Zhurikova et al., VEGETOS 2018, 31:2
DOI: 10.4172/2229-4473.1000414

Vegetos: An International Journal of Plant Research

Review Article

A SCITECHNOL JOURNAL

The Carbonic Anhydrases in Higher Plant Chloroplasts

Zhurikova E[1], Rudenko N[1], Ignatova L[1] and Ivanov B[1*]

[1]*Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Russia*

[*]**Corresponding author:** Ivanov Boris, Institute of Basic Problems of Biology, Russian Academy of Sciences, Pushchino, 142290 Russia, Tel: +7(4967) 732448; E-mail: borpushchin@gmail.com

Rec date: Mar 20, 2018 **Acc date:** Apr 20, 2018 **Pub date:** Apr 27, 2018

Abstract

The aim of this review is to analyze the participation of carbonic anhydrases in the reactions proceeding in the higher plant chloroplasts. The short description of the catalytic mechanisms, inhibitors and activators of carbonic anhydrases belonging to α- and β- families, the representatives of which were found in chloroplasts is given. The current data about the proteins with carbonic anhydrase activity and about already identified enzymes in both chloroplast stroma and thylakoids are presented. The dependencies of carbonic anhydrase gene expression levels on the light intensity during growth are described for enzymes situated in these parts of the chloroplasts of Arabidopsis plants. We also present the results of our studies being conducted with mutants with knocked out genes encoding carbonic anhydrases. We hypothesize on the role of thylakoid carbonic anhydrases in the processes regulating the development of non-photochemical quenching of leaf chlorophyll fluorescence. The significance of the presence of the number of carbonic anhydrases in the chloroplast thylakoids is discussed.

Keywords: Carbonic anhydrase; Higher plants; Chloroplast; Thylakoids

Introduction

Carbon atoms are skeleton basis for the most of organic compounds, proteins, lipids, carbohydrates, while inorganic carbon is the participant in whole series of the important metabolic reactions in all cellular organisms. The concentration of inorganic carbon in the form of CO_2 is near 400 ppm in the present earth atmosphere. The dilution of CO_2 in water solutions results in the appearance of carbonic acid, which is promptly dissociated into bicarbonate, HCO_3^-, and H^+. Being the products of many biochemical reactions in cell, CO_2 and HCO_3^- can be reagents in other reactions. The most important biosphere reaction with participation of inorganic carbon is the reaction of its inclusion into organic molecules in course of photosynthesis.

In plants with C4- and CAM- types of photosynthesis, the primary fixation of inorganic carbon the reaction of bicarbonate with phosphoenolpyruvate, while the final fixation resulting in creation of stable organic molecule (carbohydrate) occurs as addition of CO_2 molecule to ribulosobisphosphate in the reaction catalyzed by Rubisco. In C3 plants and in eukaryotic algae there is no preliminary binding of bicarbonate to phosphoenolpyruvate, and CO_2 fixation catalyzed by Rubisco is primary reaction of inorganic carbon inclusion into organic matter; in these photosynthetic organisms this reaction takes place in the chloroplasts, where ATP and NADPH necessary for proceeding of Calvin cycle regenerating ribulosobisphophate are produced at the expense of photosynthetic electron transport.

Obviously, the efficient productive photosynthesis, i.e. the efficient CO_2 fixation requires the permanent CO_2 inflow from external medium, air or water, in terrestrial plants and algae, respectively, to chloroplast. It is considered, that to accomplish the way through biological membranes and water phases, inorganic carbon should change its form, emerging either as CO_2 or as bicarbonate. The change of the form is especially important in the chloroplast stroma, where inorganic carbon is present mostly in the form of bicarbonate, while the substrate of Rubisco is CO_2. So, the high rates of photosynthesis require the quick interconversion of HCO_3^- and CO_2. As usually consider, the spontaneous rates of both CO_2 hydration and bicarbonate dehydration reactions,

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \quad (1)$$

It cannot ensure the observed rates of photosynthesis. Therefore, during many years, the scientists study the participation in photosynthetic processes of carbonic anhydrases (CAs), which are able to increase the rates of these reactions many times over.

The participation of CAs in the interconversion of HCO_3^- and CO_2 was initially discovered in the animals, and later, CAs were detected in all cellular organisms, including photosynthetic ones, not only within cells, but also in peryplasmic space of some algae [1]. All enzymes, which by now were identified as CAs were divided into seven families (α, β, γ, δ, ζ, ε, η), taking into account the conservative sequences in genes encoding CAs. The data about the properties of CAs of different families, their roles in the processes in animals and human, their participation in the mechanism of CO_2 concentration in algae and bacteria may be found in the reviews [1-5]. Only CAs of α-family is present in cells of animals and human. In higher plant cells, the representatives of α-, β-, and γ-families were discovered. The enzymes related to other four families were found in bacteria, some algae and protozoa. In one plant cell and even within one cell compartment, several CAs, which belong to the indicated three families, can exist. In plant mitochondria, for instance there are six CAs belonging to β- and γ-families [6].

In this review, the current information about the composition of the chloroplast CAs of higher C3-plants and the established and proposed functions of these CAs will be presented. It is necessary to note here that from the data accumulated now, the functions of chloroplasts CAs seem not limited by the assumed feed of CO_2 to carboxylation centers of Rubisco in the chloroplast stroma.

Comparison of structure and catalytic mechanism of α- and β-CAs

Since the representatives of only a- and b-families of CAs were found in chloroplasts of higher plants, the structures and catalytic mechanisms of the enzymes related to these families are shortly characterized at first. It is noteworthy, that the enzymatic activity of CAs has an amazing symmetry of kinetic properties of direct and reverse reactions. The hydration reaction, which is catalyzed by the CAs proceeds via two stages. In the first stage CO_2 molecule attacks $Zn\text{-}OH^-$, to produce $Zn\text{-}HCO_3^-$ bond. HCO_3^- is then replaced by the

H_2O molecule, releasing HCO_3^- [7]. The second stage of catalysis is water protolysis in the active site of the enzyme with release of H^+. The rate of proton transfer to water to water from the group with pK of 7 is about 10^3-10^4 s^{-1}, which is 2-3 times slower than in reaction catalyzed by enzyme. Water is a poor proton acceptor under physiological conditions. Thus, it is suggested that H^+ is transferred not to water molecule, but to buffer [7].

