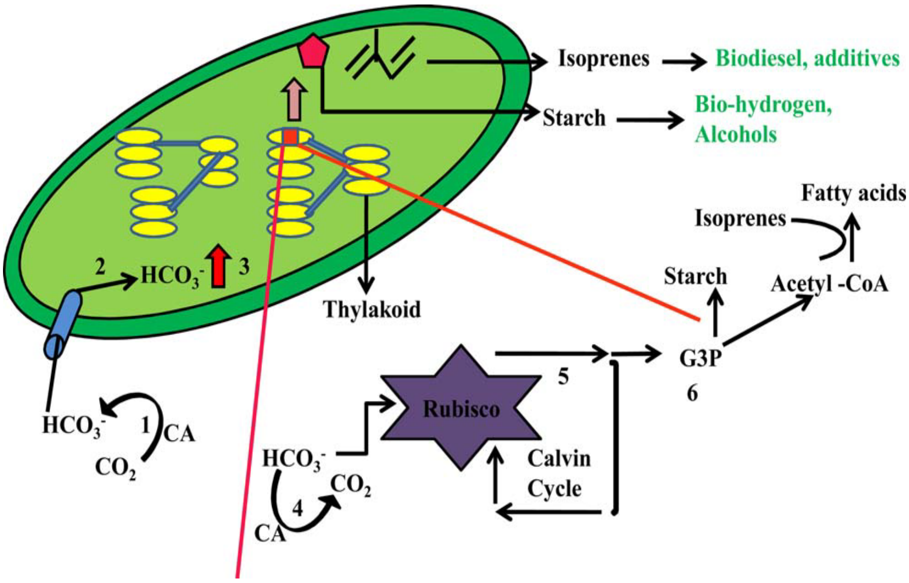

Figure 1: A generic model for the operation of a CCM showing the potential roles of CA in the process

anhydrases (α), beta anhydrases (β), gamma anhydrases (γ) and delta anhydrases (δ). These are found majorly, whereas, there also exist two more class of anhydrases called epsilon anhydrases (ε) and zeta anhydrases (ζ), which are limited to the bacterial kingdom and protists, respectively. The α-class is found throughout the animal kingdom, in the periplasm. The ɑ-carbonic anhydrases were widely identified in vertebrates, algae [8] and in eubacteria as reported by Chirica et al. [9]. The β-carbonic ahydrases are mostly found in higher plants [10]; micro-algae [11]; Eubacteria [12]; Archaebacteria [13]; Cyanobacteria and Fungi [14] .The γ-carbonic anhydrases are discovered in the algae [15]. δ-carbonic anhydrases have only been described in some diatoms. The δ-carbonic anhydrase families appears to be a case of convergent evolution with almost no sequence similarity with the ɑ-, β- or γ-carbonic anhydrase types [16]. ε-carbonic anhydrase is part of the carboxysome shell and has additional domains that serve the function in bacteria [17]. ζ-carbonic anhydrase is limited to maline protists and resembles the β-carbonic anhydrase family, with other metals such as Cd or Co substitute for Zn [18].

Isozymes

Isozymes or isoenzymes are basically enzymes that only differ in the sequences of amino acid but catalyze the same chemical reaction. Carbonic anhydrase has various isozymes that play a great role in various physiological processes of higher plants.

Zinc as a cofactor of beta (β) carbonic anydrase

A co-factor is a non- proteinacious chemical compound or metallic ion that is required for assisting a smooth biochemical transformations and biological activities. "Carbonic anhydrase a metalloenzyme has property to convert CO_2 to HCO_{3-} reversibly". The Zn acts as a cofactor in carbonic anhydrase and plays an important role in carboxylation. The presence of Zn ion (Zn^{2+}) on active site which binds with the enzyme and causes reduction in pKa value and finally, allows the nucleophilic attack on CO_2.

For the activation of catalytic site, water bounds to Zn, which can be ionized to form a hydroxide bridge (carbonic anhydrase), polarized by a base to generate nucleophilic catalysis or displaced by the substrate (Figure 2).

Different gene family of carbonic anydrases in higher plants

Higher plants contain various carbonic anhydrases which shows variations in their distribution in terms of the number of genes present in each family. For example, Arabidopsis has 19 carbonic anhydrase genes (8ɑ-CA, 6β-CA, 5γ-CA) [10], rice also have a similar number of genes present in them [19]. The alpha-carbonic anhydrase (ɑ-CA), beta-carbonic anhydrase (β-CA) and gama-carbonic anhydrase (γ-CA) isoforms share the same general catalytic mechanism in higher plants, despite their structural difference [20].

