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BIOCATALYTIC CARBON DIOXIDE CAPTURE PROMOTED BY CARBONIC ANHYDRASE

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The rapid and steady increase in the concentration of CO_2, the most abundant greenhouse gas in the atmosphere, leads to extreme weather and climate events. Due to the burning of fossil fuels (oil, coal and natural gas), the concentration of CO_2 in the air has been increasing in recent decades by more than 2 ppm per year, and in the last year alone — by 3.29 ppm. To prevent the "worst" scenarios of climate change, immediate and significant reductions in CO_2 emissions through carbon management are needed.

Aim. Analysis of the current state of research and prospects for the use of carbonic anhydrase in environmental decarbonization programs.

Results. Carbonic anhydrase (CA) is an enzyme that accelerates the exchange of CO_2 and HCO_3^- in solution by a factor of 10^4 to 10^6. To date, 7 types of CAs have been identified in different organisms. CA is required to provide a rapid supply of CO_2 and HCO_3^- for various metabolic pathways in the body, explaining its multiple independent origins during evolution. Enzymes isolated from bacteria and mammalian tissues have been tested in CO_2 sequestration projects using carbonic anhydrase (CA). The most studied is one of the isoforms of human CAz — hCAII — the most active natural enzyme. Its drawbacks have been instability over time, high sensitivity to temperature, low tolerance to contaminants such as sulphur compounds and the impossibility of reuse. Molecular modelling and enzyme immobilisation methods were used to overcome these limitations. Immobilisation was shown to provide greater thermal and storage stability and increased reusability.

Conclusions. Carboanhydrases are involved in biological CO_2 assimilation. Therefore, the study of such enzymes and the conditions of their participation in atmospheric CO_2 sequestration provides a basis for the development of biocatalytic means to enhance atmospheric decarbonization.

Key words: climate; decarbonization; biosequestration of CO_2; carbonic anhydrase; immobilization of enzymes.

The processes of carbon sequestration, deposition and release from biological and geological sources are well balanced, thanks to which the balance of the ecosystem is maintained [1]. With the beginning of industrialisation, the global climate balance was disturbed, the concentration of CO_2 in the atmosphere began to increase rapidly [2]. At the end of the 19th century, the Swedish scientist Svante Arrhenius was one of the first to study the relationship between the level of atmospheric carbon dioxide (CO_2) and the Earth's temperature, focusing on understanding the role of CO_2 in climate regulation on a geological time scale. His work played an important role in the development of modern climatology [3]. The concept of anthropogenic global warming emerged much later, mainly in the second half of the 20th century, as a result of the development of climatology and the accumulation of factual data.

In the 1960s, Charles David Keeling demonstrated that the amount of anthropogenic carbon dioxide emitted into the air was sufficient to cause global warming [3]. and a broader understanding of the greenhouse effect. The increase in greenhouse gas (GHG) levels in the atmosphere over the past five decades is considered to be the main cause of global warming. Although carbon dioxide is not the only greenhouse gas in the atmosphere, its share is 65% of the total volume of global emissions, it is the largest contributor to the total GHG in the atmosphere and is responsible for 60% of the effects of global warming.

Today, 22 billion tonnes of CO_2 are emitted into the atmosphere from anthropogenic sources. The emission of GHG as a result of the use of fossil fuels is the main reason why the concentration of CO_2 has increased from the pre-industrial level of 270 ppm to the current level of 420 ppm. Over the next hundred years, energy demand is expected to more than double, which experts predict will lead to a doubling of CO_2 emissions by 2050 [4]. Data from ice cores in Greenland and Antarctica show that such high concentrations of CO_2 have not been observed for more than 400,000 years. The consequences of such an unprecedented increase in CO_2 concentrations may include: ocean acidification, sea level rise, climate disruption and ecosystem destruction [5–7].

Currently, CO_2 concentrations continue to increase by about 2 ppm per year, and by 2.53 ppm in the last year alone: from 417.15 ppm in August 2022 to 419.68 ppm in August 2023 according to https://gml.noaa.gov/ccgg/trends/. In order to reduce GHG emissions into the atmosphere, there is an urgent need for strategies to mitigate the consequences of CO_2 emissions. GHG emissions can be reduced by reducing the use of fossil fuels and promoting CO_2 sequestration [2, 8]. Atmospheric CO_2 can be sequestered using physical, chemical and biological approaches [1]. Depending on the chosen conversion method, CO_2 is captured and converted into value-added products [9, 10]. One of the most effective approaches for sustainable development is the biological sequestration of CO_2. Plants and microorganisms can capture atmospheric CO_2 using the carbon concentration mechanism (CCM) and convert the captured carbon into bioproducts [11]. However, these biological accumulators are only temporary carbon sinks, since CO_2 is released again during respiration, decomposition of dead organisms, and also during fires.

Decarbonisation programmes are being developed to remove carbon from the atmosphere and sequester it for indefinite or very long periods (thousands to millions of years) [4]. These include chemical adsorption from flue gases followed by storage in deep geological repositories, or conversion of CO_2 to insoluble carbonate salts [12–15].

The conversion of CO_2 into mineral carbonates offers the prospect of a safe, stable and environmentally benign product for long-term carbon sequestration [12, 16, 17], as significant reservoirs of carbonate minerals have existed for millions of years.

Under natural conditions, carbon sequestration in mineral carbonates is a very slow process. To speed up the reaction, decarbonisation technologies use an enzyme known as carbonic anhydrase (CA), which radically accelerates CO_2 hydration [18, 19].