The most active α-CA was found in human blood, so-called CAII, having a turnover number of 1.3-1.9 x 10^6/s under physiological conditions [8]. This CA is a monomer with molecular mass of 29 kDa consisted of 10 twisted β-fold structures. The active site is located at the bottom of 15 Å depth conical cavities, directed towards the protein [9]. Three histidine residues, His94, His96 and His119 forming distorted tetrahedron coordinate zinc in the active site of α-CA. Water is the fourth ligand, the ionization of which is critical in catalysis. His64 that was found only in the α-CAs located between the zinc and the protein surface participates in proton transfer step, as its imidazole side facing the cavity of the active site [10]. Its pK is about 7.1, which corresponds to proton transfer by zinc-bound water with pK 7 in both hydration and dehydration directions, and His64 acts as a temporary "station" for protons [11].

The molecular mass of β-CAs varies within a wide range, from 45 to 200 kDa [12]. Dimers (or pseudodimers) are blocks in structure of these CAs, which associate to form tetramers (the most common form of the molecule) or octamers with one zinc in each reaction site [13]. β-CAs in dicotyledonous plants are high molecular mass (180-270 kDa) proteins with oligomeric or, more often, octameric structure, like stromal β-CA from pea with molecular mass of 194 kDa [10]. β-CAs in monocotyledons are dimers with a lower molecular mass than β-CAs in dicotyledonous [14].

β-CA of pea is almost as fast and as efficient as human α-CAII. Historically, the study of β-CAs has evolved from the point of view of the mechanism of α-CAs functioning. Only later, it had been discovered that these two enzymes were not homologous [15]. The investigation of β-CA active site structure has shown that for representatives of these two families the environment of zinc in active site cavities did not coincide. Zinc ligands in β-CAs active site are cysteine (Cys160, Cys223) and histidine (His220) residues with water, acetate or acetic acid as the fourth ligand [16]. Although this active site structure of β-CAs is different from those of α-CAs, superposition of the mirror image of the structure of the active site of α-CAs onto active site of β-CA demonstrates that their functionally equivalent groups have a very similar geometric arrangement. Catalytic mechanisms of α-CAs and β-CAs are also similar to each other, differing in some details, namely, amino acids residues located near the active site, have different functions in carrying out the catalytic reaction of these enzymes [10,12]. The path of proton from active site during the second stage of reaction is not clear for β-CAs. There are no close situated residues that may function as a proton shuttle like His64 in α-CAII [17]. The proton shuttle of α-CAs is located in the active site at the bottom of a funnel with a depth of 15 Å. In β-CAs, which active site is much closer to protein surface, at a distance of 12 Å, the effective proton transfer may not require such a device. Most likely, that a proton is directed immediately to buffer molecule [7].

Inhibitors and activators of CAs

Another difference between α- and β-CAs is their reaction to the chemical compounds that cause the inhibition or stimulation of their activity. In early 1930s, researchers discovered that compounds capable of binding metals in complex, especially anions such as cyanide, sulfide, azide, thiocyanate and 2,3-dimercaptoethanol-1, are potent inhibitors of α-CAII from red blood cells. Mann et al. [18] first discovered a strong inhibition of this enzyme by sulfonamides. This is a group of compounds having an aromatic or heterocyclic radical attached to sulfonamide group. Sulfonamides are inhibitors of all CAs, regardless of the structure of the active site. This property is a feature of CAs, since none of the known enzymes are also inhibited by these compounds also specifically. In this case, the inhibitors form complexes in a ratio of 1: 1 with each independent site of the enzyme by replacement water Stimulation of CA activity by a number of substances, in particular by azoles, a group of compounds to which acetazolamide belongs to, is caused by the acceleration of proton withdrawal. Proton transfer is a limiting stage of CA reaction [7], and low-concentration azoles are able to play a role of proton transporter [19].

Representatives of different CA families differ in degree of inhibition by one or another class of compounds. The binding of sulfonamides, for example, to stromal pea β-CA is about 100 times weaker than that to human α-CA [20], at the same time; this β-CA is much more strongly inhibited by anions [21]. Due to the variety of β-CA structures, inhibitors also act on their activity in different ways. These differences in sensitivity to inhibitors are associated with differences in structure of the tunnel leading to the active site. A narrow hydrophobic channel in active site of β-CAs does not pass over molecules bigger than water or anion molecule. Sulfonamides are too large to adapt to narrow cavity of active sites of β-CAs, this leads to a weaker inhibition effect [10].

The effect of increasing the activity of CA with histamine and histidine was detected simultaneously with the effect of inhibition in the 40s of the last century [18]. Nowdays it is known that the activity of a number of CAs of β-family increases with interaction with the same activators as the activity of α-CAs [22]. Studies of CA activators, natural and synthetic (biogenic amines, amino acids and their derivatives, oligopeptides and some pharmacological agents) have shown that they contain elements possessing proton accepting properties (primary or secondary amino groups) attached to bulky aromatic/heterocyclic rings via an aliphatic linker of carbon chain [23].

Data from spectroscopy and crystallography showed that the CA activators bind near the surface of the cavity of the active site and participate in the process of proton transfer between the active site and the reaction medium, thereby strengthening the rate of the determinative stage of the catalytic cycle [24].

CAs of chloroplast stroma

The chloroplast stromal CA, which averages from 1 to 20% of total leaf protein [25], being the most abundant leaf protein after Rubisco, responses to a major part of CA activity of leaf. Illumination of leaves led to increase of pH in chloroplast stroma up to 7.6-7.7, at which a value of HCO_3^- concentration in stroma reaches 230 μM as compared with 11 μM CO_2 concentration there at atmospheric CO_2 level in air. Some early studies showed that in the presence of CA in vitro Km (CO_2) decreased, and in plants grown at elevated CO_2 in air the activities of both CA and Rubisco dropped. The inhibition of added CA resulted in the deceased of carboxylation, but oxygenation activities of Rubisco increased [26].

