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Review

The multifunctional face of plant carbonic anhydrase

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ABSTRACT

Although most studies on the ubiquitous enzyme carbonic anhydrase (CA) have indicated its significant role in plants to facilitate the diffusion of CO_2 to the site of inorganic carbon fixation, it is becoming increasingly likely that carbonic anhydrase isoforms also have diverse unexplored functions in plant cells. This review lays emphasis on additional roles of CA associated with many physiological, biochemical and structural changes in plant metabolism. The presented findings have revealed essential functions of CA isoforms in plant adjustment to both abiotic and biotic agents and developmental stimuli. However, sometimes it is difficult to separate the non-photosynthetic from the photosynthetic-related role of CAs during post-stress impaired metabolism, and the preventive CA outcome might be due to the effect of these enzymes on improvement of photosynthetic capacity. Finally, taking into account the experimental evidence, the direct and indirect functional roles of CAs in mitigating negative effects of environmental conditions are presented.

Contents

1. Introduction

Carbonic anhydrases (CAs; EC 4.2.1.1) are metalloenzymes mostly containing zinc ligands that catalyze the reversible hydration of carbon dioxide to bicarbonate. Carbonic anhydrases are among the most efficient enzymes, and act over a very broad spectrum of acidity. The catalytic rate of some CA isoforms exceeds 1×10^6 s^{-1} (Chegwidden and Carter, 2000). At least five (Yu et al., 2007) to six (Kaul et al., 2011) genetically distant families (α, β, γ, δ, ε and ζ) of CA isoforms exist and are selectively distributed among living organisms. The representatives of α-CAs family are the best known, found mainly in animals (including the 16 active human isoforms) and also discovered in higher plants, algae and bacteria (Esbaugh and Tufts, 2006; Capasso and Supuran, 2015; DiMario et al., 2017). The β-CAs dominate in photosynthetic organisms and in some invertebrates but they also were found in representatives of bacteria and fungi (e.g. Moroney et al., 2001; Innocenti et al., 2008; Joseph et al., 2010; Syrjänen et al., 2010). The γ-CAs have been detected in archaea and some bacteria and recently have been found in plants (Parisi et al., 2004). Finally, the δ-CAs and ε-CAs have been mainly detected in marine diatoms (Burnell et al., 1990; Hewett-Emmett and Tashian, 1996; Lane et al., 2005; Xu et al., 2008; Gilmour, 2010; Ludwig, 2012; Wang et al., 2012; Fromm et al., 2016a); however, ε-CAs were identified also in chemolithoautotrophic bacteria and marine cyanobacteria (So et al.,

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2004). Interestingly, the latter class of CAs naturally uses cadmium as its catalytic metal ion (Lane et al., 2005). Crystallographic studies revealed that α-CAs are mainly monomeric or multimeric, and the other classes belong to dimeric (δ-CAs and ε-CAs) through trimeric (γ-CAs and ζ- CAs) to different oligomeric (β-CAs) states (Liljas et al., 1972; Peters et al., 2008; Zimmerman and Ferry, 2008; Rowlett, 2010). Moreover, these authors document that despite structural differences between α-CAs and β-CAs or γ-CAs a similar mechanism is involved in the two-step reversible hydration of CO_2 to bicarbonate. Briefly, this ping-pong mechanism is activated by the nucleophilic attack of a zinc-bound hydroxyl ion on CO_2, which results in a metal-bound bicarbonate. The product is replaced by a H_2O molecule, and the enzyme is regenerated when a proton is transferred to the bulk solution via a histidine residue. The histidine was firstly postulated as a proton shuttle in α-CAs by Steiner et al. (1975).

Our knowledge of carbonic anhydrase activity in plants has expanded significantly since the first scientific report was published 78 years ago (Neish, 1939). During this time an overwhelming majority of studies have focused on the key role of CAs in inorganic carbon fixation, respiration and CO_2 transport and electrolyte secretion among other things (Burnell et al., 1990; Badger and Price, 1994; Moroney et al., 2001). It was found that CAs participate in the regulation of chloroplast pH and protect stroma enzymes against denaturation during rapid and drastic changes in light conditions (Reed and Graham, 1981). The most extensive study was devoted to β-CA involvement in the CO_2 concentrating mechanism (CCM), which increases the content of this gas in close proximity to Rubisco and consequently decreases photorespiration (Badger, 2003). Comprehensive coverage of older and recent research regarding the structure and biochemistry of CAs can be found in a number of interesting review articles (Burnell, 2000; Kimber and Pai, 2000; Tripp et al., 2001; Tiwari et al., 2005; Gilmour, 2010; Ludwig, 2012; Wei-Hong et al., 2014; Fromm et al., 2016a).

Carbonic anhydrase is present in a variety of plant tissues. In C_4 plants, CA activity is mainly located in the cytosol of mesophyll cells, where it catalyzes hydration of atmospheric CO_2 to bicarbonate for phosphoenolpyruvate carboxylase (PEPC) (Hatch and Burnell, 1990). In C_3 plant mesophyll, CA is located mainly in the chloroplast stroma fine-tuned with Rubisco (ribulose-1,5-bisphosphate carboxylase) and in the cytosol (e.g. Fett and Coleman, 1994; Rumeau et al., 1996; Burnell, 2000; Wei-Hong et al., 2014; DiMario et al., 2016).