Carbonic anhydrases catalyze the reaction of the reversible hydration of carbon dioxide:

$$CA + CO_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CA{\cdot}CO_2 \underset{k_{-2}}{\overset{k_{cat}}{\rightleftharpoons}}$$

$$\underset{k_{-2}}{\overset{k_{cat}}{\rightleftharpoons}} CA + HCO_3^- + H^+ \qquad (1)$$

The rate of interconversion of CO_2 and HCO_3^- in the absence of enzyme is relatively low: the value of the rate constant for the non-catalytic hydration of CO_2 is ~ 0.037 s^{-1} at 25 °C and an ionic strength of 0.2 [20]. This reaction has a second order rate constant k_1 of 0.0027 $M^{-1} \cdot s^{-1}$ at 37 °C and neutral pH, which corresponds to a pseudo first order rate constant of 0.15 s^{-1}. The dehydration reaction constant k_{-1} is equal to 50 s^{-1}. These constants determine the $[CO_2]/[H_2CO_3]$ ratio in aqueous solutions of 340 : 1. Carbonic acid H_2CO_3 dissociates in aqueous solution with pK 1 6.35 and pK 2 10.25, i.e. at neutral pH inorganic carbon in the form of bicarbonate ion (HCO_3^-) predominates. Carbonic anhydrases (CA) significantly accelerate both reactions, especially the hydration reaction. The most active human carbonic anhydrase II enzyme has a k_{cat} of the CO_2 hydration reaction of more than $10^6 \cdot s^{-1}$, and the rate constant of the dehydration reaction is $2.5–10^5 \cdot s^{-1}$ [21] (Table 1).

Carbonic anhydrases are ubiquitous in both prokaryotes and eukaryotes [22, 23]. They play important roles in numerous physiological processes such as respiration, pH and CO_2 homeostasis, secretion and gluconeogenesis. There are seven different classes of CAs (α,

β, γ, δ, ζ, η, θ, ι) that differ in their role in various important physiological processes, amino acid sequences and three-dimensional tertiary structure [24]. Despite structural differences, all CAs share the same catalytic mechanism involving a central metal atom (most commonly zinc).

Recently, CAs have been the target of environmental studies as enzyme accelerators to significantly improve CO_2 capture in the aqueous phase [25, 26].

Types of Carbonic Anhydrases and Their Features

α-Carbonic anhydrases

α-CAs are widespread and are found in vertebrates [27], algae [28, 29], higher plants [30, 31], eubacteria [32–34], some species of fungi and protozoa, as well as some bacteria and cyanobacteria [35, 24]. However, α-CA has not been detected in archaea [36]. Most α-CAs are monomers with a molecular mass of about 30 kDa (Fig 1, *A*). Most α-CAs are active as approximately 30 kDa monomers with three histidines coordinating the zinc atom [30] (Fig. 1, *B*).

Historically, α-CAs were the first carboanhydrases to be described, isolated from animal erythrocytes. They are considered to be the evolutionary youngest group of CAs [39] found in mammals, including humans, and have a limited distribution in prokaryotes [22].The alpha class includes all 16 mammalian CA isoforms with different organ-tissue distribution and subcellular location, 15 of which have been found in humans (HCAs) [24], and several new isoenzymes have also been identified in non-mammalian vertebrates, among which HCAII is the best studied and characterised CA. The tertiary structure of HCAII consists of a unique domain containing ten β-bands that twist to form a β-sheet (eight of which are antiparallel, the other two parallel). Around these β-sheets are up to eight more α-helices on the surface of the protein (Fig. 1, *A*).

The α-CA isoenzymes differ in their kinetic properties, tissue distribution and subcellular localisation, and sensitivity to different inhibitors. In general, there are three distinct groups of CA isoenzymes in the α-CA gene family. One of these groups contains cytoplasmic CA, which includes mammalian CAs I, II, III, V, VII, and XIII. These isoenzymes are found in the cytoplasm of various tissues, with the exception of mitochondrial CA V. Another group of isoenzymes called membrane bound CAs consists of mammalian CAs IV, IX, XII, XIV and XV [40]. These isoenzymes are associated with the plasma membranes of many different tissue types. The latter group contains some very unusual isoenzymes, CA VIII, X and XI, which have lost the classical CA activity of CO_2 hydration/dehydration and are referred to as CA-related proteins [41].

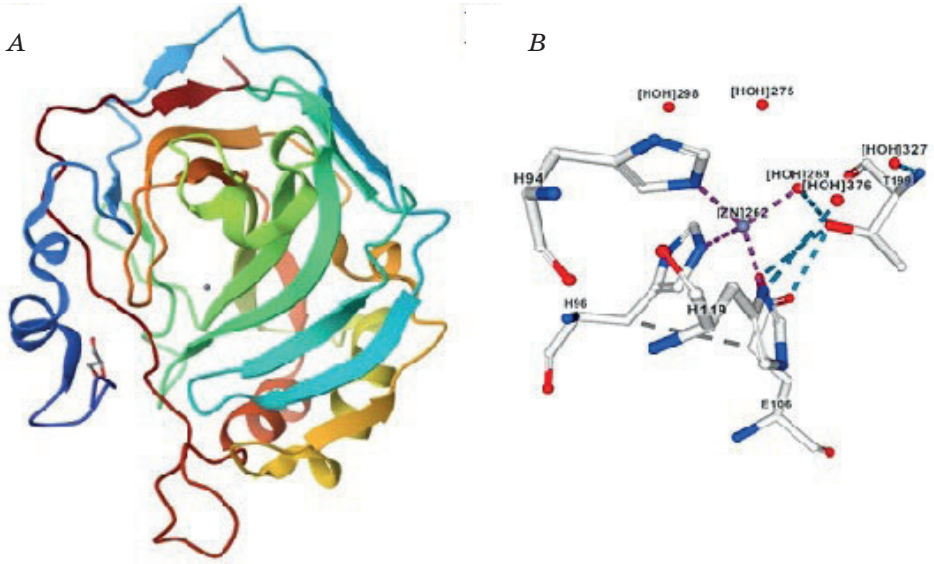

Fig. 1. **Structure of human CAII enzymes retrieved from PDB 3U45 (*A*). The human CAII monomer mostly consists of beta strands (*B*) a single active site with three zinc-coordinating histidine residues [37, 38]**

