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Carbonic Anhydrase Genes Network: Key Role Players in pH Flux and Abiotic Stress Tolerance

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ABSTRACT

The persistent change in climatic conditions increasing the exiting stress conditions for plants resulting the internal fight to overcome stress challenges. Under normal condition CO_2 fixed in cells enters either c3 or c4 cycle. Whereas, any imbalance/stress leads to the accumulation of carbonic acid and hydrogen peroxide; even, at extreme condition cyanide accumulation leads to cell death. Identification of interacting molecules by network analyses will help in translational research and network rewiring in developing adaptations to abiotic stress conditions. In this report, we tried to elucidate the existing carbonic anhydrase network of Glycine max and its relationship with abiotic stress condition.

Keywords: Abiotic stress tolerance, Carbonic anhydrase, Genes network, pH flux

INTRODUCTION

The Carbonic anhydrases (CAs) are ubiquitous metalloenzymes existingmanifoldin any genome. These enzymes are encoded by three distinct evolutionary unrelated gene families: (i) α-CAs (in vertebrates, bacteria, algae and cytoplasm of green plants), (ii) β-CAs (predominantly in bacteria, algae and chloroplasts of both monocots and dicotyledons) and (iii) γ-CAs (mainly in archae and some bacteria) (Chirica *et al*., 2001, Hewett, 2000, Smith and Ferry , 2000, Supuran *et al*.,2003 and Supuran and Scozzafava, 2002). There are 14 α-CA isozymes or CA- related proteins (CARPs) in arabidopsis with very diverse subcellular localizations and tissue distributions. There are various cytosolic forms (CAs I-III, CA VII), four membrane-bound isozymes (CA IV, CAIX, CA XII and CA XIV), one mitochondrial form (CA V) and a secreted CA isozyme (CAVI) (Supuran *et al*., 2003 and Supuran and Scozzafava, 2002). These enzymes catalyze a very simple physiological reaction, the interconversion of the carbon dioxide and the bicarbonate ion, and are thus involved in important physiological processes associated with respiration and transport of CO_2 /bicarbonate between metabolizing tissues, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues and organs, biosynthetic reactions (such as gluconeogenesis and lipid and urea synthesis), calcification and many other physiological or pathological processes. Already we have predicted the mechanism of acid tolerance in cells its relationship in abiotic stress tolerance and pH flux (Yasin *et al*., 2016 and Yasin, 2015). Inhibitors for applied research were designed using many of these isozymes as important targets.

Carbonic anhydrase: Structure

Carbonic anhydrase (CA; carbonate hydro-lyase) is a zinc-containing; zn dependent enzyme that catalyzes the reversible hydration of carbon dioxide:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

In human system, the enzyme is a target for drugs, such as acetazolamide, methazolamide, and dichlorphenamide, for the treatment of glaucoma. α, β, and $\gamma$are three evolutionary distinct CA families. All known CAs from the animal kingdom are of the α type, whereas plants contain all these types. Of seven types of mammalian CA isozymes are there with different tissue distributions and intracellular locations, CA I–VII. Crystal structures of human CA I and II, bovine CA III, and murine CA V have been determined. Many of the CA present in plants don't have an experimentally validated structure and hence, it possible to predict only by homology based modelling using available animal CA protein structures. All of them have the same tertiary fold, with a central 10-stranded β-sheet as the dominating secondary structure element. The zinc ion is located in a cone-shaped cavity and coordinated to three histidyl residues and a solvent molecule. Inhibitors bind at or near the metal center guided by a hydrogen-bonded system comprising Glu-106 and Thr-199. The catalytic mechanism of CA II has been studied in particular detail. It involves an attack of zinc-bound OH− on a CO_2 molecule loosely bound in a hydrophobic pocket. The resulting zinc-coordinated HCO_3^- ion is displaced from the metal ion by H_2O. The rate-limiting step is an intramolecular proton transfer from the zinc-bound water molecule to His-64, which serves as a proton shuttle between the metal center and buffer molecules in the reaction medium (Lindskog and Coleman, 1973).

Carbonic anhydrase inhibitors

Two main classes of carbonic anhydrase inhibitors (CAIs) are known: the metal-complexing anions and the unsubstituted sulfonamides, which bind Zn (II) ion of the enzyme either by

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substituting the nonprotein zinc ligand or add to the metal coordination sphere creating trigonal-bipyramidal species. Sulfonamides, the most important CAIs, bind in a tetrahedral geometry of the Zn (II) ion in a deprotonated state, with the nitrogen atom of the sulfonamide moiety coordinated to Zn (II) and a stretched network of hydrogen bonds involving the residues Thr 199 and Glu 106, also participating in anchoring the inhibitor molecule to the metal ion (Steiner *et al*., 1975). The aromatic/heterocyclic part of the inhibitor (R) interacts with hydrophilic and hydrophobic residues of the cavity. Anions might bind either in tetrahedral geometry of the metal ion or as trigonal-bipyramidal adducts, such as the thiocyanate adduct.