In other studies, the role of CA in promotion of CO_2 fixation was not supported. The transformation of tobacco plants with antisense

constructs of stromal CA gene led to 1-2 % level of CA, but there were no serious changes in the rate of photosynthesis, Rubisco activity, chlorophyll content, and stomata conductance [27].

Both antisense and gene knockout Arabidopsis thaliana plants with reduced levels of β-CA1 demonstrated lower survivability. But it could be restored either by inserting sucrose in cultivation medium or by increase CO_2 content in air during planting [28]. The capacity for light-dependent CO_2 assimilation rate in β-CA1 deficient seedlings was diminished, and the development of the first true leaves was reduced as a result. However, the survived mature plants of both types of β-CA1 deficient mutants did not exhibit any phenotypic distinction from the wild type plants. This may be considered as the evidence of β-CA1 failure to participate in photosynthesis in mature leaves [28].

Recently, one more stromal CA, α-CA1, has been found in Arabidopsis [29]. In plants with knocked out gene encoding α-CA1 a decrease in photosynthetic activity and the ability to accumulate starch was found. A phenotype of 4-week β-CA1 knockouts had no significant differences with wild type plants [28], whereas in α-CA1 mutant plants CO_2 uptake was reduced to 40% with a concomitant decrease in the rate of growth and starch accumulation. Also in α-CA1 mutant plants the level of photosynthetically synthesized soluble carbohydrates was changed. These data indicate that α-CA1 is involved somehow in the process of photosynthesis and, perhaps, it is the one, which supplies the substrate for Rubisco.

About 60% of Rubisco bound with thylakoid membranes [30]. Later, it was found that one of the stromal CA isoforms was associated with Rubisco on the outer side of the thylakoid membrane [31]. Ethoxyzolamide, a membrane-penetrating specific CA inhibitor, decreased the rate of CO_2-dependent oxygen release in pea protoplasts what confirm the need of CA for effective CO_2 fixation [32,33]. The marked suppression of pea protoplasts photosynthesis by the inhibitors of photorespiration suggests that photorespiratory CO_2 is also an important source of CO_2 within mesophyll protoplasts [33].

The effect of light intensity on the expression level of the genes encoding chloroplastic CAs was dependent on the duration of daylength [34]. The content of transcripts of all stromal CA genes in plants grown under “short day” conditions (8 h day/16 h night) increased with adaptation to high light intensity. At the longer daylength (16 h/8 h days) the exceptions were found. In Arabidopsis leaves, there are two forms of RNA of At3g01500 gene encoding β-CA1 [34]. In “long day” plants, the expression level of one of the alternate β-ca1 gene forms decreased with adaptation to high light illumination, while the expression level of the other form increased, as well as the intensity of expression of the gene coding another CA of chloroplasts, α-CA1. The opposite effect of light intensity under "long" day conditions on the content of two forms of the transcripts of the β-ca1 gene indicates different functions of the proteins encoded by these genes. A similar increase of the expression level of the gene coding another form of β-CA1 and of the gene coding α-CA1, after adaptation to increased illumination, suggests their cooperation in supply CO_2 to Rubisco [34].

There are many studies on the role of stromal CAs in the protection of plants under stress. It has been revealed that stromal CA can bind salicylic acid. The last one is an important signal component that causes a cascade of reactions leading to an increase in gene expression and post-translational activity of proteins involved in protecting plants from oxidative stress in viral infections [35]. There are data that the soluble CAs from chloroplasts of C3 plants, including β-CA1, is the part of a protective mechanism, which is induced after penetration of different pathogens [36]. Recombinant A. thaliana inbred lines with resistance to herbivores insect Plutella xylostella possessed at least a twofold increased content of stromal β-CA1 and plasmalemmal β-CA4 [36]. In Oryza sativa seedlings the salinity and osmotic stress has led to an increase in total CA activity and to an increase in the level of mRNA of the gene encoding soluble stromal CA of β-family [37]. Overexpression of CA in Arabidopsis resulted in the improved growth of mutant plants on salt containing medium, compared to the wild type [37].

The increase in atmospheric CO_2 level causes the closure of stomata in leaves and, thus, affects the inflow of CO_2 in plants tissues. However, any CO_2-binding proteins that would control this response were unknown. It was shown that in A. thaliana, with knocked out genes At3g01500 and At1g70410, encoding β-CA1 and β-CA4, respectively, CO_2-dependent regulation of stomatal movements was disrupted [38]. Thus, there is a plenty of studies that demonstrate the importance of stromal CAs for plant cell metabolism, but the exact role of the most abundant CA, β-CA1, in photosynthesis has not yet been established.

Thylakoid CAs of chloroplasts

The presence of CA situated in thylakoid membranes of higher plant was at first reported in the study conducted with bean chloroplasts [39]. After destruction of thylakoid membrane by Triton X-100, the CA activity could be visualized in gel by detection of bromothymol blue color change, which resulted from CO_2 hydration reaction. Besides the discovering the CA activity of thylakoid membrane, this study showed that the treatment of these membranes with detergents permitted to isolate the active enzyme. Later, it was found that the CA activity of thylakoids strongly differed from activity of soluble stromal CA by a variety of properties [40,41]: the CA activity of soluble CA did not depend on pH, while this activity of thylakoids had maximum at pH 6.8; $Km(CO_2)$ of stromal CA was 20 mM, and $Km(CO_2)$ of thylakoids was 9 mM; the CA activity of thylakoids was stimulated at submicromolar concentrations of such inhibitors of CAs as acetazolamide and azide. It was also shown that thylakoid CA from pea chloroplasts did not demonstrate cross-reaction with antibodies against stromal CA from spinach, while stromal CA did [42].