β–*Carbonic anhydrases*

β-CAs were first identified as carbonic anhydrases in higher plants [42, 43]. Subsequently, β-CAs were discovered in cyanobacteria [28, 44], microalgae [45], eubacteria [46], archaeubacteria [22] and fungi [47]. This CA family is not represented in any vertebrate genome. The active site of β-CAs contains histidine and two cysteine residues that act as zinc ligands [48, 49]. β-CAs are usually active as dimers or multimers. In *Pisum sativum*, CA dimers form tetramers (Fig. 2, 3), which are held together by their C-termini to form octamers [50]. An active center is formed at the interface between the two subunits.

β-CA's were discovered after α-CAs in plants and other organisms. The quaternary organisation of β-CAs is more diverse: they can form different oligomeric structures such as dimers, tetramers and octamers. The molecular masses of these oligomers range from 45 to 200 kDa. Among them, the dimer is the catalytically active unit responsible for the carboanhydrase activity of the enzyme. Individual β-CA subunits have molecular masses of 25 to 30 kDa (Fig. 2). These subunits have a unique α/β folded structure that allows them to associate and form dimers, which are the main structural units in the catalytic mechanism. The structure of the β-CA monomer consists mainly of helices surrounding a β-sheet of four parallel β-strands. A fifth C-terminal β-strand is also involved in the oligomerisation of β-CA [5].

Initially, it was thought that the β-class of CAs consisted exclusively of enzymes localised in the chloroplasts of higher plants, and they were commonly referred to as 'plant CAs'. However, with the accumulation of new data since the 1990s, it has become clear that β-CAs are much more widespread and are found in different organisms in all three superkingdoms: eukaryotes, bacteria and archaea. β-CAs have been identified in a wide range of organisms including microalgae, cyanobacteria, bacteria, fungi, archaea, higher C3 and C4 plants and invertebrates.

This wide distribution suggests that β–CAs play an important role in a variety of biological processes in different life forms. Phylogenetic analyses by Smith and Ferry [22] of 76 β-CAs from a variety of organisms belonging to different domains of life revealed distinct patterns. Unicotyledonous and dicotyledonous plants formed monophyletic groups, suggesting a common evolutionary origin. However, the remaining β-CAs formed four distinct subgroups or clades, including two clades formed by enzymes from Eukarya and Bacteria, and two exclusively prokaryotic clades: one comprising enzymes from Gram-negative bacteria, and the other consisting predominantly of sequences from Archaea and Gram-positive bacterial species.

The functional unit of β-CA is the dimer (Fig. 2), although the most common oligomeric form of β-CA is the tetramer [50, 53]. The β-CA dimer is formed by extensive interactions created by the two N-terminal α-helices of one monomer wrapping around the second monomer and a minor hydrogen bond between the second β-helices of each monomer [50]. Tetramers are formed by interactions mainly through the fifth, C-terminal β-helix [50]. In pea, chloroplast β-CA forms an octamer. For some β-CA, dicots have a unique C-terminal extension of the fifth β-sequence, whereas unicots do not [50, 53]. Octamers are formed by slightly different interactions with these fifth β-band extensions [50, 53].

The structural diversity observed in β-CA reflects the adaptability of these enzymes to different environments and physiological

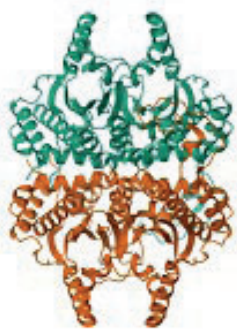

Fig. 2. **X-ray structure of a β-carbonic anhydrase from the red alga, *Porphyridium purpureum* R-1 [51]**

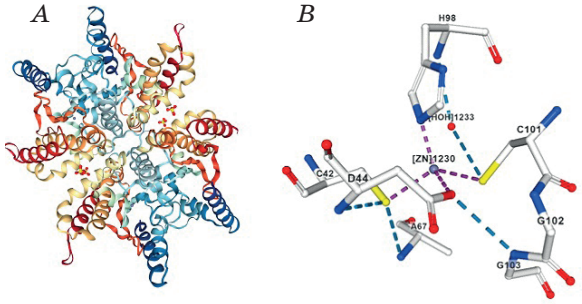

Fig. 3. **Ribbon diagram of the β-CA native tetramer of Haemophilus influenzae β-CA retrieved from PDB 2A8C (*A*) [52].**
The active site of β-CAs containing one histidine and two cysteine residues that act as zinc ligands (*B*)

roles. Understanding the structural features of β-CAs can provide valuable information on their catalytic mechanisms, regulation and potential applications in biotechnology and medicine.

γ-Carbonic anhydrases

γ-CAs were first discovered in the archaea Methanosarcina thermophila [54]. Plants [55] and photosynthetic bacteria [56, 57] also contain γ-carboanhydrases, while reports detailing γ-CA in animals have not appeared. The amino acid sequence of Cam showed no significant homology with known representatives of α- or β-class carboanhydrases [55].

Similar to the active centre of α-CAs, the active centre of Archaebacterium γ-carboanhydrases has zinc in the active centre coordinated by three histidine residues and one water molecule [59] (Fig 4, *B*). However, in contrast to the structure of carboanhydrases, which are monomers, the functional unit of γ-carboanhydrases is a trimer with three active centres spanning the monomer-monomer interfaces.