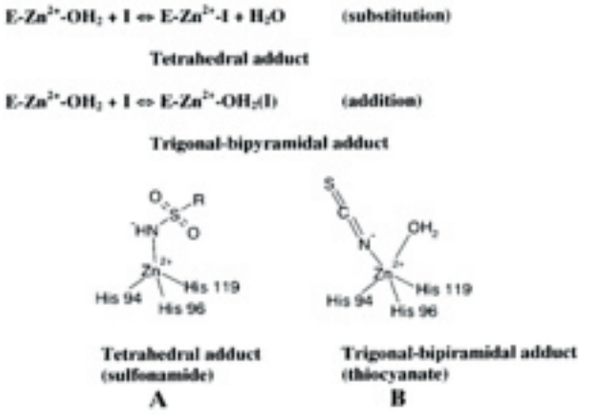


Fig 1: CA inhibition mechanism by sulfonamide, thiocyanate and anionic inhibitors

Redrawn and adapted from Bertini *et al*., 1982 and Lindskog and Coleman, 1973.

The classical CA inhibitors (CAIs) are the primary sulfonamides, RSO2NH2, which are in clinical use for more than 50 years as diuretics and systemically acting antiglaucoma drugs.In fact, there are around 30 clinically used drugs (or agents in clinical development) belonging to the sulfonamide or sulfamate class, of types 1–25, which show significant CA inhibitory activity. Failure in fixing carbon trapped in the form of carbonic acid under stress condition will lead to decline in pH leading to cell death (Yasin *et al*., 2016). In addition to the established roles of these CAIs in animal system, there is no such established corrective measures reported for plants unless there are adaptations (Chaves *et al*., 2009 and Yasin *et al*., 2016). However, critical barriers to the design of CAIs as therapeutic agents are related to the high number of isoforms in humans (16 CAs, of which 13 have catalytic activity); their rather diffuse localization in many tissues/organs, and the lack of isozyme selectivity of the presently available inhibitors of the sulfonamide/sulfamate type. Such kind of studies are lacking in plants.

Mechanism

An inverse relationship between the pH dependencies of the rates of hydration of CO_2 and dehydration of HCO_3^- by carbonic anhydrase is a direct consequence of the thermodynamic equilibrium between CO_2 and $HCO3^-$ and independent of any assumptions about the catalytic mechanism. It is further known that proposed mechanisms for carbonic anhydrase involving $HCO3^-$ as the substrate in the dehydration reaction and a proton transfer reaction,

$$EH^+ \rightleftharpoons E + H^+$$

as an obligatory step during catalysis obey the rule of microscopic reversibility (Supuran *et al*., 2004 and Casini *et al*., 2003). This includes mechanisms in which the proton dissociation is from a zinc-coordinated water molecule. Such mechanisms can be in accord with the observed rapid turnover rates of the enzyme, since rapid proton exchange can occur with the buffer components,

$$EH^+ \rightleftharpoons E + H{+}BH^+.$$

Mechanisms in which H_2CO_3 is the substrate in dehydration avoid the proton-transfer step, but require that H_2CO_3 combines with enzyme more rapidly than in a diffusion-controlled reaction (Bertini *et al*., 1982).

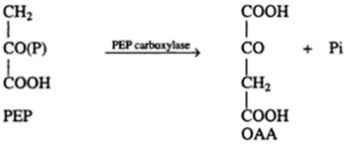
Mechanism of breakdown of carbonic acid in plants

CO_2 enters water through interface with the atmosphere and the biological processes of organic carbon digestion and photosynthesis. Gaseous carbon dioxide, CO_2, reacts with water forming carbonic acid H_2CO_3 (aq). Carbonic acid may lose protons to form bicarbonate HCO_3^- and carbonate CO_3^{2-}. In this case the proton is liberated to water, decreasing pH. The complex chemical equilibria are described using two acid equilibrium equations. The first acid equilibrium constant accounts for the $CO_2 - H_2CO_3$ (aq) equilibrium. It consequently seems to have a high pKa. The fraction of the inorganic carbon in a particular form is called the "alpha" explained as an equation (Chaves *et al*., 2009).