The next step in the studies of thylakoid CAs was the localization of their positions in this structure. The inhibition of thylakoid CA activity by inhibitors of photosystem II (PSII) operation, diuron and hydroxylamine [43], as well as the inhibition of PSII operation by CA inhibitors, anions formiate, nitrate, azide, and imidazole [44] pointed to close coupling between CA and PSII in thylakoid membrane. Moreover, using antibodies against thylakoid CA Cah3 from C. reinhardtii, CA in the thylakoid membrane fragments enriched with PSII (PSII membranes) isolated from pea [45] and maize [46] has been revealed.

Lu et al. [46] detected two sources of CA activity in the PSII membranes isolated from maize. The authors designated one of them as ‘extrinsic’ as it can be extracted by K, Na and Ca salts in high concentrations. The same relocation of part of CA activity of BBA-particles into solution after treatment with salts was also observed in the study with pea [42]. The other source of CA activity, which remained in PSII-membrane after above described treatments, was designated by the authors of study [46] as ‘intrinsic’. The study [47], which showed that the core complex of PSII is responsible for at least part of the CA activity of PSII-membrane from pea and wheat can be considered as indicative of nature of the ‘intrinsic’ CA activity.

Other studies revealed the presence of the low-molecular and high-molecular mass sources of CA activity in PSII-membrane from pea [48] and Arabidopsis [49]. These proteins were identified in gel by color reaction after native electrophoresis, and the eluates of corresponding bands of the gel demonstrated the CA activity measured by standard method, tracking pH change in the course of hydration reaction in the solution. Tentatively, the high-molecular source may be paralleled with intrinsic one in the study [46] and related to the core complex of PSII [47]. In [50], it was supposed that the source of CA activity in core complex of PSII is not CA of any family, but it may be a complex protein construction operating as CA. It is noteworthy; the acetazolamide in 10^{-7} M concentration stimulated the CA activity of the above mentioned low-molecular mass source of this activity from both pea and Arabidopsis, inhibiting it gradually in higher extent with increasing concentration. Such stimulation, which was also observed in [40] with whole thylakoids, led to an assumption that this source of CA activity in PSII is genuine CA of α-family, seeing such phenomenon is distinguishing feature of CAs of this family in animals [19] (see above).

The possible participation of CAs in the processes determining PSII operation was suggested, basing on the data evidencing the necessary of HCO3– for reactions of electron transfer at both acceptor and donor sides of this photosystem [51-53]. The mechanism of HCO_3^- influence on electron transfer at acceptor side, so-called 'bicarbonate effect', was extensively studied, and as thought the role of this anion consists in facilitation of electron transfer from Q_A to Q_B [54]. The discussion on possible mechanisms of HCO_3^- inclusion in the reactions of water oxidation and electron transfer at donor side of PSII may be found in the studies [55,56]. The data about the CA activity of the proteins of water-oxidation complex were obtained [46,57]. However, the properties of their activity were atypical comparing with properties of known CAs, e.g. those activities require Mn^{++} and were very low sensitive to acetazolamide and ethoxyzolamide [58]. In the review, these data were disputed.

The data about the presence of CA in the vicinity of photosystem I (PSI) became appear later than about CA connected with PSII. The fragments of thylakoid membrane enriched with PSI (PSI-membranes) possessing CA activity did not demonstrate a cross-reaction with antibodies against Cah3, CA from C. reinhardtii as it was observed with PSII-membranes [45]. It was also shown that the influence of temperature and sulfonamide inhibitors acetazolamide and ethoxyzolamide on CA activity was highly different in PSI and PSII membranes. Maximum of the CA activity of eluate from gel after native electrophoresis of PSI-membranes was observed at 10°C, whereas the CA activity from PSII-membranes changed weakly and remained high even after heating at 90°C. The low molecular mass CA of PSII-membranes from pea and Arabidopsis leaves was very sensitive to ethoxyzolamide with I50 of 10^{-9} M [48,49], and the CA activity of PSI-membranes from pea leaves was equally sensitive to lipophilic and hydrophilic sulfonamide inhibitors of CA, ethoxyzolamide and acetazolamide, with I50 of 10^{-6} M [48].

It is important that the CA activity of PSI-membranes, being calculated either on chlorophyll or protein basis was estimably higher than the activity of PSII-membranes [48]. The dissimilarity between the CA activity sources situated in thylakoid membrane near PSI or PSII was clearly ensued from effects of detergent treatment of pea thylakoids and their fragments enriched with one of photosystems: two maxima of the CA activity of thylakoids were observed at Triton X-100/chlorophyll ratios of 0.3 and 1.0, and only one maximum was observed in PSII-membranes at this ratio of 1.0, and in PSI-membranes at this ratio of 0.3 [50]. The same picture was observed in PSI- and PSII-membranes from Arabidopsis leaves [49].

After complete destruction of pea thylakoids by detergent, the soluble protein possessing CA activity was discovered [50]. The proposition, which was brought forward in that study that this CA was situated in thylakoid lumen, was supported by disclosure of such protein in the thylakoids isolated from Arabidopsis [59]. In this case, not only wild type plants, but also Arabidopsis mutants with knocked out gene encoding the most abundant stromal β-CA1 were studied; using of mutants prevented contamination of thylakoids by that soluble enzyme. Some properties of the lumenal CA, namely, the increase of its activity in response to addition of dithiotreitol, a rather high molecular mass close to 130 kD, the character of influence on its activity of sulfonamide inhibitors pointed out that this protein belonged to β-family of CAs [50,59]. We propose that lumenal CAs β-CA5, which was identified in chloroplasts [6,60], but without ascertainment of its precise position within the organelle.

The possible functions of thylakoid CA

The first attempt to envision the possible functions of thylakoid CAs was undertaken by Stemler many years ago [53]. The research of characteristics of mutants lacking the specified CA, as well as the studies of effects of environmental factors on the levels of CAs gene expressions enabled now bringing forward more reasoned assessment of these functions.