The zinc ion is coordinated by his residues represented by two different subunits [59]. The β-chain region dominates the structure of γ-carboanhydrase and consists of seven complete turns forming a left-handed β-helix [59]. Each complete turn contains three β-chains, making the β-helix look like an equilateral triangle when viewed from above [59]. In photosynthetic organisms, γ-carboanhydrase may contain additional domains, as seen in the cyanobacterial CcmM proteins of cyanobacteria, which have two or three repeating C-terminal domains with high similarity to the *Rubisco* small subunit [60]. In cyanobacteria, CcmM sometimes acts as an active carboanhydrase, but some CcmM proteins lack activity [57, 61]. However, CcmM is thought to organise the packaging of Rubisco in the carboxysome even though it does not have carboanhydrase activity.

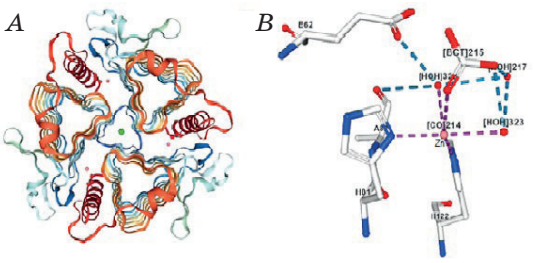

Fig. 4. **Structure of γ-CA from *Methanosarcina thermophila* 1QRE [58, 59]**

It has been shown that γ- or γ-like CAs are part of complex I of the mitochondrial electron transport chain in plants and algae [62, 63].

δ-Carbonic anhydrases

To date, δ-CAs have only been described in a few diatoms [64]. Like the first three CA families, the δ-CA families represent a case of convergent evolution, with almost no sequence similarity to α-, β- and γ–CAs [65]. The structure and geometry of the ~34 kDa enzyme was found to be similar to that of α- and γ-CA [66].

ζ-Carbonic anhydrases

This family of CA genes, also restricted to marine protists, is somewhat similar to the β-CA family [67–69]. What distinguishes this CA family from the β-CA family is that other metals, such as Cd or Co, can replace Zn in the active site [67–69].

ζ-CA, also called cadmium CA, was first isolated from the marine diatom *T. weissflogii* [68]. Seawater is mainly depleted in Zn^{2+} ions. Therefore, under strict Zn^{2+} limitation, the activity of the Zn-dependent δ-CA TWCA1 decreases sharply and is replaced by another CA using Cd^{2+} ions in its active centre. The amino acid sequence of CDCA and its exact molecular mass (69 kDa) were later determined. CDCA had no significant homology to proteins from other known classes of CAs and was therefore assigned to the new ζ-class CAs. Genes encoding CDCA homologues have been found in a number of other diatoms [67]. CDCA is a monomer with an amino acid sequence of consists of three repeats (R1-R3) that share 85% identity, while the coding DNA sequences share a much lower percentage of homology [69]. Each of the R1-R3 repeats contains an active site that structurally mimics the type I β-CA site [70].

η-Carbonic anhydrases

The η-family has been found in the protozoan parasite, *Plasmodium falciparum*, which causes human malaria [71]. This is a group of enzymes previously thought to belong to the alpha family of CAs, but it has been demonstrated that η-CAs have unique features, such as the structure of metal ion coordination. De Simone et al. [72]. showed that the metal ion coordination structure of this CA is unique among all six other gene families encoding such enzymes. In the active centre of η-CA, zinc is coordinated by two His residues and one Gln, in addition to a water molecule/hydroxide ion (Fig. 5), which acts as

a nucleophile in the catalytic cycle. Despite the fact that η- and α-CAs share the same three-dimensional structure, strongly suggesting the evolutionary origin of the former from the latter, there are significant differences between the two families, which will allow the design of selective inhibitors of the parasite to be optimised without harming the host enzymes.

ι-Carbonic anhydrases

The iota class, the newest class of CAs, was discovered in the marine diatom *Thalassiosira pseudonana* and is widespread in marine phytoplankton [73]. In diatoms, ι-CA is localised in chloroplasts and is only expressed at low CO_2 concentrations, suggesting an important role for this enzyme in CO_2 concentration mechanisms. Unlike other classes, ι-CA can use manganese instead of zinc as a metal cofactor, which has potential ecological significance since Mn^{2+} is more abundant in the ocean than Zn^{2+}. Its homologues are present in several sequenced diatoms and other algae, bacteria and archaea. Homologues of ι-CA have also been identified in Gram-negative bacteria, where they may be present as the protein homodimer CA. Thus, ι-CA is phylogenetically widespread, as confirmed by analysis of the Tara Oceans database [73]. This suggests that CAs are likely to play an important role in the global biogeochemical carbon cycle.

Structure of the active center and catalytic mechanism of carbonic anhydrases

The catalytic mechanism of CA was first described for human α-CA II (hCA II) [74]. The discovered catalytic mechanism was termed 'Zn hydroxide' because the catalytically active group of hCA II is Zn-bound water ionised to a hydroxide ion. The metal ion itself is not a nucleophile to act as a catalyst. It was later found that all classes of CAs utilise a metal hydroxide mechanism, using divalent metal hydroxide derivatives as catalytically active structures [24].

Crystallographic studies have shown that the active centres of CAs are usually a tetrahedral cavity formed by three amino acid residues and a deprotonated water molecule (hydroxide ion), which act as coordinating ligands for Me^{2+} (Fig. 5). The metal ion (usually Zn^{2+}) is hydrogen-bonded to the amino acids surrounding the active site [24].

