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The γ-carbonic anhydrase from the pathogenic bacterium *Vibrio cholerae* is potently activated by amines and amino acids

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ABSTRACT

The γ-class carbonic anhydrase (CAs, EC 4.2.1.1) from the pathogenic bacterium *Vibrio cholerae*, VchCAγ, was investigated for its activation with a panel of natural and non-natural amino acids and amines. The enzyme was effectively activated by L-tryptophan, 1-(2-minoethyl)-piperazine and 4-(2-aminoethyl)-morpholine, in the low nanomolar range (K_As 8–71 nM). In contrast, L-/D-Phe, L-/D-DOPA, D-Trp, L-/D-Tyr, 4-amino-L-Phe, histamine, dopamine, serotonin, some pyridyl-alkylamines, as well as L-adrenaline were submicromolar activators (K_As between 0.10 and 0.73 μM). L- and D-His were the least effective VchCAγ activators (K_As of 1.01–14.2 μM). The activation of CAs from bacteria have not been considered to date for possible biomedical applications. It would be of interest to study in more details the role of CA activators in processes connected with the virulence and colonization of the host by pathogenic bacteria, such as *Vibrio cholerae*, which is highly dependent on the concentration of bicarbonate in tissues.

1. Introduction

The γ-class carbonic anhydrases (CAs, EC 4.2.1.1) were discovered more than two decades ago by Ferry's group [1–3] and were less investigated compared to other members of this superfamily of metalloenzymes (i.e., α-, β-, δ-, ζ-, η- and θ-CAs) that are known to date [4–12]. By catalyzing the reversible interconversion of CO_2 to bicarbonate and hydronium ions, CA enzymes are involved in a plethora or biologically essential processes related to pH regulation, electrolyte secretion, metabolic processes important in carboxylation/decarboxylation reactions, photosynthesis, and more [13–15]. γ-CAs were originally discovered in Archaea, and subsequently identified to be widely distributed in bacteria and the mitochondria of plants [1–3,9,11,12]. Regulation of CA activity using either inhibitors or activators has pharmacologic and potentially also environmental applications [7,8]. Indeed, the CA inhibitors (CAIs) have applications as drugs for the management of many diseases in which the activity of these enzymes is upregulated/imbalanced, such as glaucoma [16], edema [17], epilepsy and obesity [18], tumors [19–21], neuropathic pain [22], and cerebral ischemia [23]. All inhibitors with pharmacological/clinical applications that have been used to date target mammalian (human) α-CAs, with 15 different isoforms known in primates [4–8]. Recently, the inhibition of CAs from pathogenic organisms (bacteria, fungi, protozoa) has emerged as a promising strategy for designing antibiotics that have a unique mechanism of action compared to the modes of pharmaceutical drugs that are currently used [14,24–27]. In contrast, CA activators (CAAs) have been less thouroughly investigated [28–32]. However, CAAs have recently been shown to be potentially useful in memory therapy and cognitive enhancement [33]. Very recently, the first activation studies of non-vertebrate CAs has been performed for several bacterial α-, β- and γ-class CA enzymes from pathogenic and non-pathogenic bacteria (such as *Burkholderia pseudomallei* [34,35] and *Sulfurihydrogenibium yellowstonense* [36]). The activation of CAs from pathogenic bacteria may be relevant for understanding the factors governing virulence and colonization of the host, because pH in the tissues surrounding the pathogens likely plays a key role in such processes and many compounds that are CAAs (amines, amino acids) are abundant in such tissues. Moreover, studies that focus on the CAA phenomena in such pathogens have not been reported previously for CAs from *Vibrio cholerae*, and have been limited exclusively to *Burkholderia pseudomallei* [34,35]. Here we present the first activation study of the γ-CA from *Vibrio cholerae* (VchCAγ) [37] using a series of amino acid and amine derivatives. This pathogen was selected because it is the etiological agent of cholera.

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2. Results and disscussion

CA activation is a well understood phenomenon, especially for the α-CAs, for which detailed kinetic, spectroscopic and X-ray crystallographic studies are available for the interaction of various enzymes with amine and amino acid activators [28,29,38–42]. The activators have a key role in the catalytic cycle for the conversion of CO_2 to bicarbonate and hydronium ions (Eqs. (1) and (2)). In the rate-determining step (Eq. (2)), the nucleophilic zinc hydroxide species of the enzyme is formed by release of a proton from a water molecule that is coordinated to the zinc ion [28,29,38–42].

$$EZn^{2+}—OH^- + CO_2 \Longleftrightarrow EZn^{2+}—HCO_3^- \overset{H_2O}{\Longleftrightarrow} EZn^{2+}—OH_2 + HCO_3^- \quad (1)$$

$$EZn^{2+}—OH_2 \overset{H_2O}{\Longleftrightarrow} —EZn^{2+}—HO^- + H_3O^+ \quad (2)$$

$$EZn^{2+}—OH_2 + A \overset{H_2O}{\Longleftrightarrow} [EZn^{2+}—OH_2 - A] \Longleftrightarrow [EZn^{2+}—HO^- - AH^+] \Longleftrightarrow EZn^{2+}—HO^- + AH^+ \quad (3)$$

This process can be assisted by active site amino acid residues which act as proton shuttles [28]. In α-CAs, one or more His residues (e.g., His64) can be involved as a proton shuttle based on kinetic and X-ray crystallographic studies [28,29,38–42]. In the presence of activators (A in Eq. (3)), an enzyme – activator complex is formed in which the proton shuttling is facilitated by the bound activator (Eq. (3)) in an intramolecular (not intermolecular) process [28,29,38–42]. X-ray crystallography results indicate that amine and amino acid activators, such as histamine, d-/l-histidine, d-/l-phenylalanine, l-adrenaline or d-tryptophan [28,29,38–42] bind at the entrance of the active site cavity in the human (h) isoforms hCA I and II, near the natural proton shuttle (His64) and participate in the rate-determining step of the catalytic cycle (Eq. (3)).

