Enzymatic Characteristics of Higher Plant Carbonic Anhydrase and Its Role in Photosynthesis

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Abstract

Carbonic anhydrase, a metalloenzyme which catalyses the reversible interconversion of HCO_3^- and CO_2, is a major protein component of higher plant tissues. It is now shown that DNA sequence and encoded proteins for the different α, β, γ, δ, ε, and ζ forms of carbonic anhydrases are present in living organisms. While there are no sequence homologies between the different type of carbonic anhydrases, all the α, β, and γ carbonic anhydrase gene family enzymes in higher plants catalyze the same chemical reaction. Its specific function is generally assumed to convert CO_2 to HCO_3^- for the phosphoenolpyruvate carboxylase reaction and convert HCO_3^- to CO_2 for the ribulose-1,5-bisphosphate carboxylase reaction in photosynthesis. Moreover, carbonic anhydrase activity in guard cells is required for CO_2-mediated stomatal regulation and carbonic anhydrases may provide an approach for plant alternatively protection against stress conditions. Recent studies on carbonic anhydrases described in this manuscript include the characterization and gene family of carbonic anhydrases, physiological function of higher plant CAs, and gene engineerings about higher plant CAs.

Keywords: higher plant carbonic anhydrase, enzymatic characteristic, photosynthetic role

1. Introduction

It is approved that yield potentials of cereal crops cannot further be increased by addition of chemical fertilizer, and photosynthesis increasing could be the major avenue to increase crop yield in next 50 years (Surridge, 2002; Reynolds et al., 2009). Plant photosynthetic organisms have developed methods for the acquisition of inorganic carbon (Ci) to aid ribulose-1,5-bisphosphate carboxylase (RuBisco) and to enhance CO_2 fixation. Carbonic anhydrases often play important roles in this process.

Carbonic anhydrase (CA, EC: 4.2.1.1), a ubiquitous zinc metalloenzyme among living organisms, catalyses the reversible interconversion with very high catalysis rates reaching $10^6 \, s^{-1}$: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$ (Raven, 1995). After CA was first discovered in red blood cells (Meldrum & Roughton, 1933), the enzyme has been found in animals, plants, archaebacteria and eubacteria. Besides carboxylase and decarboxylase rates are influenced by CAs, the processes of pH fluctuations, ion regulation, ion exchange and inorganic carbon diffusion are also influenced by CAs (Raven, 1995).

CAs has been characterized at catalytic, cellular and tissue levels in all life forms. Based on their amino acid sequences, living organism CAs can be categorized into six independent families named as α, β, γ, δ, ε, and ζ (Moroney, Bartlett, & Samuelsson, 2001). CA isozymes are localized in plasmalemma (Badger and Price, 1994), chloroplast (Husic & Markus, 1994), mitochondria (Eriksson et al., 1996), and cytoplasm (Hiltonen et al., 1998).

There is a high degree of structural homology among CAs from different higher plants. In plant leaves, CA concentration is 1-20% of total soluble protein and is next only to RuBisco in chloroplast (Fett & Coleman, 1994). Plant CAs facilitate CO_2 supply to phosphoenolpyruvate carboxylase (PEPC) in C_4 and CAM plants, and facilitate CO_2 supply to RuBisco in C_3 plants (Tiwari et al., 2005). The higher plants have developed their versions of photosynthetic CO_2 concentrating mechanisms (CCMs) to aid Rubisco in CO_2 capture. An important aspect of CCMs is the critical roles of CAs play in the overall process, participating in the interconversion of CO_2 and HCO_3^- both inside and outside the cell. The capacity to restrict the photorespiration loss of carbon could be one of important trait to develop plants with efficient photosynthetic capacity and could be a good option to

enhance crop productivity. So, it would be one important research subject to enhance the CO_2 assimilation intensity of C_4 pathway in C_3 plants by the artificially regulation of CA expression.

2. Enzymatic Characterization of Higher Plant CAs

2.1 General Enzymatic Characterization of Higher Plant CAs

There are distinct differences in primary protein structures of the various CAs, and their secondary and tertiary structures must be somewhat different. CAs shows a striking similarity in their metal-coordinating sites (Kimber & Pai, 2000). CA is a metalloenzyme requiring Zn^{2+} for its activity, besides, some CAs containing Co and Cd were also published (Price & Morel, 1990; Morel et al., 1994). CA exhibits diverse compartmentalization among organs, tissues, and cellular organelles commensurate with different physiological roles. The CA activities in different plants are varies (Wu et al., 2006). The CA enzyme activity follows pattern in tobacco: leaves>stem>pods (Majeau, Arnoldo, & Coleman, 1994), and the enzyme has been found in plant roots and fruits (Diamantopoulos et al., 2013).