These ligands, as well as the amino acids involved either in catalysis or in forming the correct structure of the active site, are strictly conserved within each class of CAs. In α-, γ- and δ-CA, three his residues coordinate the Me^{2+} ion and the fourth ligand is a hydroxide ion.

All β-CAs are divided into two types depending on the nature of the fourth ligand in the active centre and the adjacent amino acid residues [24, 75] (Fig. 5). In type I β-CAs (so-called "open active site" enzymes), Me^{2+} is coordinated by one His, two Cys and one hydroxide ion. Type II β-CAs (enzymes with a "closed active site") use an Asp instead of a hydroxide ion (Fig. 5). These enzymes are only active at pH above 8.3. It is this environment that favours the transition from a "closed active site" to an "open site" where the water molecule (hydroxide ion) occupies the position of the fourth ligand required for catalysis. All β-cases require dimerisation for catalysis [75], so a single catalytically active subunit has two active centres, each carrying Me^{2+}.

The structure of the ζ-CAS active centre is similar to that of the type I β-CA active centre [76]. In η-CA, Me^{2+} is coordinated by two His residues and one Gln residue in addition to the hydroxide ion [72].

The catalysis takes place in two steps [77]. In the first step, CO_2 is converted to HCO_3^-.

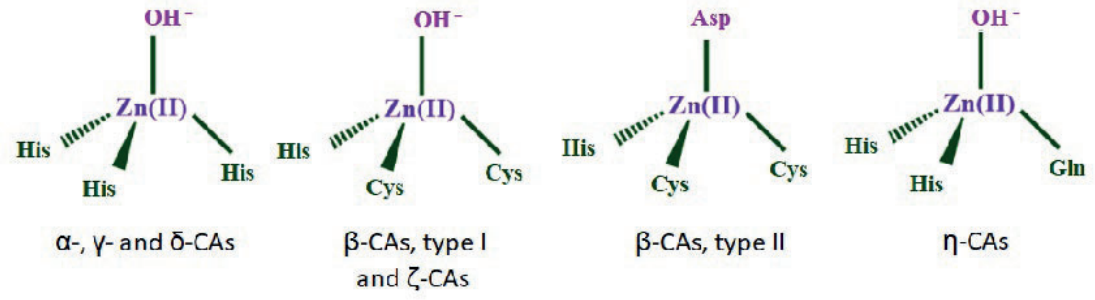

Fig. 5. **Schematic representation of active sites of different classes of CAs. Ligands coordinating metal ion (Zn^{2+}) in the active centre of enzymes are shown**

The metal hydroxide derivative in the active site of the enzyme (E) is a strong nucleophile that attacks the CO_2 molecule. This step results in the formation of a bicarbonate ion bound to Me^{2+} (Equation 2). The bicarbonate ion is replaced by a water molecule and released into solution.

$$E\text{-}Me^{2+}\text{-}OH^- + CO_2 \rightleftharpoons E\text{-}Me^{2+}\text{-}HCO_3^- \overset{H_2O}{\rightleftharpoons} \rightleftharpoons E\text{-}Me^{2+}\text{-}H_2O + HCO_3^- \quad (2)$$

This results in the formation of an inactive acid form of the enzyme in which water is coordinated to Me^{2+}.

In the second step, the active basic form of the enzyme is regenerated by ionisation of the water molecule bound to the metal and removal of a proton from the active centre to an external buffer (Equation 3).

$$E\text{-}Me^{2+}\text{-}H_2O \rightleftharpoons E\text{-}Me^{2+}\text{-}OH- + H^+ \quad (3)$$

In some cases, this step is facilitated by individual amino acid residues close to the active site of the enzyme that act as intermediates in the proton transfer [23]. The removal of protons from the active site and the reduction of the metal-bound hydroxide is the limiting step in the catalytic process.

In the highly active hCA II, the proton-translocating residues are complemented by a His cluster protruding from deep within the active centre to the enzyme surface. This cluster promotes even more efficient proton removal.

This makes hCA II the most active enzyme known to date — the rate of its catalysis is essentially limited only by the rate of ion diffusion [78].

A characteristic feature of all CAs, at least of those proteins, is the "bipolar" architecture of the active site: one half of the active site is covered with hydrophobic residues and the other half, the opposite half, is covered with hydrophilic amino acids [79].

This dual nature is due to the fact that the enzyme substrates, CO_2 and HCO_3^-, have different chemical properties. At the beginning of the reaction, the CO_2 molecule binds to a specific part of the hydrophobic region of the active centre of the CA site (hydrophobic pocket]; this facilitates the subsequent nucleophilic attack [79]. The hydrophilic moieties ensure regeneration of the active site by removing a proton from the zinc-bound water and releasing it to the external environment by means of proton-translocating groups. Table 1 summarizes typical kinetic parameters of the many carbonic anhydrase isozymes.

The data, which include enzymes from all six CA families, show that hCA II (α-class CA) or ZnCA1-R1 (ζ-class CA) are among the enzymes with the highest turnover numbers described so far.