However, for γ-CAs, no X-ray crystal structures of enzyme-activator complexes have been reported and very few X-ray crystal structures of such enzymes are available [1–3]. However, site directed mutagenesis and kinetic experiments suggest that at least two glutamate residues, Glu84 and Glu62 (*Methanosarcina thermophila* γ-CA (Cam) numbering system [43]), can act as proton shuttle residues for these enzymes. Moreover, these two residues are conserved in all γ-CAs for which the amino acid sequences are available [43]. Enzyme mutants in which Glu84 and Glu62 were substituted by alanine residues that cannot participate in proton transfer processes resulted in a decrease in k_{cat} of up to 10 times compared to the wild-type enzyme and the activity could be largely recovered by addition of imidazole (CAA) to the buffer solution [43,44]. That is, in γ-CAs, the two glutamate residues (presumably through their COO^- moieties) take part in the proton shuttling process (Eq. (3)), in the same fashion in which His64 plays this function in the α-CAs [44]. Thus, we have included

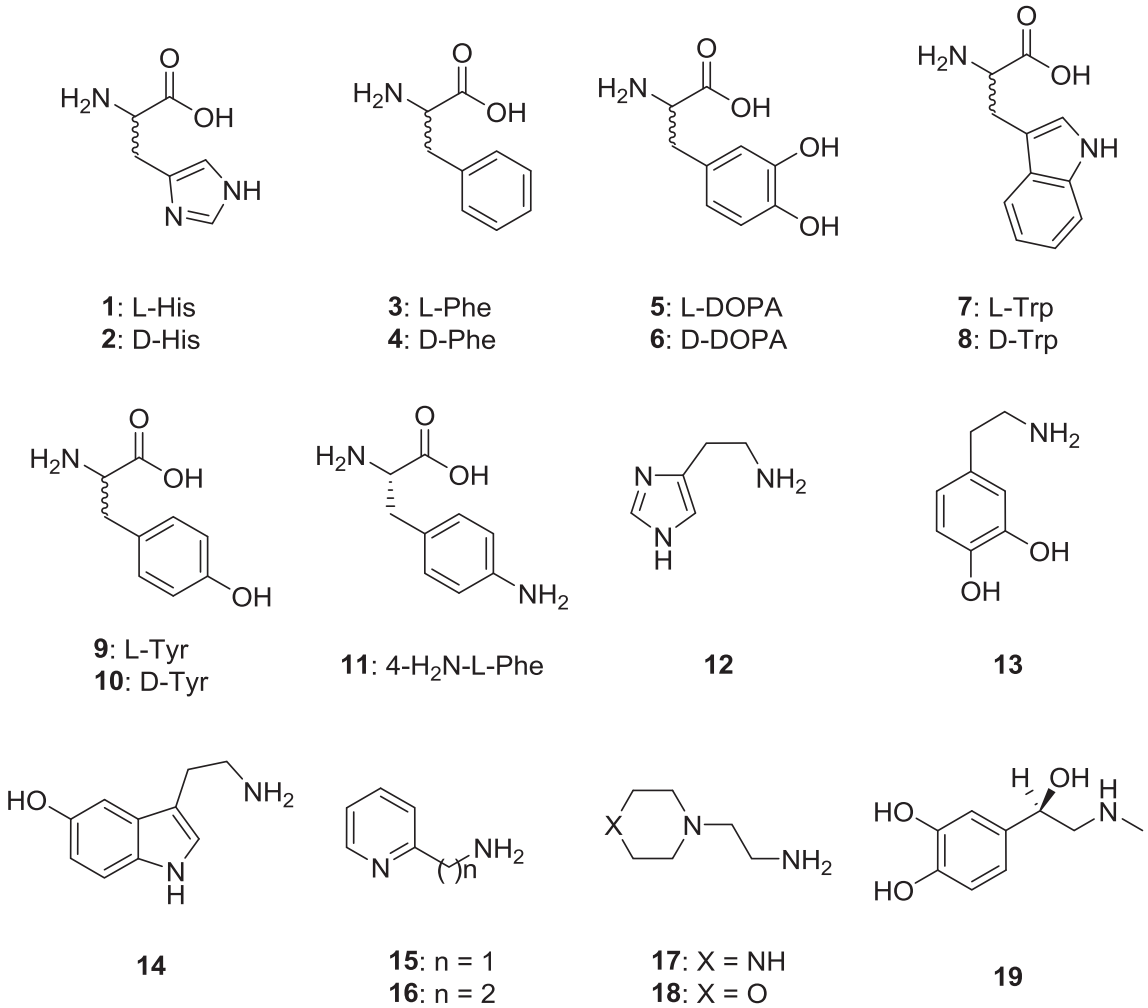

Fig. 1. Amino acids **1**–**11** and amines **12**–**19** investigated as VchCAα/β activators.