Enzymatic characterization of carbonic anhydrases in higher plants

Primary protein structures of the various carbonic anhydrases have distinct differences, but their secondary and tertiary

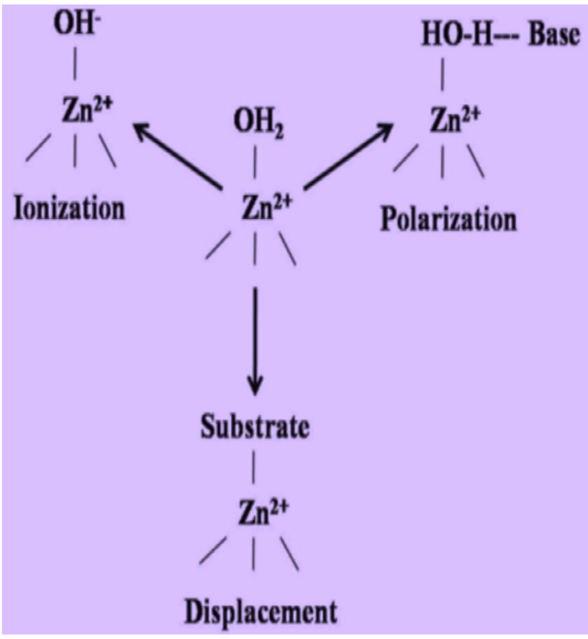

Figure 2: Simplified configuration of zinc reactions with water

structures must be fairly different. Carbonic anhydrases show a prominent similarity in their metal-coordinating sites [21]. As carbonic anhydrase is a metalloenzyme, it requires Zn^{2+} for its activity. But some carbonic anhydrases require cobalt (Co) and cadmium (Cd) for their activity as reported by Morel et al. [22].

The activity of carbonic anhydrase varies in different plants [23]. In tobacco the activity of carbonic anhydrase enzyme follows pattern: leaves>stem>pods [24], also the enzyme has been found in plant roots and fruits [25]. Diverse compartmentalization among organs, tissues, and cellular organelles is being exhibited by Carbonic anhydrase with different physiological roles. Chloroplastic carbonic anhydrase contents in higher plants are saturated while the cytoplasmic carbonic anhydrase contents are inadequate [26].

Higher plants show similarity between cytoplasmic and chloroplastic carbonic anhydrases in kinetic properties, affinity for CO_2 and sensitivity to inhibitors [27]. It has been shown that from the purification of the Solanum cytosolic isoform that it is structurally and biochemically similar to the chloroplastic form, although its monomeric mass is larger [28]. The difference of expression between cytoplasm and chloroplast carbonic anhydrase because difference of promoter region between the two kinds of carbonic anhydrases [29]. The bicarbonate (HCO_3^-) pools are maintained by cytoplastic carbonic anhydrases, and compensate leakage of free CO_2 from the cytoplasm. Chloroplast carbonic anhydrases appear to be associated with other enzymes of the Calvin cycle in a large multienzyme complex [30]. Chloroplast fraction in C3 is associate with the carbonic anhydrase activity, there are at least 10 to 15% of the total activity is cytoplastic [28].

Importance of carbonic anhydrase

- Carbonic anhydrases (CAs; EC 4.2.1.1) is a metalloenzymes with zinc ligands that catalyze the reversible hydration of carbon dioxide to bicarbonate. Among the most efficient enzyme, carbonic anhydrases acts over a broad spectrum of pH. Knowledge of carbonic anhydrase activity in plants has expanded significantly since the first scientific report was published 78 years ago [31]. During this time majority of studies have focused on the key role of carbonic anhydrases in inorganic carbon fixation, respiration and CO_2 transport and electrolyte secretion among other things [32] and it was found that carbonic anhydrases helps in the regulation of chloroplast pH and protect stroma enzymes against denaturation during rapid and drastic changes in light conditions. Beta-carbonic anhydrase involvement in the CO_2 concentrating mechanism (CCM), which increases the content of this gas in close proximity to RuBisCO and consequently decreases photorespiration [26].

