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Supporting information for article:

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Supporting Information

Structural Insights into Activity Enhancement and Inhibition of H64A Carbonic Anhydrase II by Imidazoles

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S1. Methods

S1.1. Enzyme kinetics measurements

^{18}O exchange assay was carried out to study the kinetics of the catalyzed reaction of the hydration and dehydration of CO_2 and HCO_3^- -and how these small imidazoles (I, 1MI, 2MI and 4MI) affect the activity of H64A CA II. The pH values were chosen based on the pK_a of these imidazoles.

The method (Tu et al., 1989) relies on the depletion of ^{18}O from species of CO_2 as measured by membrane inlet mass spectrometry using an Extrel EXM-200 mass spectrometer (Pastorek et al., 1994). In the first stage of catalysis, the dehydration of labeled bicarbonate has a probability of labeling the active site with ^{18}O (eq 1). In a following step, protonation of the zinc-bound ^{18}O-labeled hydroxide results in the release of $H_2{}^{18}O$ to the solvent and loss of signal from the isotopic species (eq 2).

$$HCOO^{18}O^- + EZnH_2O \rightleftarrows EZnHCOO^{18}O^- \rightleftarrows COO + EZn^{18}OH^- \qquad (1)$$

$$H^+His64\text{-}EZn^{18}OH^- \rightleftarrows His64\text{-}EZnH_2{}^{18}O \rightarrow His64\text{-}EZnH_2O + H_2{}^{18}O \qquad (2)$$

This approach yields two rates: The R_1, the rate of CO_2 and HCO_3^- interconversion at chemical equilibrium (eq 1), as shown in Equation 3, and R_{H2O}, the rate of release from the enzyme of water with labeled substrate oxygen (eq 2).

$$R_1/[E] = k_{cat}^{ex}\,[CO_2]/(\,K_{eff}^{CO2} + [CO_2]) \qquad (3)$$

In Equation 3, k_{cat}^{ex} is a rate constant for maximal interconversion of CO_2 and bicarbonate, K_{eff}^{CO2} represents a binding constant for the substrate to enzyme. The ratio $k_{cat}^{ex}/K_{eff}^{CO2}$ is considered equivalent in value to k_{cat}/K_M from steady state experiments, and is a measure of the successful binding and interconversion of substrate and product.

The second rate, R_{H2O}, is the component of the ^{18}O exchange that is dependent upon the donation of protons to the ^{18}O-labeled zinc-bound hydroxide. In such a step, His64 as a predominant proton donor in the catalysis provides a proton (Equation 2). The value of R_{H2O} can be determined and considered as the rate constant for proton transfer from His64 to the zinc-bound hydroxide according eq 4, in which k_B

is the rate constant for proton transfer to the zinc-bound hydroxide and $(K_a)_{donor}$ and $(K_a)_{ZnH2O}$ are ionization constants of the proton donor, His64, and zinc-bound water. The least-squares determination of kinetic constants of Equation 3 and Equation 4 was carried out using Enzfitter (Biosoft).

$$R_{H2O}/[E] = k_B/([1 + (K_a)_{donor} /[H^+]][1 + [H^+]/(K_a)_{ZnH2O}]) \qquad (4)$$

The uncatalyzed and carbonic-anhydrase-catalyzed exchanges of ^{18}O between CO_2 and water at chemical equilibrium were measured in the absence of buffer (to prevent interference from the second intermolecular proton transfer reaction) at a total substrate concentration of 25 mM and 25 °C.

References

Pastorek, J., Pastoreková, S., Callebaut, I., Mornon, J. P., Zelník, V., Opavský, R., Zat’ovicová, M., Liao, S., Portetelle, D., & Stanbridge, E. J. (1994). *Oncogene*. **9**, 2877–2888.

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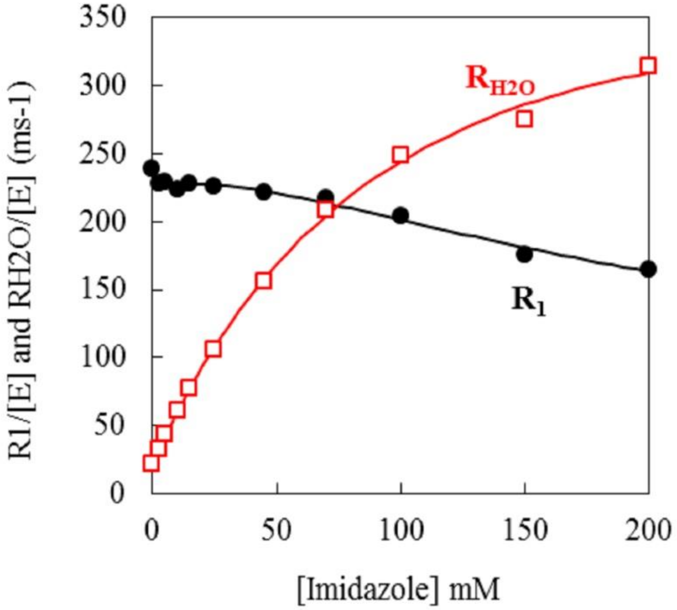
Figure S1 (a) The effect of imidazole (I) on R1/[E] (black) and R_{H2O}/[E] (red) catalyzed by H64A CA II. (b) Crystal structure of H64A CA II in complex with imidazole, showing the site of inhibition (site 3). His64 from wt-CA II is superposed and shown as yellow sticks for orientation perspective.