Until now, the only one CA, α-CA4 was identified among the proteins of thylakoid membranes in Arabidopsis [60]. The Arabidopsis mutant with knocked out gene encoding this CA had higher fresh weight of leaf rosette, highly increased starch content, decreased CO_2 assimilation rate, and higher production of H_2O_2 in leaves under illumination as compared with wild type (WT) plants [61,62]. In the mutant, the non-photochemical quenching of leaf chlorophyll fluorescence (nPQ) was appreciably suppressed, and this suppression was revealed to be the result of from almost exclusively considerable decrease of energy-dependent component of nPQ [62,63]. The latter fact led to proposition that α-CA4 was situated near PSII, participating in local protonation of PsbS protein, the conformation change of which was responsible for development of energy-dependent quenching of chlorophyll fluorescence. Thus, the release of protons may be the main function of this CA and not the transformation of CO_2 to HCO_3^- in the hydration reaction per se.

The development of nPQ is one of the mechanisms protecting photosynthetic apparatus from photo inhibition, and we observed that mutant lacking α-CA4 lost photosynthetic activity in high light faster than WT plant [62]. It is important that under increased level of illumination during growth the mutant contained fewer amounts of proteins of light-harvesting complex, Lhcb1 and Lhcb2, than WT plants [63]. This may be considered as an adaptation change since the decrease of light-harvesting antenna size can help to defend photosynthetic structures from photoinhibition when nPQ decreased.

In WT plants adapted to high intensity of light, a significant increase in the level of expression of α-ca4 gene has been observed [34]. This effect is consistent with the data about decrease of energy-dependent nPQ in the adapted to high illumination plants with knocked out gene encoding α-CA4 [62,63].

Arabidopsis mutants with knocked out gene encoding α-CA2, the position of which in plant was not determined in the previous studies,

differed from WT plants for a variety of characteristics: fresh weight of leaf rosette and starch content were lower, while CO_2 assimilation rate was appreciably higher, by 30-40% [62]. Remarkably, the absence of α-CA2 resulted in the increase of nPQ as compared with WT plants. It is seen that α-CA2 and α-CA4 knockouts differed from WT plants in the opposite direction. This permitted to suppose that α-CA2 was situated in thylakoid membrane as well as α-CA4. The function of α-CA2 may be the control of proton outflow from lumen, and in this case, these protons should be used in the HCO_3^- dehydration reaction. The tentative scheme of possible locations and functions of α-CA2 and α-CA4 are presented in Figure 1.

The content of transcripts of the gene encoding α-CA2 became higher under the "short day" conditions with increasing of plant age [34]. The adaptation of plants to high illumination under these conditions has led to additional increase in the level of α-ca2 transcription, in the same way as for other genes of chloroplast CAs. In "long day" conditions, the expression level of this gene did not change. The difference in the level of expression of α-ca4 and α-ca2 genes under increasing illumination in "long day" conditions is additional argument supporting the assumption of the opposite functioning of α-CA4 and α-CA2. The changes in the ratio of the content of these proteins may provide that balance of their operations which is necessary under some or other physiological conditions. It was previously found, that the expression level of the gene encoding α-CA2 increased under plant growth at low CO_2 concentration in air (150 ppm) [6]. This possibly reflects the necessity of this CA for outflow of excess of protons from lumen under their high accumulation there due to Calvin cycle limitation.

The opposite directions of the reactions catalyzed by α-CA2 and α-CA4 can be consequence of the different orientation of cavity of their catalytic centers in thylakoid membrane. We can't say now, what the CA activity sources being discussed above correspond to these two CAs. Tentatively, α-CA4 may be compared with low-molecular mass source of CA activity in PSII, while α-CA2 with the source of this activity situated near PSI (Figure 1).

When plant were adapted to high illumination, the contents of transcripts of the only one gene of chloroplasts, β-ca5, under both lengths of light period, "short" and "long", decreased. These data are in agreement with our assumption about the location of β-CA5 in thylakoid lumen and about its role in the acceleration of proton diffusion to ATP synthase channel, with the participation of CO_2/HCO_3^- buffer [34]. This acceleration is important just under low illumination, since proton flux in lumen is low, and protons can "get lost" along the way to ATP synthase.

The interesting demonstration of the activity of thylakoid CA has been recently found. The stimulating effect of bicarbonate addition on the rate of phosphorylation in isolated thylakoids is known for many years [64]; however, it had not satisfactory explanation. It was proposed [65], that CA can in some way be engaged in this stimulation. In our study, we had shown that hydrophilic inhibitor of CA, mafenide, which suppressed the CA activity of thylakoids abolished only the phosphorylation stimulation by bicarbonate in the presence of phenazinmetasulfate and methyl viologen (MV), but did not affect both the rate of electron transport with MV and phosphorylation rate in the absence of bicarbonate [66]. It was found [48], that activity of CA situated near PSI was inhibited equally by both acetazolamide poorly penetrating into lipid membranes and lipophilic ethoxyzolamide (see above), indicating the position of this CA at the stromal surface of thylakoid membrane, where it was equally accessible to these inhibitors. We implied that the stimulation of phosphorylation by HCO_3^- was the result of its dehydration catalyzed by this CA, which in isolated thylakoids faced the reaction medium, and CO_2 molecules emerged in this reaction at membrane surface layer could partly inflow to thylakoid lumen where their hydration, with possible participation of luminal CA, led to an increase of proton concentration, and accordingly, to increase of phosphorylation rate. This mechanism is able to explain the other effects of HCO_3^- addition into thylakoid suspension, namely, a decrease of electron transport rate under coupling conditions, an increase of phosphorylation rate after addition of bicarbonate into reaction medium containing an uncouple ammonium in low concentrations [66]. Described mechanism may also be a reason of a shift of medium pH, at which the maximal phosphorylation rate can be reached to more acidic values in the presence of bicarbonate [65,67].