Carbonic acid fixation is a major step in plants in which mechanism involves series of following events: (i) Carboxylation (ii) Reduction (iii) Regeneration of PEP (iv) Decarboxylation (v) Refixation of CO_2 and fate of pyruvate. The series of events that follow in this process occur in mesophyll cells as well as in bundle sheath cells. The first three events i.e. Carboxylation, Reduction and Regeneration of PEP occur in mesophyll cells and the other two i.e. Decarboxylation and Refixation of CO_2 and fate of pyruvate occur in bundle sheath cells (Sinha, 2004).

Carboxylation involves diffusion of atmospheric CO_2 to mesophyll cells via stomata, where carbonic acid is formed by dissolving in water. The reaction is catalyzed by the enzyme Carbonic anhydrase.

Carbonic acid gets ionized to form bicarbonate anion and H^+ cation. Bicarbonate is accepted by CO_2 acceptor molecule- PEP (Phosphoenol pyruvate) to form oxaloacetic acid- a four carbon compound. The reaction is catalyzed by the enzyme PEP carboxylase



The reaction takes place in cytosol of mesophyll cells. The next events occur in mesophyll cells and bundle sheath cells respectively.

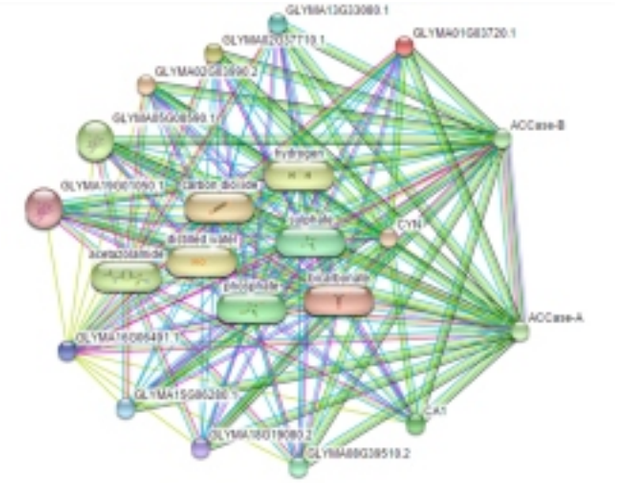
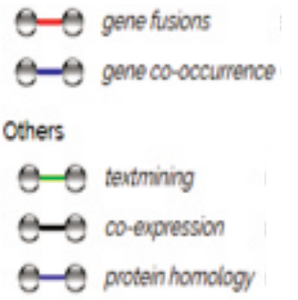


Fig. 2: Carbonic anhydrase network in *Glycine max*

Fig. 3: Carbonic anhydrase network in *Arabidopsis thaliana*

Figure Legend



CA network in plants

Acetyl-CoA carboxylase is a biotinylated enzyme that catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA. This is a two-step, reversible reaction, consisting of the ATPdependent carboxylation of the biotin group on the carboxyl carrier domain by the biotin-carboxylase activity, followed by the transfer of the carboxyl group from biotin to acetyl-CoA by the czarboxyl-transferase (CT) activity (Delye *et al*., 2005).

The network is predicted (Fig. 1 and Fig. 2) based on functional relationship using networking tool, illustrates that several biochemical compounds regulates the carbonic anhydrase activity both in normal and stress conditions. The network signifies multitude interactions; experimentally verified, computationally predicted, co-expression, annotation, text mining, gene neighborhood and homology significantly. Sulfate and phosphate being the most common compounds contribute in salt stress as well. Cyanide compounds and hydrogen co-express themselves in association with ACCase-A, ACCase-B and CA1. Phosphate, sulfate and biocarbonate are experimentally verified connections with photolysis and effect the same in stress condition by acting in-accordance with carbonic acid and hydrogen / proton to form H_2O_2 and CYN in extreme conditions. Acetazolamide has been known for its inhibitory action in against CA in human. Annotation, text mining and gene neighborhood based network wiring demonstrates that 11 carbonic anhydrase genes were identified in glycine max including CA1, GLYMA01G03720.1,GLYMA02G37710.1 and GLYMA02G03990.2 contributing substantially in stress, associating with ACCase-A, ACCase-B and CA1;under stress conditions resulting excessive CYN and H_2O_2 production, accumulation of carbonic acid in cells will be lethal to plants. These experimentally validated steps of predicted network rewiring needs to be implemented to narrow down the gene association network in order to understand the evolved mechanism in abiotic stress condition in a systems biology approach.

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