The table shows data on α-class CAs: human cytosolic isoenzyme hCA II; bovine (wtbCAII) and the bacterium SazCA (from SulfuriHydrogenibium azorense); the β-class includes the enzyme Can2 from the fungus

Table 1. **Kinetic parameters for the CO_2 hydration reaction catalysed by various CAs belonging to the various families**

	Class	Organism	k_{cat} (s^{-1})	k_{cat}/KM [$(Ms)^{-1}$]	References
hCA I	α	Human	2.0×10^5	5.0×10^7	[78]
hCA II	α	Human	1.4×10^6	1.5×10^8	[70]
wtbCAII	α	Bovini	9.3×10^4	1.198×10^8	[80]
SazCA	α	Bacterium	4.4×10^6	3.5×10^8	[81]
Can2	β	Fungus	3.9×10^5	4.3×10^7	[82]
FbiCA1	β	Plant	1.2×10^5	7.5×10^6	[83]
PgiCA	γ	Bacterium	4.1×10^5	5.4×10^7	[84]
CdCA1-R1	ζ	Diatom	1.5×10^6	1.4×10^8	[85]
ZnCA1-R1	ζ	Diatom	1.4×10^6	1.6×10^8	[70]
TweCA	δ	Diatom	1.3×10^5	3.3×10^7	[76]
PfCA	η	Protozoa	1.4×10^5	5.4×10^6	[71]

Cryptococcus neoformans FbiCA1 from the plant *Flaveria bidentis*; γ-class enzyme — PgiCA from the anaerobic bacterium *Porphyromonas gingivalis*; δ- and ζ-class enzymes from the diatom *Thalassiosira weissflogii*; ζ-class CA with zinc (ZnCA1-R1) and cadmium (CdCA1-R1); η-CA (PfCA) from *Plasmodium falciparum*.

As the most active enzyme, hCA II is often tested in CO_2 capture projects from industrial gas emissions [20, 25, 77]. Carbonic anhydrase from bovine erythrocytes, although less active than the hCA II isoform, is one of the most studied forms of CA and is promising for use in flue gas decarbonisation programmes [86–88].

The combustion of fossil fuels produces not only CO_2 but also nitrogen and sulphur oxides, from which nitrates, nitrites, sulphates and sulphites are formed in the aquatic environment. The influence of these anions on the activity of CA is of interest when designing reactors for CO_2 sequestration. The results of the study of these effects show that the enzymatic activity of hCA II is only slightly inhibited by NO_3^-, NO_2^-, SO_4^{2-}, HSO^{3-} [89]. η-CA (PfCA) proved to be much more sensitive to these anions [71].

Other impurities of flue gases are heavy metal ions that inhibit to varying degrees carbonic anhydrases hCAII, hCAI and α-CA from different vertebrate and invertebrate species [90].

Comparative analysis of the results obtained when studying the effects of heavy metals on plants [91] and multiple forms of plant carbonic anhydrases [92–94] allows us to conclude that plant β-carbonic anhydrases are more sensitive to the inhibitory effects of heavy metals than animal α-CAs. The reason for the high susceptibility of β-CAs to Me^{2+} seems to be the presence of SH-containing amino acids (cys) in their active centre (Fig. 5), which have a high affinity for heavy metal ions. Obviously, these characteristics should be taken into account in the design of reactors, depending on the composition of the flue gases to be decarbonised, for which one or other CA will be used.

Influence of temperature on the structure and activity of carbonic anhydrases

At high temperature, enzymes lose their biological activity and are irreversibly denatured by denaturation. This inactivation by thermal denaturation has a profound effect on enzyme productivity [95]. The temperature limitation of enzymes is an important parameter for industrial applications, affecting the cost of the process if the enzyme cannot be reused. Lavecchia and Zugaro studied the effect of thermal denaturation on the catalytic properties of carbonic anhydrase from bovine erythrocytes [96]. It was found that at temperatures below 60 °C the enzyme is very stable, whereas at temperatures between 60 and 65 °C there is a sharp decrease in biological activity followed by irreversible loss of activity.

Below 60 °C the enzyme recovers almost completely its biological activity, whereas in the range 60–65 °C a considerable decrease in the catalytic activity takes place, above 65 °C the residual activity drops to zero after few minutes. Thermal transition between native and partially unfolded carbonic anhydrase is a highly cooperative process. The observed loss of activity appears to be a consequence of large conformational changes affecting all the molecule and causing the unfolding of the protein. When the enzyme solution is cooled down to 25 °C in order to measure the catalytic activity, the structural rearrangements of the polypeptide chain are likely to result in an uncorrected reconstitution of the active site region.

As the turnover rate of hCA II is much higher than that of other types of CAs, it is attracting increasing attention in CO_2 capture projects. However, its hydratase activity is very sensitive to temperature, and the enzyme is inactivated at 45 °C [97]. This greatly complicates its use as a potential catalyst for accelerating CO_2 absorption from flue gases [98], i.e. at high temperatures and in the presence of trace amounts of pollutants [16, 99].

Recently, many authors have reported a decrease in enzyme activity with prolonged exposure to high temperatures [89, 100, 101]. It became clear that the development of enzymatic CO_2 capture technologies would be impossible without solving the problem of the thermal stability of CAs.

To solve this problem,

1) In thermophilic organisms, enzymes are being sought that maintain high activity as the temperature rises [22, 55, 58, 59, 95, 97, 102, 103];

2) Molecular modelling methods are used with directed replacement of critical amino acids in the CA molecule [72, 77, 95, 99, 101, 104, 105];

3) Developing methods and carriers for immobilizing CA to increase its thermal stability, improve enzyme recovery and reuse, and reduce the overall cost of the process [15, 100, 106–108];

4) Scaling up research to develop enzymatic CO_2 sequestration technologies for subsequent industrial implementation [107, 109].

CAs from *Methanobacterium thermoautotrophicum* (CAB) and *Caminibacter mediaatlanticus* DSM 16658 are able to retain more than 40% of their initial hydratase activity at 85 °C when reduced to 1.7×10^4 s^{-1} [22]. Enzymes from *Bacillus subtilis* and *Citrobacter freundii* are stable at ≤ 60 °C but lack hydratase activity [17].

In recent years, four highly thermally stable α-CAs, SspCA from *Sulfurihydrogenibium yellowstonense* YO3AOP1, SazCA from *Sulfurihydrogenibium azorense*, *Thermovibrio ammonificans* (TaCA) and *Persephonella marina* EX-H1 (PmCA), have been extensively studied.