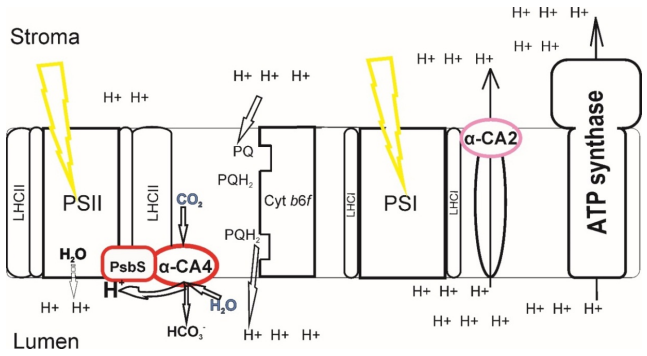

Figure 1: The tentative scheme of the locations and functions of α-CA4 and α-CA2 in the thylakoid membrane of chloroplasts of higher plants. Stroma, the chloroplast space, excluding thylakoids; lumen, the space within thylakoid; LHCI and LHCII, light-harvesting complexes I and II, respectively; PSII, pigment-protein complex caring electron-transfer centers of Photosystem II, b6f-complex, protein complex acting as a plastoquinol–plastocyanin reductase, and containing cytochromes b6 and f and Rieske iron-sulfur center; PSI, pigment-protein complex caring electron-transfer centers of Photosystem I; PQ and PQH_2, plastoquinone and plastohydroquinone, respectively; PsbS, the protein of LHCII, the proton activated conformation change of which is responsible for development of energy-dependent quenching of chlorophyll fluorescence; ATP synthase, the enzyme that syntheses ATP, using a proton gradient across the thylakoid membrane.

Conclusion

It is noteworthy that information about the functions of CAs in higher plants is accumulated relatively slowly. In last years, only using of mutants added noticeable input in understanding of the role of these enzymes in higher plant life. However, this role turned out educible when the plants were placed in stressful conditions, or with using of double mutants. The above may be evidence that the integrated CA system including several these enzymes operates in higher plant cells and in their compartments, and just such system provides the proceeding or regulation of some or other physiological process. The presence of several CAs in such compartments as mitochondrion and chloroplast permits probably to support the processes of reversible conversion of CO_2 and bicarbonate in the event of inhibition of any

enzyme of the system. Such inhibition is possible due to the environment changes leading to changes of ion balance, or owing to input of substances-inhibitors of CAs from air or soil. The participation of CAs in the regulation of physiological processes in plants bases first of all on their ability to affect pH in cell compartments at the expense of catalysis of the dehydration and the hydration reactions. At this, as our data showed for α-CA2 and α-CA4 operations, the CA system of pH regulation can use oppositely directed influences (Figure 1). The presence of CAs in all cellular organisms in the Earth is obviously explained by inevitable presence in them of bicarbonate buffer system, which originated there due to presence of CO_2 gas in the atmosphere. The scientists revealed the key role of CAs in the providing of photosynthesis of algae, where these enzymes are the essential part of the CO_2 concentration mechanism in the cell, and possibly, soon the important role of CAs in great part of physiological processes in higher plants will be determined.