There are similarity between cytoplasm CA and chloroplast CA in higher plants including CA kinetic properties, affinity for CO_2 and sensitivity to inhibitors (Hatch & Burnell, 1990). It is shown 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 (Rumeau et al., 1996). The difference of promoter region between cytoplasm and chloroplast CA induces the difference of expression between the two kinds of CAs (Badger & Price, 1994). The cytoplastic CAs, maintains HCO_3^- pools and compensates leakage of free CO_2 from the cytoplasm. Chloroplast CAs appear to be associated with other enzymes of the Calvin cycle in a large multienzyme complex (Jebanathirajah & Coleman, 1998). Although the bulk of the CA activity is associated with the chloroplast fraction in C_3 plants, there are at least 10 to 15 % of the total activity is cytoplastic (Rumeau et al., 1996). Chloroplast CA contents in higher plants are saturated and cytoplasm CA contents are inadequate (Badger, 2003), and it is important to study the function and application of cytoplasm CAs in higher plant resistance because the CA activity was up-regulated by salts and osmotic stresses (Yu et al., 2007).

2.2 Different Gene Family of CAs in Higher Plants

With regard to higher plants, the distribution of the number of CA genes in each gene family also varies. Arabidopsis has 19 CA genes (8α-CA, 6β-CA, 5γ-CA) (Arabidopsis Genome Initiative, 2000), and rice (Yuan et al., 2005) have a similar number. Despite their structural difference, the α-CA, β-CA and γ-CA isoforms in higher plants share the same general catalytic mechanism (Lindskog, 1997).

The α-CAs were widely identified in vertebrates (Meldrum & Roughton, 1933), algae (Fujiwara, Ishida, & Tsuzuki, 1996), higher plants (Arabidopsis Genome Initiative, 2000), and eubacteria (Chirica, Elleby, & Lindskog, 2001). Most a-CAs is monomers of about 30 kDa with at least three histidines. The Arabidopsis α-CA molecular structure is dominated by antiparallel β- sheets forming a spherical molecule with two halves and the active site is located in a funnel shaped crater with the zinc atom (Moroney, Bartlett, & Samuelsson, 2001). It is shown from EST sequence information in Arabidopsis that there are three α-CA coding sequences are expressed.

The β-CAs are found in cyanobacteria (Fukuzawa et al., 1992), micro-algae (Eriksson et al., 1996), eubacteria (Hewett & Tashian, 1996), fungi (Gotz, Gnann, & Zimmermann, 1999), archaebacteria (Smith & Ferry, 2000), and higher plants (Arabidopsis Genome Initiative, 2000). This CA family does not be represented in any vertebrate genome. plant β-CAs are oligomers ranging in size from 42 to 220 kDa depending on the species. It is shown that all of the β-CA coding sequences in Arabidopsis are expressed. The similarity between the different dicot and monocot β-CAs is about 70%, whereas the similarity within each group is up to 80% (Hewett & Tashian, 1996). The β-CA in *F. Bidentis* are expressed in roots, leaves and flowers (Ludwig, 2012).

The γ-CAs are discovered in the archaebacterium (Alber & Ferry, 1994), algae (Klodmann et al., 2010), eubacteria and plants (Newman, 1994). Recent work has indicated that γ- or γ-like CAs were part of Complex I of the mitochondrial electron transport chain in plants and algae, and plant γ-CA might play roles in CO_2 cycles of photorespiration, but chloroplastic γ-CA has not yet been reported (Martin et al., 2009).

δ-CAs have only been described in some diatoms. The δ-CA families appears to be a case of convergent evolution with almost no sequence similarity with the α-, β-, or γ-CA types (Roberts, Lane, & Morel, 1997). The ε-CA family is limited to bacteria containing a-type carboxysomes and has not been found in eukaryotes. ε-CAs is part of the carboxysome shell and has additional domains that serve the function in bacteria (Tanaka et al., 2008). ζ-CA is limited to maline protists and resembles the β-CA family, with other metals such as Cd or Co substitute for Zn (Lane et al., 2005).

3. Physiological Functions in Photosynthesis of Higher Plant CAs

As CA appears to be found in all organisms studied, the enzyme would seem to indicate important physiological functions. The connection between CA and photosynthesis is perhaps the most widely understood roles of plant CA. This role is most important because the uncatalyzed interconversion between CO_2 and HCO_3^- is 10^4 times slower compared with the flux of CO_2 in photosynthesis (Badger & Price, 1994). CA is the only enzyme of photosynthetic carbon metabolism, and any change in CA activity directly affects the rate of photosynthetic CO_2 fixation under CO_2 limiting conditions.

General physiological roles of plant CA is given below:

One role is to supply CO_2 or HCO_3^- for some metabolic reactions, such as HCO_3^- for the PEPC reaction in C_4 photosynthesis and CO_2 for the Rubsico reaction in photosynthesis, requiring the intervention role of CA enzyme. Even within the C_3 mesophyll cell cytosol, CA may be required for the provision of HCO_3^- for PEPC which plays a compensating role. Besides Rubisco and PEPC, a number of other biological reactions require either CO_2 or HCO_3^- which can be supplied by CA. Take the acetyl CoA carboxylase for example, which catalyzes the initial step in fatty acid biosynthesis and uses only CO_2 (Vats, Kumar, & Ahuja, 2011).