Capasso et al, [110] reported a highly thermally stable recombinant α-CA (SspCA), which was identified and characterised from the thermophilic bacterium *Sulfurihydrogenibium yellowstonense* sp. YO3AOP1. It retains its high catalytic activity for the CO_2 hydration reaction even after heating at 70 °C for several hours.

Russo et al, [100] developed a novel recombinant CA, SspCA, isolated from the thermophilic bacterium *Sulfurihydrogenibium yellowstonense* sp. YO3AOP1. They also reported that the half-life of the enzyme at pH ≈ 8.3 was 53 days at 40 °C and 8 days at 70 °C.

De Luca et al. and Vullo et al. [102] reported the biochemical properties, thermostability and inhibition of a novel α-CA enzyme, SazCA, obtained by translational analysis of the genome of the thermophilic bacterium *Sulfurihydrogenibium azorense*. The SazCA enzyme is highly thermostable, withstanding incubation temperatures of 90–100 °C for several hours without loss of activity. However, SazCA is highly sensitive to most inorganic anions (except sulphate), which inhibit the enzyme at concentrations of about 1 mM.

So for today, SazCA has the highest catalytic hydratase activity of 3.5×10^8 $M^{-1}\cdot s^{-1}$ at 20 °C [102], which is more than twice the activity of hCA II. The enzyme retains high hydratase c after 3 h incubation even at 100 °C, which may be due to the substitution of two amino acids involved in proton transfer: Glu2 for His2 and Gln207 for His207 [72]. These two mutations could affect the pKa of the proton shuttle and consequently its ability to transfer the proton [72].

Using the methods of molecular modeling, it was possible to increase the thermal stability of the spacecraft due to such strategies as increasing the stiffness of the surface loop [104] and compactness of the surface [105, 111], reducing surface hydrophobicity [99] and introducing a conservative disulfide bridge [77].

Enzyme immobilisation

CA enzymes are rather unstable molecules with a limited range of working conditions, and their use in their free form is not recommended because they cannot be extracted from the reaction environment [108], which severely limits their large-scale industrial applications. Also, the use of free enzyme dissolved in the solution phase is not favourable due to the significant amount of enzyme required in enzymatic processes. Enzyme denaturation is another challenge, resulting in the loss of enzyme CO_2 hydration activity over time. Enzyme immobilisation allows mobile enzymes to be confined by attaching them to an inert, insoluble material. This can provide increased resistance to changes in pH or temperature. It also allows the enzyme to remain in a stabilised state throughout the reaction, after which it can be easily separated from the products and reused. This greatly increases the efficiency of the process and is therefore widely used in industry for reactions dependent on enzymatic activity [112]. Therefore, enzyme immobilization is a rational approach to overcome these limitations and develop a viable CO_2 capture system using aqueous solvents.

Immobilisation of carbonic anhydrase can offer several advantages, including improved stability, reusability and easier separation of the enzyme from the reaction medium. Various supports and immobilisation strategies have been explored to immobilise carbonic anhydrase with the aim of achieving good activity and stability compared to free enzyme in solution [15, 107].

The immobilisation strategy is crucial for maintaining the activity and stability of immobilised carbonic anhydrase. Various strategies such as physical entrapment (encapsulation), covalent binding and adsorption have been used to immobilise carbonic anhydrase [112, 113].

Physical entrapment involves the encapsulation of the enzyme within the carrier matrix, which provides protection and maintains the enzyme activity. Enzyme encapsulation is an irreversible immobilisation method in which enzymes are entrapped within the fibre, either in polymer membranes or in lattice structures of the material, which supply the substrate and remove products from the enzyme. Capture involves the physical confinement of the enzyme within a limited

network space. Mechanical stability, enzyme leaching and chemical interaction with the polymer are generally improved when the enzyme is immobilised by encapsulation. This method modifies the encapsulating material to provide an optimal microenvironment for the enzyme. An ideal microenvironment with optimal pH, polarity or amphiphilicity can be achieved by using different support materials [113].

Adsorption. Adsorption involves the non-covalent binding of the enzyme onto the surface of the carrier, which can be reversible and allows for enzyme release. This method is based on physical interactions between proteins and solid support surfaces through van der Waals forces, hydrogen bridges and electrostatic interactions. Adsorption immobilisation of enzymes is relatively simple and can have higher commercial potential, lower cost and higher efficiency with relatively weak (non-covalent) binding of the enzyme.

However, the physical binding is generally too weak for the enzyme to remain attached to the support and not be subject to enzyme leaching, resulting in significant substrate contamination [108, 113].

Covalent binding and cross-linking. Covalent binding involves the formation of covalent bonds between the enzyme and the carrier, which provides strong attachment and stability. The technique of covalent enzyme immobilisation is one of the most prominent. The formation of covalent bonds is required for more stable binding, and these are generally formed by reaction with functional groups present on the protein surface. The functional groups that contribute to enzyme binding include side chains of lysine (amino group), cysteine (thiol group) and aspartic and glutamic acids (carboxyl group) [114]. The activity of the covalently bound enzyme depends on the coupling method, the composition of the carrier material, as well as its size and shape and the specific conditions during coupling [15,107].

Support materials for CA immobilization. The choice of the support material is an important factor which must be considered, as it has an important effect on the performance of biocatalytic system [108]. Some features should be considered for support material selection such as availability, low cost, functional group availability, mechanical stability, and compatibility with the enzyme.

1. *Polymer-based materials:* Polymers such as polyvinyl alcohol (PVA), polyvinyl acetate (PVA), polyethyleneimine (PEI), polyurethane (PU), and polyethyleneglycol (PEG)have been widely used as support materials for CA immobilization. These polymers have high mechanical stability, tunable porosity and can provide a stable matrix for the enzyme.

