Esterase activity of Carbonic anhydrases serves as surrogate for selecting antibodies blocking Hydratase activity

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Supporting information

The enzymes (CA9 & CA12) were titrated with substrate (*p*NPA) from 0.3mM to 35mM in acetone due to very limited solubility of *p*NPA in aqueous solution (1-4) in the absence or presence of 0.3μM AZA, which is greater than the K_d value of AZA (0.02μM for CA2 and 0.04μM for CA4) (5, 6). The V_{max} , K_m and the apparent K_m (K_m^{app}) were calculated according to the Michaelis-Menten equation, curves were plotted using least squares non-linear fitting in Prism6 software (Figure S1). As expected for a competitive inhibitor (7-11), the inhibitor (AZA) increases the measured apparent K_m without affecting the V_{max} to a great extent. The K_m values are normally high for the esterase activity of carbonic anhydrases and in agreement with the published data for other CA isoforms (5, 12, 13). The standard deviation of K_m^{app} is very high and cannot be measured accurately under these experimental conditions, i.e. high AZA concentration (0.3μM) and at the same time not being able to reach the saturating concentrations of the substrate due to limited solubility. From the K_m and K_m^{app} values, the inhibition constant (K_i) was derived using the Michaelis-Menten equation, $K_m^{app} = K_m (1 + [I]/K_i)$. Kinetic and inhibition data are summarized in table S2. Esterase K_i values 26nM (CA9) and 12.5nM (CA12) are comparable with the published hydratase K_i values of CA9 (14) and CA12 (15).

The esterase activity of Carbonic anhydrases is in general very slow and requires high concentration of enzyme to perform the enzymatic assay (6, 16). Here we presented the esterase enzyme activity of CA9 and CA12, which cannot be reliably detectable at a final concentration lower than 0.025μM (Figure S3). Therefore, four times higher concentrations of CA9 (0.093μM) and CA12 (0.114μM) have been implemented for all the assays in order to facilitate robust quantification of the inhibition.

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Figures & Table for Supporting information

Figure S1: Esterase activity of CA9 (A) and CA12 (B) at different concentrations of the substrate (pNPA). AZA is Acetazolamide, 0.3 μ M. Enzyme concentrations are 0.093 μ M (CA9) and 0.114 μ M (CA12). Final concentration of acetone in the reaction is 10% (V/V) for substrate concentrations from 0.3mM to 23mM and 20% at 29mM and 35mM substrate concentrations. The data is representative of two independent experiments in triplicates.

Table S2: Kinetic and inhibition data of CA9 and CA12 esterase activity. Substrate (pNPA) concentrations range from 0.3mM to 35mM. AZA is Acetazolamide, 0.3 μ M. Enzyme concentrations are 0.093 μ M (CA9) and 0.114 μ M (CA12).

	$V_{\max}$ (nmoles/minute)	With 0.3 μ M AZA $V_{\max}^{\text{app}}$ (nmoles/minute)	K_m (mM)	With 0.3 μ M AZA K_m^{app} (mM)	AZA K_i (nM)
CA9	0.67 ± 0.04	1.88 ± 01.56	12.05 ± 1.59	150.2 ± 146.5	26.18
CA12	1.44 ± 0.09	6.53 ± 11.43	13.43 ± 2.04	335.3 ± 634.3	12.52

Figure S3: Esterase activity (1 mM pNPA) at different concentrations of CA9 (A) and CA12 (B) over time. The data is representative of two independent experiments in triplicates. pNP is *para* Nitrophenyl over time.