- Carbonic anhydrase plays an important role in the mitigation of climate change in response to increasing concentration of CO_2 in the atmosphere.

- So far, a multiplicity of carbonic anhydrase isoforms in Arabidopsis has been found, with eight αCA (AtaCA1-8) and six βCA genes (AtbCA1-6). The physiological role of alpha-carbonic anhydrase (AtαCA1) was widely expressed in the aboveground tissues; it has been shown to be localized to the chloroplast stroma following transport through the secretory pathway and N-glycosylation [33]. Gama- carbonic anhydrase (γ-CAs) play a biological role in mitochondrial physiology [34], including experimentally proven the importance of gama-carbonic anhydrases for complex I assembly [35]. Moreover, γ-CAs is implicated in male sterility [36], plant growth and embryogenesis [37].

•Molecular, biochemical and genetic studies of carbonic anhydrases analyzed in various tissues of many organs and plant species suggested an effect of carbonic anhydrase on a wide range of diverse biological processes, including pH regulation, gas and ion exchange, the provision of bicarbonate for anaplerotic reactions, and fatty acid biosynthesis [38].

•The increased amount of two carbonic anhydrases identified with MALDI-TOF MS could be involved in freezing tolerance during cold acclimation in the purified plasma membrane fraction from Arabidopsis leaves [39].

•There is evidence that during the immune response of plants to pathogens, carbonic anhydrase plays a very important role, acting as a salicylic acid binding protein3 (SABP3) [40]. Experimentally, this dual activity of chloroplastic carbonic anhydrases has been observed in tobacco. Finally, the carbonic anhydrase function in plant immunity has been defined enigmatically as oxidative stress protection.

Role of beta (β) carbonic anhydrase in photosynthesis

Plants need various enzymes for carrying out photosynthesis. The rate of photosynthesis and CO_2 fixation (under limited CO_2 condition) gets directly affected due to any change in activity of carbonic anhydrase. It is the only carbon metabolism enzyme which shows fluctuations in the activity among different species with varying CO_2 concentration in the environment. Its activity gets to decrease rapidly into the air bubbles at low CO_2 emissions while its activity increases at high CO_2 emissions. This has shown a strong co-relation between the activity of carbonic anhydrase and CO_2 concentration, and therefore, this enzyme is critical for carrying the process of photosynthesis.

Functions of beta (β) carbonic anhydrase

The functions of Carbonic anhydrase in photosynthetic CO_2 fixation are:

(1) Beta (β) carbonic anhydrase helps In C3 plants to raise the concentration CO_2 in the chloroplast, which plays a significant role as a substrate by the RuBisCO enzyme, therefore increasing its carbon fixation (carboxylation) rate and hence participates in the reaction by incorporating CO_2 into carbohydrates during photosynthesis where CO_2 can be used only as Carbon and not the carbonic acid or the bicarbonate ion.

(2) Similarly, in C4 plants carbonic anhydrase associates with phosphoenolpyruvate (PEP) carboxylase (PEPC) provides continuous supply of HCO_{3-} at the site of carbon fixation [41]. CO_2 hydration to form HCO_{3-} is used as a substrate by phosphoenolpyruvate carboxylase (PEPC) in C4 and CAM plants. This is supported by the fact that carbonic anhydrase of C4 leaves is entirely confined to the cytosol of mesophyll cells while bundle sheath cells contain very low or no carbonic anhydrase activity [4]. Thus, carbonic anhydrase in mesophyll cells rapidly converts diffusing atmospheric CO_2 to HCO_{3-} at rates that are compatible with those for photosynthesis. In C4 plants (Figure 3), Carbonic anhydrase catalyzes the first critical step of C4 photosynthesis, the hydration of CO_2 to bicarbonate, which PEPC uses as the substrate for carboxylation of PEP to oxaloacetate in the cytosol of mesophyll cells [29]. The inorganic carbon substrate HCO_{3-} of this enzyme was recognized as being supplied by carbonic anhydrase [27].

(3)The carbonic anhydrase helps in diffusion of CO_2 through the plasma membrane and the chloroplast.