2. *Silica-based materials:* Silica-based materials, such as silica gels, mesoporous silica, and silica nanoparticles, have been extensively studied for CA immobilization. They have a high surface area, which provides a large amount of immobilization sites for the enzyme. Silica-based materials also have good compatibility with the enzyme and can provide a stable environment for its activity.

Inorganic materials, such as silica nanoparticles, gold nanoparticles, and titanium dioxide nanoparticles, offer unique properties for enzyme immobilization. They provide stability and controlled release of the enzyme, which can enhance the overall performance of the CO_2 capture process.

3. *Metal-organic frameworks (MOFs):* MOFs are a class of porous materials composed of metal ions connected by organic ligands. These materials have shown great potential as support materials for CA immobilization due to their high porosity and tunable structures. MOFs can provide a controlled environment for the enzyme and improve its stability and catalytic activity.

4. *Mesoporous materials*: Mesoporous materials, such as silica, alumina, and zeolites, have attracted attention for enzyme immobilization. These materials provide a high surface area and a well-defined pore structure, allowing for efficient loading and dispersion of CA enzyme [115].

5. *Magnetic nanoparticles*: Nanoparticles, such as magnetic nanoparticles and gold nanoparticles, have been explored as carriers for immobilizing carbonic anhydrase. These nanoparticles can provide high surface area-to-volume ratios and can be easily separated from the reaction medium using external magnetic fields or centrifugation.

6. *Carbon-based materials*: Carbon-based materials, such as activated carbon, carbon nanotubes, and graphene oxide, have been investigated for CA immobilization. These materials have a large surface area, good mechanical stability, and are chemically inert, making them suitable support materials for enzyme immobilization.

Conclusions

Numerous studies in recent years have shown that the use of carbonic anhydrase for enzymatic CO_2 capture has significant potential

to create a cost-effective technology. However, the industrial application of enzymes in carbon capture processes is limited by their high cost, low catalytic activity, poor stability over time, high sensitivity to temperature, low tolerance to pollutants such as sulphur compounds and reusability. To overcome these limitations, further developments are needed to improve the economics. Carbonic anhydrases are found in all organisms, from protozoa to humans. Not all of them have been studied, so the search for new stable enzymes among organisms with enormous genetic diversity remains an urgent task. The use of molecular modelling has already led to impressive successes in the design of thermostable enzymes, and further developments in this area are expected. Enzyme immobilisation offers great potential for the industrial production of carbonic anhydrase in captured CO_2. Advances in support materials, immobilisation strategies and reactor design could lead to the development of efficient and cost-effective enzymatic CO_2 capture systems.

Significant progress is also expected in areas such as the conversion of captured carbon dioxide into chemicals such as carbon monoxide, methanol, formic acid, glucose or methane, and fuels by chemical, electrochemical and enzymatic methods.

Few laboratory processes have been tested on a large scale, and refinement of their details on an industrial scale is necessary to create sustainable technologies.

In summary, the research carried out confirms the remarkable potential of some CAs as biocatalysts and gives hope for the feasibility of introducing biomimetic processes into CO_2 sequestration technology.

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БІОКАТАЛІТИЧНЕ УЛОВЛЮВАННЯ ВУГЛЕКИСЛОГО ГАЗУ ЗА УЧАСТЮ КАРБОНГІДРАЗИ

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Швидке та постійне зростання концентрації CO_2, найпоширенішого парникового газу в атмосфері, призводить до екстремальних погодних та кліматичних явищ. Унаслідок спалювання викопного палива (нафти, вугілля та природного газу) концентрація CO_2 в повітрі зростала упродовж останніх десятиліть більше ніж на 2 ppm на рік, і лише за останній рік — на 3,29 ppm. Для запобігання «найгіршим» сценаріям зміни клімату, необхідно негайно та суттєво скоротити викиди CO_2 шляхом управління викидами вуглецевого газу.

Мета. Аналіз сучасного стану досліджень та перспектив використання карбоангідрази в програмах декарбонізації навколишнього середовища.

Результати. Карбоангідраза це — ензим, який прискорює обмін CO_2 та HCO_3^- у розчині від 10^4 до 10^6 разів. На сьогоднішній день в різних організмах ідентифіковано 7 типів карбоангідраз. Карбоангідраза потрібна для забезпечення швидкого постачання CO_2 та HCO_3^- для різних метаболічних шляхів в організмі, що пояснює її багаторазове незалежне походження під час еволюції. Ензими, виділені з бактерій і тканин ссавців, були протестовані в проектах секвестрації CO_2 з використанням карбоангідрази. Найбільш вивченою є одна з ізоформ карбоангідрази людини — hCAII — найбільш активний природний ензим. Його недоліками були нестабільність у часі, висока чутливість до температури, низька толерантність до забруднень, таких як сполуки сірки, і неможливість повторного використання. Найбільш вивченою в цьому відношенні є одна з ізоформ карбоангідрази людини — hCAII — найбільш активний природний ензим. Для подолання цих обмежень використовували методи молекулярного моделювання та іммобілізації ензимів. Показано, що іммобілізація забезпечувала більшу термічну стабільність і стабільність під час зберігання, а також підвищувала можливість повторного використання.

Висновки. Карбоангідрази беруть участь у біологічній асиміляції атмосферного CO_2. Тому, вивчення таких ензимів та умов, необхідних для їх активації та ефективного поглинання CO_2, створює підґрунтя для розроблення біокаталітичних засобів підвищення декарбонізації атмосфери.

Ключові слова: зміна клімату; декарбонізація; біосеквестрація CO_2; карбоангідраза; іммобілізація ензимів.