So far, a multiplicity of CA isoforms in *Arabidopsis* has been found, with eight αCA (AtaCA1-8) and six βCA genes (AtbCA1-6) (Villarejo et al., 2005; Fabre et al., 2007; Burén et al., 2011). The experimental approach with green fluorescent reporter protein genes fused with cDNA sequences coding for βCAs showed that βCAs were targeted to specific subcellular compartments: βCA1 and βCA5 were present in the chloroplast, βCA2 and βCA3 in the cytosol, βCA4 in the plasma membrane and βCA6 in the mitochondria. The physiological role of αCAs is very puzzling. 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 (Villarejo et al., 2005). The expression of AtαCA2 and AtαCA3 was restricted to conducting organs and flowers, suggesting rather specific functions for these two AtαCAs; however, mRNA of both AtαCA2 and AtαCA3 were detected also in *Arabidopsis* leaves at low CO_2 concentration, implicating a specific function under CO_2 limitation (Fabre et al., 2007). In turn, γ-CAs play biological role in mitochondrial physiology (Perales et al., 2005), including experimentally proved importance of γ-CAs for complex I assembly (Meyer et al., 2011). Moreover, γ-CAs are implicated in male sterility (Villarreal et al., 2009) plant growth and embryogenesis (Wang et al., 2012; Fromm et al., 2016b; Córdoba et al., 2016).

While taking into account and not undermining the principal engagement of CA in photosynthesis, our report focuses on the implications of CAs in modulating plant adjustment to various environmental and developmental conditions.

2. Carbonic anhydrase under abiotic factors and developmental stimuli

In C_4 and Crassulacean acid metabolism (CAM) plants, the role of carbonic anhydrase as a potent donor of bicarbonate for PEPC to produce oxaloacetic acid is well defined and documented (e.g. Jenkins et al., 1989; Badger and Price, 1994; Hatch and Burnell, 1990; Ludwig, 2012). The organ-specific expression patterns of the β-CAs gene family for cytosolic and chloroplastic isoforms in *Flaveria bidentis* (C4 plant) were previously analyzed by Tetu et al. (2007). Three distinct β-CAs were identified: CA1, which has non-photosynthetic functions, including lipid biosynthesis and antioxidant activity; CA2, which provides bicarbonate to various metabolic pathways, and is present in the cytosol of cells in both photosynthetic and non-green tissues; and CA3, prevalent in the cytosol of mesophyll cells and providing a substrate for PEPC. In turn, it may be expected that the main functions of CAs in C_3 photosynthesis are to facilitate the equilibration of the inorganic carbon pool and the transfer of CO_2 across the chloroplast membrane as well as the maintenance of CO_2 supply for Rubisco in the stroma (Burnell, 2000). Genetic evidence for the existence of thylakoid-associated CA (tCA) localized near photosystem II (PSII) of *Chlamydomonas reinhardtii* (Karlsson et al., 1998) and pea (Moskvin et al., 2000) revealed a contribution of tCA to the light intensity-dependent removal of overproduced protons from the lumen. A complementary proposal is that PSII-associated CA is involved in preventing damage of the water-oxidizing complex by the stabilization via bicarbonate of the manganese cluster (Villarejo et al., 2002). However, in the spectrum of other findings the emphasized role of CA in C_3 photosynthesis is rather unclear and incomplete.

Molecular, biochemical and genetic studies of CAs analyzed in various tissues of many organs and plant species suggested an effect of CA 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 (Chollet et al., 1996; Hoang and Chapman, 2002; Tetu et al., 2007; Wei-Hong et al., 2014).

Many years ago, various experimental approaches with CA inhibitors (Swader and Jacobson, 1972) and with limiting zinc nutrition revealed a decrease in CA activity with no significant reduction of the photosynthetic rate in C_3 plants (Edwards and Mohamed, 1973; Randall and Bouma, 1973). In addition, no significant impact on net CO_2 assimilation was observed in antisense CA transgenic tobacco with a 99% reduction of CA activity (Price et al., 1994; Majeau et al., 1994; Williams et al., 1996). However, in CA antisense plants stomatal conductance and susceptibility to water stress increased in response to the decline of CA activity (Majeau et al., 1994). Subsequently, it was found that in *Arabidopsis Atβ-CA1* knock-out mutants and in plants with an antisense construct targeting *Atβ-CA1* mRNA, CA activity is not engaged in the photosynthesis of the mature leaves, in contrast to the cotyledons (Ferreira et al., 2008). However, using pharmacological approach Riazunnisa et al. (2006) suggested photorespiration as a significant source of CO_2 for photosynthesis in pea mesophyll protoplasts at limiting CO_2 and indicated CA as an essential link to concentrate or retain CO_2 in pea mesophyll cells.

Paradoxically, the level of CA activity is similar in C_3, C_4 and CAM