References

1. Mitra M, Mason CB, Xiao Y, Ynalvez RA, Lato SM, et al. (2005) The carbonic anhydrase gene families of Chlamydomonas reinhardtii. Can J Bot 83: 780-795.
2. Smith KS, Ferry JG (2000) Prokaryotic carbonic anhydrases. FEMS Microbiol Rev 24: 335-366.
3. Ivanov BN, Ignatova LK, Romanova AK (2007) Diversity in forms and functions of carbonic anhydrase in terrestrial higher plants. Russ J Plant Physiol 54: 143-162.
4. Rudenko NN, Ignatova LK, Fedorchuk TP, Ivanov BN (2015) Carbonic anhydrases in photosynthetic cells of higher plants. Biochem (Mosc) 80: 674-687.
5. DiMario RJ, Clayton H, Mukherjee A, Ludwig M, Moroney JV (2017) Plant carbonic anhydrases: structures, locations, evolution, and physiological roles. Mol Plant 10: 30-46.
6. Fabre N, Reiter IM, Becuwe-Linka N, Genty B, Rumeau D (2007) Characterization and expression analysis of genes encoding α and β carbonic anhydrases in Arabidopsis. Plant Cell Environ 30: 617-629.
7. Silverman DN (1991) The catalytic mechanism of carbonic anhydrase. Can J Bot 69: 1070-1078.
8. Khalifah RG (1971) The carbon dioxide hydration activity of carbonic anhydrase I. Stop-flow kinetic studies on the native human isoenzymes B and C. J Biol Chem 246: 2561-2573.
9. Sjoblom B, Polentarutti M, Djinović-Carugo K (2009) Structural study of X-ray induced activation of carbonic anhydrase. Proc Natl Acad Sci 106: 10609-10613.
10. Kimber MS, Pai EF (2000) The active site architecture of Pisum sativum β-carbonic anhydrase is a mirror image of that of α-carbonic anhydrases. EMBO J 19: 1407-1418.
11. Isaev A, Scheiner S (2001) Proton conduction by a chain of water molecules in carbonic anhydrase. J Phys Chem B 105: 6420-6426.
12. Rowlett RS (2010) Structure and catalic mechanism of the β-carbonic anhydrase. Biochim Biophis Acta 1804: 362-373.
13. Tripp BC, Smith K, Ferry JG (2001) Carbonic anhydrase: new insights for an ancient enzyme. J Biol Chem 276: 48615-48618.
14. Atkins CA, Patterson BD, Graham D (1972) Plant carbonic anhydrases II. Preparation and some properties of monocotiledon and dicotiledon enzyme types. Plant Physiol 50: 218-223.
15. Fawcett TW, Browse JA, Volokita M, Bartlett SG (1990) Spinach carbonic anhydrase primary structure deduced from the sequence of a cDNA clone. J Biol Chem 265: 5414-5417.
16. Provart NJ, Majeau N, Coleman JR (1993) Characterization of pea chloroplastic carbonic anhydrase. Expression in Escherichia coli and site-directed mutagenesis. Plant Mol Biol 22: 937-943.
17. Björkbaska H, Johansson IM, Forsman C (1999) Possible roles for His 208 in the active-site region of chloroplast carbonic anhydrase from Pisum sativum. Arch Biochem Biophys 361: 17-24.
18. Mann T, Keilin D (1940) Carbonic anhydrase Purification and nature of the enzyme. Biochem J 34: 1163-1176.
19. Ilies M, Banciu MD, Ilies MA, Scozzafava A, Caproiu MT, et al. (2002) Carbonic anhydrase activators: design of high affinity isozymes I, II, and IV activators, incorporating tri-/tetra-substituted-pyridinium-azole moieties. J Med Chem 45: 504-510.
20. Reed ML, Graham D (1981) Carbonic anhydrase in plants: Distribution, properties and possible physiological roles. Progr Phytochem 7: 47-94.
21. Johansson IM, Forsman C (1993) Kinetic studies of pea carbonic anhydrase. Eur J Biochem 218: 439-446.
22. Innocenti A, Durdagi S, Doosdar N, Strom TA, Supuran CT (2010) Nanoscale enzyme inhibitors: fullerenes inhibit carbonic anhydrase by occluding the active site entrance. Bioorg Med Chem 18: 2822-2828.
23. Supuran CT (1992) Carbonic anhydrase activators Part 4 A general mechanism of action for activators of isozymes I, II and III. Rev Roum Chim 37: 411-421.
24. Briganti F, Iaconi V, Mangani S, Orioli P, Scozzafava A, et al. (1997) A ternary complex of carbonic anhydrase: X-ray crystallographic structure of adduct of human carbonic anhydrase II with the activator phenylalanine and the inhibitor azide. Inorganica Chim Acta 275/276: 295-300.
25. Okabe K, Yang SY, Tsuzuki M, Miyachi S (1984) Carbonic anhydrase: its content in spinach leaves and its taxonomic diversity studied with anti-spinach leaf carbonic anhydrase antibody. Plant Sci Lett 33: 145-153.
26. Porter MA, Grodzinski B (1984) Acclimation to high CO2 in bean carbonic anhydrase and ribulose bisphosphate carboxylase. Plant Physiol 74: 413-416.
27. Price GD, von Caemmerer S, Evans JR, Yu JW, Lloyd J, et al. (1994) Specific reduction of chloroplast carbonic anhydrase activity by antisense RNA in transgenic tobacco plants has a minor effect on photosynthetic CO2 assimilation. Planta 193: 331-340.
28. Ferreira FJ, Guo C, Coleman JR (2008) Reduction of plastid-localized carbonic anhydrase activity results in reduced Arabidopsis seedling survivorship. Plant Physiol 147: 585-594.
29. Villarejo A, Buren S, Larsson S, Dejardin A, Monne M, et al. (2005) Evidence for a protein transported through the secretory pathway en route to the higher plant chloroplast. Nature Cell Biol 7: 1224-1231.
30. Anderson LE, Gibbons JT, Wang X (1996) Distribution of ten enzymes of carbon metabolism in pea (Pisum sativum) chloroplasts. Int J Plant Sci 157: 525-538.
31. Lazova GN, Stemler AJ (2008) A 160 kDa protein with carbonic anhydrase activity is compexed with rubisco on the outer surface of thylakoids. Cell Biol Int 32: 646-653.

