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The first activation studies of the η-carbonic anhydrase from the malaria parasite *Plasmodium falciparum* with amines and amino acids

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ABSTRACT

The first activation study of a η-class carbonic anhydrase (CAs, EC 4.2.1.1) is reported. A panel of 24 natural and non-natural amino acids and amines was used to explore the activation profile of *Plasmodium falciparum* CA (PfACA). The most effective activators belonged to the amino acid chemotype, with D-Glu, L-Asp, L-/D-Phe and L-/D-DOPA possessing activation constant in the range of 82 nM–0.75 μM, whereas L-/D-His, L-Tyr, 4-amino-L-Phe and L-Gln were slightly less effective (K_A in the range of 1.00–2.51 μM. The only amine with submicromolar activating properties was 1-(2-aminoethyl-piperazine) with a K_A of 0.71 μM, whereas histamine, dopamine and serotonin showed K_A ranging between 7.18 and 9.97 μM. As CA activators have scarcely been investigated for their interaction with protozoan CAs, this study may be relevant for an improved understanding of the role of this enzyme in the life cycle of the malaria producing organisms belonging to the genus *Plasmodium*.

1. Introduction

Malaria represents one of the most widespread infections worldwide, leading to serious morbidity and mortality mainly within but not limited to the developing countries [1,2]. The extensive drug resistance problems and the climate change are only some of the factors contributing to the intensification of the crisis, with no new drugs available for a long period and with the diffusion of the insect vectors (mosquitoes) also in parts of the world which normally did not experience this disease. This is in fact common to other diseases produced by parasitic protozoa, with *Plasmodium* spp. infection responsible for the highest number of deaths, but *Trypanosoma* spp., *Leishmani* spp and other protozoans are rapidly diffusing from their original part of the world to new territories, such as Europe, North America and Australia [3–5]. The lack of efficient treatment options for most of these protozoan infections makes the research of possible, alternative drug targets for fighting malaria and similar infections quite challenging, considering also the undeserved "orphan" status for many of these diseases [5–7].

We have reported several years ago that *Plasmodium falciparum*, the malaria parasite producing the worst type of malaria, encodes for a genetically distinct class of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1), more specifically the η-CA family (the enzyme was denominated PfaCA) [4,8]. Unlike the remaining 6 genetic families of these enzymes (α-, β-, γ-, δ-, ζ- and θ-CAs), the zinc coordination pattern of PfaCA is unique, with the catalytically crucial zinc ion being coordinated by two His and one Gln residues [8]. The fourth zinc ligand is a water molecule/ hydroxide ion, acting as nucleophile for the CO_2 hydration reaction to bicarbonate and protons, which is the physiological reaction catalyzed by these enzymes. This reaction affords bicarbonate, which is then used in the biosynthesis of pyrimidines by the parasite, in a biochemical pathway unique to the protozoan and different from that of vertebrates [9]. It has been in fact demonstrated that sulfonamide inhibitors of P. falciparum CA (considered in that period to belong to the α-CA class) do show powerful antimalarial effects in vitro and in vivo [9]. Thus, investigation of PfaCA for its interaction with inhibitors may lead to a new approach for inhibiting the growth of this widespread parasite. However, neither PfaCA nor any other η-CAs were yet investigated for their interaction with CA activators (CAAs), the other class of relevant modulators of the activity of these enzymes [10–12]. Indeed, CAAs belonging to various classes were extensively

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investigated for the activation of the mammalian (h, human; m, murine) CA isoforms known to date, CA I – XV [13–17]. Several drug design studies for CAAs belonging to the amine and amino acid classes have also been reported, and led to the discovery of the activation profile of the different isoforms with many classes of activators [13–18], and the mechanism of activation is also well understood at least for the α-CAs, for which many adducts of various isoforms with amine and amino acid activators were reported [10,13,18]. The activators bind at the entrance of the CA active site and through moieties able to participate in proton shuttling facilitate the rate-determining step of the catalytic cycle, the transfer of a proton from the water coordinate to the metal ion to the reaction medium, which in the wild type enzyme is assured by an active site residue, such a histidine placed in the middle of the active site (His64, hCA I numbering is the most frequent proton shuttle residue for the human (h) CAs, such as isoforms hCA II, IV, VII, IX, etc) [10,13].

Recently, the potential of CAAs as pharmacological agents for the therapy of memory disorder and cognition impairment has also been demonstrated [18]. However, unlike CAIs, which are clinically used as diuretics [19], or for the management of glaucoma [20], obesity [21], hypoxic tumor [22], neuropathic pain [23], or arthritis [24], there are no clinically approved CAAs for the moment.