(4)There are various carboxylation enzymes which help in photosynthesis but among all Carbonic anhydrase plays a terrific role in photosynthesis because it has property to convert reversibly CO_2 to HCO_{3-}. Due to participation of active CO_2 transport through the plasma of the membrane by converting CO_2 into HCO_{3-} is how Carbon enters the cell [42]. Due to the increase in activity of this enzyme, the rate of fixation of carbon in plants also increases. Carbonic anhydrase holds a special significance for global forests because it sequesters about 90% of terrestrial carbon pool [43], which can lower the impact of growing global warming [44]. The above functions make carbonic anhydrase a biochemical marker for carbon sequestration which helps in the mitigation of global warming.

Role of beta (β) carbonic anhydrase in stomatal closure

The stomata are a minute opening or pore through which exchange of gases takes place. They are mostly found on under-surface of plant leaves. The enzyme carbonic anhydrase plays a great role in stomatal closure as explained below:

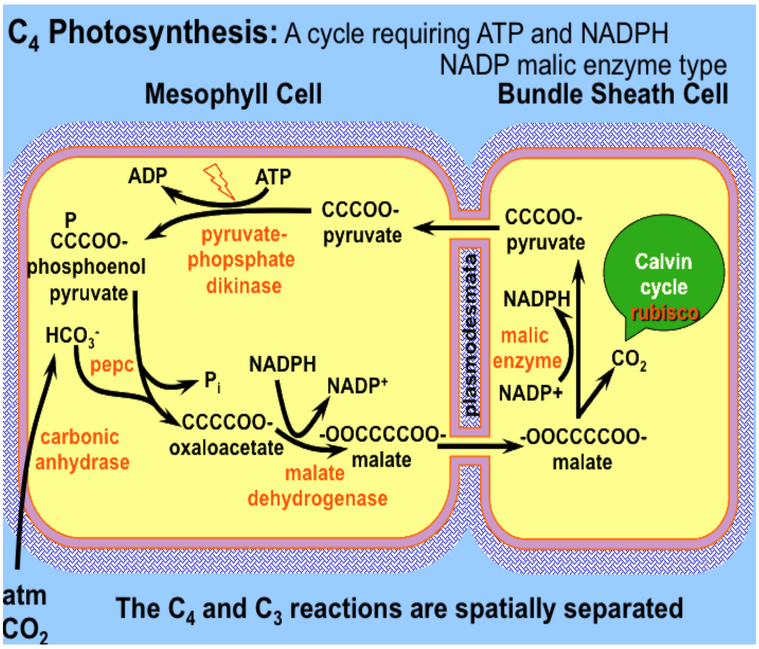

Figure 3: Simplified diagram of the C4 and C3 reactions

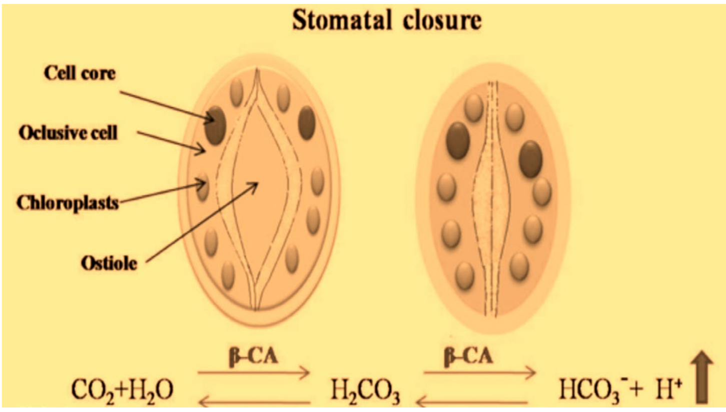

Figure 4: Simplified diagram of the central reaction for stomatal closure

During the dark phase of photosynthesis, the amount of CO_2 fixation gets depleted while the process of respiration continues which leads to increase in CO_2 level by shifting the central reaction (Figure 4) towards the right which results in a decrease of pH. The decreasing pH causes the inactivation of various enzymes such as amylase, which stops the mechanism of hydrolysis of starch and glucose, resulting in a decrease of the osmotic gradient in occlusive cells causing loss of water and closure of ostiole [45].

CONCLUSION

Carbonic anhydrase plays critical role in all photosynthetic CO_2 concentrating mechanisms. Carbon dioxide being main culprit behind the global warming is cause of concern for present generation as well as for the future generations. Therefore, need of hour is to think of the present and sustaining of the future. This review attempts to present a picture of the physiological role of carbonic anhydrase in plants, in the fixation of greenhouse gas like CO_2 from the atmosphere and its role as biochemical marker for carbon sequestration.

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