32. Ignatova LK, Moskvin OV, Ivanov BN (2001) Effects of carbonic anhydrase inhibitors on proton exchange and photosynthesis in pea protoplasts. Russ J Plant Physiol 48: 467-472.
33. Riazunnisa K, Padmavathi L, Bauwe H, Raghavendra AS (2006) Markedly low requirement of added CO2 for photosynthesis by mesophyll protoplasts of pea (Pisum sativum): possible roles of photorespiratory CO2 and carbonic anhydrase. Physiol Plant 128: 763-772.
34. Rudenko NN, Vetoshkina DV, Fedorchuk TP, Ivanov BN (2017) Effect of light intensity under different photoperiods on expression level of carbonic anhydrase genes of the α-and β-families in Arabidopsis thaliana leaves. Biochem (Mosc) 82: 1025-1035.
35. Slaymaker DH, Navarre DA, Clark D, del Pozo O, Martin GB et al. (2002) The tobacco salicylic acid binding protein 3 (SABP3) is the chloroplast carbonic anhydrase which exhibits antioxidant activity and plays a role in the hypersensitive defense response. Proc. Natl Acad Sci USA 99: 11640-11645.
36. Collins RM, Afzal M, Ward DA, Prescott MC, Sait SM et al. (2010) Differential proteomic analysis of Arabidopsis thaliana genotypes exhibiting resistance or susceptibility to the insect herbivore Plutella xylostella. PLoS One 5:e10103.
37. Yu S, Zhang X, Guan Q, Takano T, Liu S (2007) Expression of a carbonic anhydrase gene is induced by environmental stresses in rice (Oryza sativa L). Biotech Lett 29: 89-94.
38. Hu H, Boisson-Dernier A, Israelsson-Nordström M, Böhmer M, Xue S, et al. (2010) Carbonic anhydrases are upstream regulators of CO2-controlled stomatal movements in guard cells. Nat Cell Biol 12: 87-93.
39. Komarova YM, Doman NG, Shaposhnikov GL (1982) Two forms of carbonic anhydrase in chloroplasts of beans. Biochemistry 47: 1027-1034.
40. Moskvin OV, Ignatova LK, Ovchinnikova VI, Ivanov BN (1995) Membrane-associated carbonic anhydrase of pea thylakoids. Biochem (Mosc) 60: 859-864.
41. Ignatova LK, Moskvin OV, Romanova AK, Ivanov BN (1998) Carbonic anhydrases in the C3-plant leaf cell. Aust J Plant Physiol 25: 673-677.
42. Moskvin OV, Shutova TV, Khristin MS, Ignatova LK, Villarejo A, et al. (2004) Carbonic anhydrase activities in pea thylakoids. Photosynth Res 79: 93-100.
43. Vaklinova SG, Goushtina LM, Lazova GN (1982) Carboanhydrase activity in chloroplasts and chloroplast fragments. CRAcad Bulg Sci 35: 1721-1724.
44. Stemler A, Murphy J (1983) Determination of the binding constant of H14CO3-to the photosystem II complex in maize chloroplasts: effects of inhibitors and light. Photochem Photobiol 38: 701-707.
45. Pronina NA, Allahverdiev SI, Kupriyanova EV, Klyachko-Gurvich GL, Klimov VV (2002) Localization of carbonic anhydrase in subchloroplastic pea particles. Russ J Plant Physiol 49: 341-349.
46. Lu YK, Stemler AJ (2002) Extrinsic photosystem II carbonic anhydrase in maize mesophyll chloroplasts. Plant Physiol 266: 16746-16754.
47. Khristin MS, Ignatova LK, Rudenko NN, Ivanov BN, Klimov VV (2004) Photosystem II associated carbonic anhydrase activity in higher plants is situated in core complex. FEBS Lett 577: 305-308.
48. Ignatova LK, Rudenko NN, Khristin MS, Ivanov BN (2006) Heterogeneous origin of carbonic anhydrase activity of thylakoid membranes. Bio chem (Mosc) 71: 525-532.
49. Ignatova LK, Rudenko NN, Mudrik VA, Ivanov BN (2011) Carbonic anhydrase activity in Arabidopsis thaliana thylakoid membrane and fragments enriched with PSI or PSII. Photosynth Res 110: 89-98.
50. Rudenko NN, Ignatova LK, Ivanov BN (2007) Multiple sources of carbonic anhydrase activity in pea thylakoids: soluble and membrane-bound forms. Photosynth Res 91: 81-89.
51. Stemler A (1980) Forms of dissolved carbon dioxide required for photosystem II activity in chloroplast membranes. Plant Physiol 65: 1160-1165.
52. Moubarak-Milad M, Stemler A (1994) Oxidation-reduction potential dependence of photosystem II carbonic anhydrase in maize thylakoids. Biochemistry 33: 4432-4438.
53. Stemler A (1997) The case for chloroplast thylakoid carbonic anhydrase. Physiol Plant 99: 348-353.
54. Govindjee, Pulles MPJ, Govindjee R, VanGorkem HJ, Duysens LNM (1976) Inhibition of the reoxidation of secondary acceptor of Photosystem II by bicarbonate depletion. Biochim Biophys Acta 449: 602-605.
55. Stemler A, Murphy J, Jursinic P (1984) Effects of flashing light and hydroxylamine on the binding affinity of H14CO3-to photosystem II in chloroplast thylakoids. Photobiochem Photobiophys 8: 289-304.
56. Klimov VV, Allakhverdiev SI, Feyziev YM, Baranov SV (1995) Bicarbonate requirement for the donor side of photosystem II. FEBS Lett 363: 251-255.
57. Shitov AV, Pobeguts OV, Smolova TN, Allakhverdiev SI, Klimov VV (2009) Manganese-dependent carbonic anhydrase activity of photosystem ii proteins. Biochem (Mosc) 74: 509-17.
58. Bricker TM, Frankel LK (2011) Auxiliary functions of the PsbO, PsbP and PsbQ proteins of higher plant photosystem II: a critical analysis. J Photochem Photobiol B 104: 165-178.
59. Fedorchuk T, Rudenko N, Ignatova L, Ivanov B (2014) The presence of soluble carbonic anhydrase in the thylakoid lumen of chloroplasts from Arabidopsis leaves. J Plant Physiol 171: 903-906.
60. Friso G, Giacomelli L, Ytterberg AJ, Peltier JB, Rudella A, et al. (2004) In-depth analysis of the thylakoid membrane proteome of Arabidopsis thaliana chloroplasts: new proteins, new functions, and a plastid proteome database. Plant Cell 16: 478-499.
61. Zhurikova EM, Ignatova LK, Semenova G, Rudenko NN, Mudrik VA, et al. (2015) Effect of knockout of α-carbonic anhydrase 4 gene on photosynthetic characteristics and starch accumulation in leaves of Arabidopsis thaliana. Russ J Plant Physiol 62: 564-569.
62. Zhurikova EM, Ignatova LK, Rudenko NN, Mudrik VA, Vetoshkina DV, et al. (2016) The participation of two carbonic anhydrases of alpha family in photosynthetic reactions in Arabidopsis thaliana. Biochem (Mosc) 81: 1182-1187.
63. Rudenko NN, Fedorchuk TP, Vetoshkina DV, Zhurikova EM, Ignatova LK, et al. (2018) Influence of knockout of At4g20990 gene encoding α-CA4 on photosystem II light-harvesting antenna in plants grown under different light intensities and day lengths Protoplasma 255: 69-78.

64. Cohen WS, MacPeek WA (1980) A proposed mechanism for the stimulatory effect of bicarbonate ions on ATP synthesis in isolated chloroplasts. Plant Physiol 66: 242-245.
65. Onoiko EB, Polishchuk AV, Zolotareva EK (2010) Stimulation of photophosphorylation in spinach isolated chloroplasts by exogenous bicarbonate: role of carbonic anhydrase. Rep Nat Acad Sci Ukr 10: 160-165.
66. Fedorchuk TP, Opanasenko VK, Rudenko NN, Ivanov BN (2018) Bicarbonate-induced stimulation of photophosphorylation in isolated thylakoids: effects of carbonic anhydrase inhibitors. Biological membranes 35: 34-41.
67. Muray T, Akazawa T (1972) Bicarbonate effect on the photophosphorylation catalyzed by chromatophores isolated from Chromatium strain D XII Structure and function of chloroplast proteins. Plant Physiol 50: 568-571.