Natural and non-natural amino acids and amines of type **1–24** represent the most investigated simple types of CAAs, and they were evaluated also in the present study for their interaction with the η-class enzyme PfaCA (Fig. 1). These compounds were investigated for their potential as CAAs against many classes of CAs, including a limited number of prokaryotic (bacterial/archaeal) CAs [25]. However, few protozoan CA enzymes have been investigated for their activation profiles to date, except the β-class enzyme from *Leishmania donovanii* chagasi (LdcCA) [26]. Thus, here we report the first activation study of PfaCA, the η-class enzyme from the protozoan *Plasmodium falciparum*,

Table 1
Activation of human carbonic anhydrase (hCA) isozymes I, II, and PfaCA with L-Tyr, at 25 °C, for the CO_2 hydration reaction. [27]

Isozyme	k_{cat}* (s^{-1})	K_M* (mM)	$(k_{cat})_{L\text{-}Tyr}$** (s^{-1})	K_A*** (μM) L-Tyr
hCA I[a]	2.0×10^5	4.0	13.9×10^5	0.020
hCA II[a]	1.4×10^6	9.3	12.8×10^6	0.011
PfaCA[b]	3.8×10^5	5.2	11.2×10^5	1.02

* Observed catalytic rate without activator. K_M values in the presence and the absence of activators were the same for the various CAs (data not shown).

** Observed catalytic rate in the presence of 10 μM activator.

*** The activation constant (K_A) for each enzyme was obtained by fitting the observed catalytic enhancements as a function of the activator concentration. [13] Mean from at least three determinations by a stopped-flow, CO_2 hydrase method. [30] Standard errors were in the range of 5–10 % of the reported values (data not shown).

[a] Human recombinant isozymes, from ref.[13]; b Protozoan recombinant enzyme, this work.

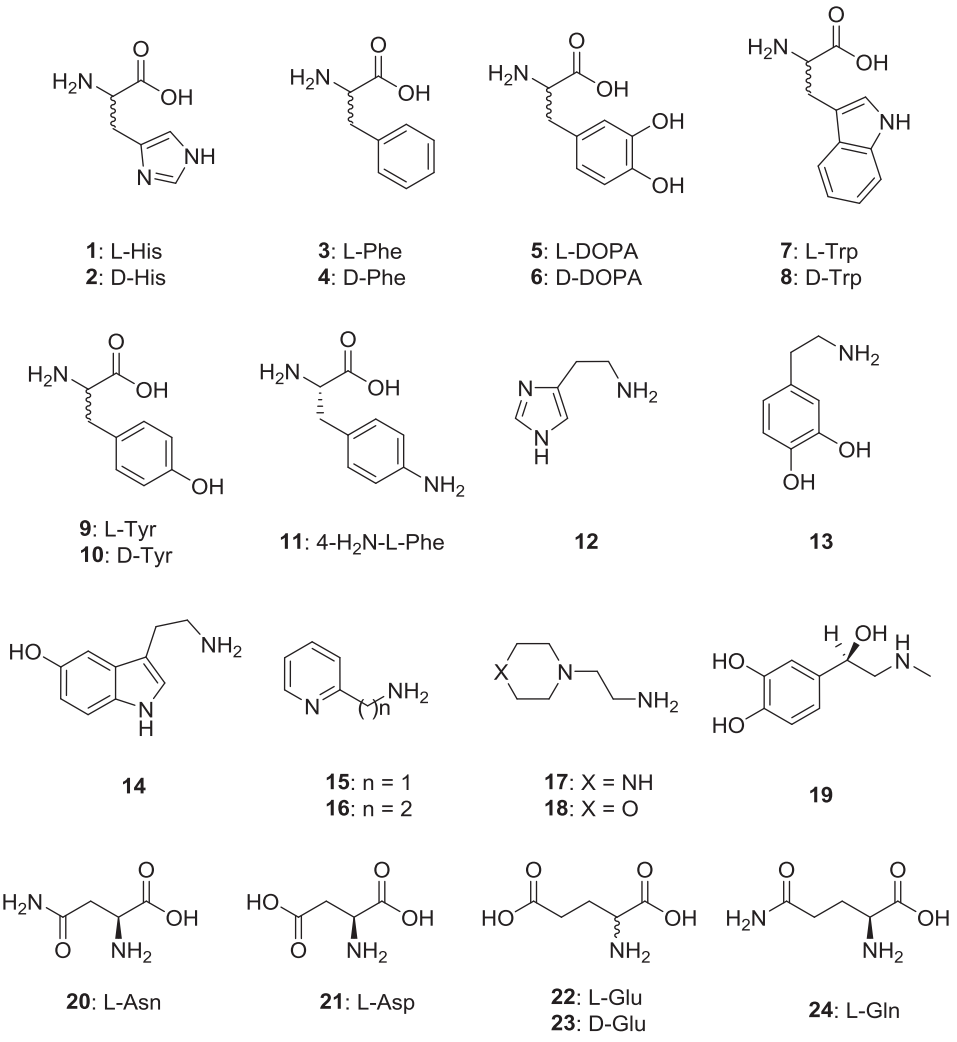

Fig. 1. Amino acids and amines **1–24** investigated as PfaCA activators.