(a)



(b)

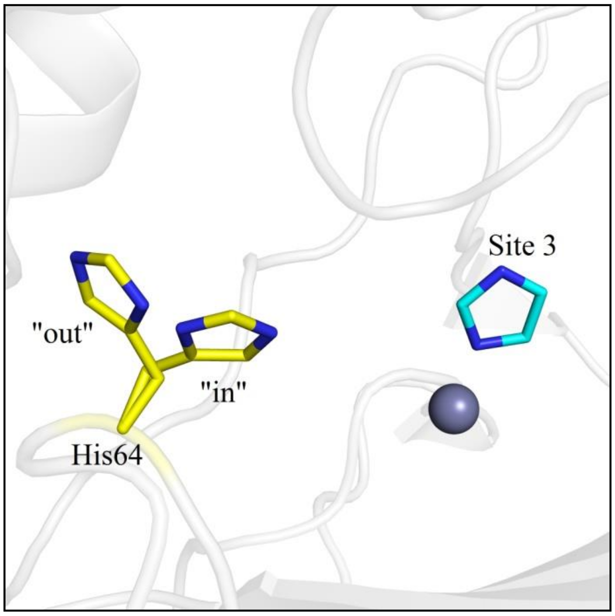
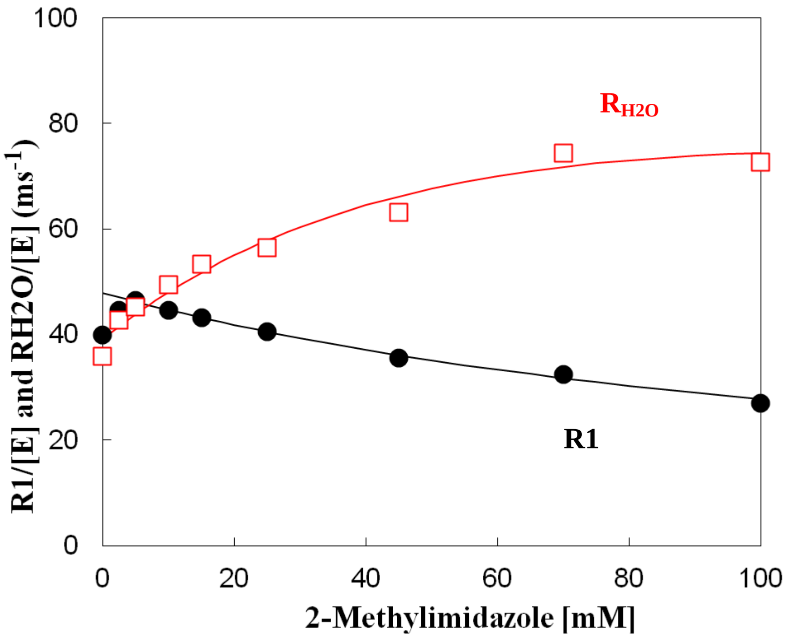


Figure S2 (a) The effect of 2-methyl imidazole (2MI) on R1/[E] (black) and R_{H2O}/[E] (red) catalyzed by H64A CA II. (b) Crystal structure of H64A CA II in complex with 2-methyl imidazole, showing the sites of inhibition (site 3 and most likely also site 4). His64 from wt-CA II is superposed and shown as yellow sticks for orientation perspective.

(a)



(b)

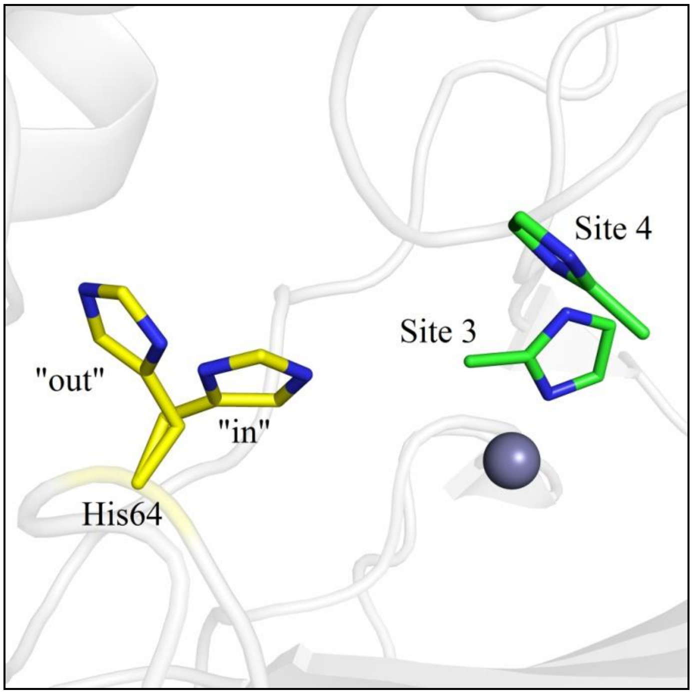


Figure S3 The activity enhancement by exogenous proton donors of R_{H2O}/[E] (s^{-1}) catalyzed by H64A CA II. The proton donors were I (black) at pH 6.7, 1MI (red) at pH 7.3, 2MI (blue) at pH 8.1, and 4MI (green) at pH 7.7. The data were obtained at 25 °C using solutions maintained at a minimal ionic strength of 0.2 M by addition of Na_2SO_4.