At the same time, CA is the delivery of Ci to the correct location within the cell and is the reduction of CO_2 leakage (Braun & Zabaleta, 2007). CO_2 which enter into cells from the external gaseous environment can pass through biological membranes and quickly leak out of the cell. Plants may deal with this problem by converting CO_2 generated by the cell to HCO_3^-. The ectopic expression of CA activity in the cytosol of bundle-sheath cells of *Flaveria bidentis* leaded to an increase of CO_2 leakage in bundle-sheath (Ludwig et al., 1998). It showed that the absence of CA from the bundle sheath cells may be limit the conversion of accumulated CO_2 to HCO_3^- and this may limit the reduction of CO_2 leakage.

The next role of CA is in the CO_2 signaling pathway. It is suggest that low chloroplastic CA plants were compensating by increasing stomatal conductance to improve CO_2 entry into the leaf (Williams, Flanagan, & Coleman, 1996). Hu et al. (2010) suggested that guard cell expression of mammalian α CAII complemented the reduced sensitivity of ca1 ca4 mutant plants, showing that CA-mediated catalysis is an important mechanism for CO_2 induced stomatal closure.

Moreover, it has also been suggested that CA plays indirect roles in photosynthesis. CA activity has been hypothesized that it is possibly required for regulating chloroplast pH during rapid changes in light intensity and photosynthetic electron transport (Stemler, 1997). Ferreira et al. (2008) study demonstrated that βCA1 plays a significant role in seedling establishment in Arabidopsis. Besides, CA is involved in a variety of biological processes including pH regulation, ion exchange, respiration, biosynthesis, antioxidation and so on (Badger & Price, 1994; Moroney et al., 2001; Tetu et al., 2007).

4. The Application of Gene Engineering About Higher Plant CAs

Biosynthesis of CA may be regulated by some environmental factors such as light, salt content, osmetic stress, photon flux density, availability of Zn and CO_2 concentration (Karlsson et al., 1998). These results suggest that the expression of CA was related to environmental stresses. Transgenic Arabidopsis over-expressing OsCA1 had a greater salt tolerance at the seedling stage than wild-type plants and the CA activity was up-regulated by salts and osmotic stresses (Yu et al., 2007). The possible mechanism of over-expressing OsCA1 had a greater salt tolerance was that plants might rely on CA to catalyze HCO_3^- rapidly transformed to CO_2 in physiological drought condition caused by salt stress, to meet the physiology needs of the plants. Inhibition of CA activity in ethoxyzolamide-infiltrated C_3 plant leaf pieces resulted in an 80-90 % inhibition of photosynthesis at low CO_2 concentrations, and indicated an essential role for CA (Badger & Pfanz, 1995). Consequently, the regulation function of CA would play important role under stress conditions.

Over-expression of two specific isozymes, βCA1 or βCA4 considerably increased instantaneous water use efficiency in Arabidopsis thaliana plants indicating that βCA expression levels are not saturated in wild-type guard cells (Hu et al., 2010). All overexpression lines showed enhanced fresh weight from excised leaves compared with wild-type plants. Therefore, guard cell-targeted overexpression of βCA was sufficient to modulate CO_2 regulation of stomatal conductance and might provide an approach for improving the water use efficiency of C_3 plants.

5. Conclusions

In general, the studies achieve the efforts to reengineer crops to improve its photosynthetic efficiency under such conditions as CO_2 availability is limited. In most of the discussions on biotechnological approaches aiming to transfer C_4-like features into C_3 plants, CA has received much less attention though CAs play important roles in

photosynthesis process (Häusler et al., 2002). CAs take part in the adaptation to low CO_2 concentration in C_3 plants and it is one important research subject that how to enhance the CO_2 assimilation intensity of C_4 pathway in C_3 plants by artificially modifying the CA promoters. It is important that the study on the differ of CAs (especialy β-CA) between C_3 plants and C_4 plants, and the application of plant CA may contributed to enhance crop photosynthetic efficiency and increase crop yields.

Some study data suggest that although increased CA levels occur, they alone are unable to maintain CO_2 concentrations within the chloroplast (Li et al., 2010; Vats et al., 2011). It is possible that an appropriate CA/Rubisco ratio would be more effective in maintaining chloroplast CO_2 levels. Moreover, identification of the molecular mechanisms of CA-participant CO_2-mediated stomatal regulation is fundamental to understanding the regulation of gas exchange between plants and the atmosphere. Meanwhile, there is a striking correlation between stomatal conductance and CO_2 assimilation rate that differs between C_3 and C_4 plants. It would be interesting to determine if similar regulatory cis elements are present in genomic promoter sequences of both CA and C_4 PEPC isoforms. The mechanism behind these relationships is not yet understood. So, it is assumed that although we believe that plant CAs are important for biological functions, more work is required to resolve the specific field of CAs in photosynthesis improving and water use efficiency controlling.

Acknowledgements

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