*--SUPPLEMENTARY INFORMATION --*

# Human Arginase II: Crystal Structure and Physiological Role in Male and Female Sexual Arousal

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Running title: Human Arginase II

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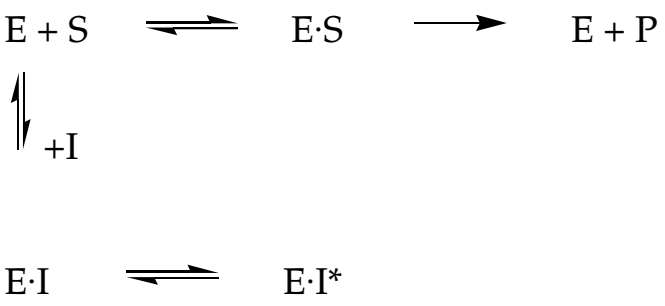
**Inhibition Assays**

Slow-onset inhibition of ΔM1-V23/ΔH331-I354 human arginase II by BEC at pH 9.5 was measured as described for the wild-type enzyme (1) by the addition of enzyme to assay mixtures containing 100 mM-CHES-KOH (pH 9.5), 100 μM $MnCl_2$, 24 mM unlabeled arginine (10 x $K_M$), 0.05 μCi L-[*guanido*-$^{14}$C]arginine (Perkin Elmer Life Sciences), and varying concentrations of BEC. Aliquots were removed at indicated times, and [$^{14}$C]urea was analyzed as described (1). Slow release kinetics were analyzed by incubating the enzyme with 30 μM BEC for 15 min at room temperature, followed by a 100-fold dilution into assay mixture. The production of [$^{14}$C]urea was monitored as described (1). The inhibition constant for BEC was determined from the ratio of $k_{off}/k_{on}$ and also estimated from the final steady-state velocities using the equation for competitive inhibition.

Progress curves for L-arginine hydrolysis in the presence of BEC at pH 9.5 are nonlinear, as shown in Figure 1. The nonlinearity of the progress curves is indicative of slow binding inhibition, typically characterized by the rapid formation of a reversible E·I complex followed by a slow isomerization or conformational change to yield the inhibitory E·I* complex (Scheme I).

*Scheme I*




Progress curves for the arginase-catalyzed production of urea in the presence of BEC at pH 9.5 were fit by nonlinear least-squares analysis to the integrated expression

$$P = v_s(t) + (v_o - v_s)(1 - e^{-k_{obs}(t)})/k_{obs} \qquad (1)$$

where P is the amount of urea formed (in cpm), $v_o$ is the initial rate of urea formation, $v_s$ is the steady-state rate of urea formation, and $k_{obs}$ is the apparent first-order rate constant for the establishment of the equilibrium between E·I and E·I* (2). Within the limitations of the assay, the initial velocities ($v_o$) appear to be independent of inhibitor concentration, suggesting that the dissociation constant for the E·I complex must be larger than the range of inhibitor concentrations used to generate the progress curves. Due to these limitations, the steady-state intermediate E·I is not observed, and therefore arginase II-BEC association is approximated as a single step process with $K_i = k_{off}/k_{on}$ (Scheme II).

*Scheme II*

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E{\cdot}I^*$$

The association rate constant $k_{on}$ was estimated from a plot of $k_{obs}$, determined from an analysis of the progress curves using equation 1, versus inhibitor concentration according to equation 2:

$$k_{obs} = k_{off} + k_{on}[I]/(1 + [S]/K_M) \qquad (2)$$

The dissociation rate constant, $k_{off}$, was determined by monitoring the rate of the return of activity from the enzyme-inhibitor complex via equation 1. A replot of $k_{obs}$ versus [BEC] yields $k_{on} = 9.63 \times 10^3\ M^{-1}\ sec^{-1}$, and the best fit of inhibitor release data (Figure 1, inset) yields $k_{off} = 2.23 \times 10^{-3}\ sec^{-1}$. The resultant $K_i$ value of 0.23 μM for BEC calculated from these rate constants is in good agreement with the value of 0.13 μM estimated from the steady-state velocities (data not shown). These $K_i$ values are approximately 4-8 fold larger than the corresponding values for the wild-type enzyme.

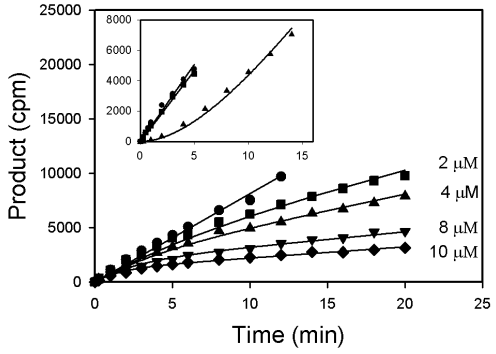



**Figure 1:** Slow-binding inhibition of ΔM1-V23/ΔH331-I354 arginase II by BEC at pH 9.5. Progress curves were generated as described in the Materials and Methods section at the indicated concentrations of BEC. Inset: Release of BEC from the arginase II-BEC complex. The straight lines correspond to a control assay performed in the absence of BEC and an assay carried out in the presence of 0.3 μM BEC. The curve represents the regain of activity following a 100-fold dilution of the preformed arginase-BEC complex to give a final concentration of 0.3 μM BEC.

**Electron Density Maps of Binuclear Manganese Cluster**

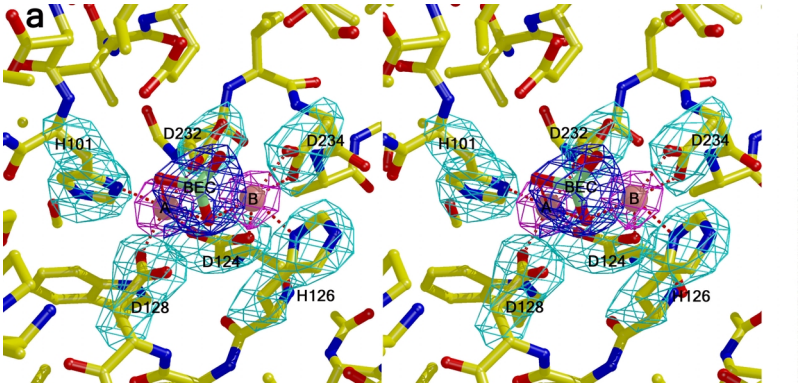



**Figure 2:** Omit electron density maps of the binuclear manganese cluster calculated with Fourier coefficients $|\mathbf{F}_o|-|\mathbf{F}_c|$ less the atoms of metal ligands (cyan, contoured at 5.2 σ), or $Mn^{2+}_A$ and $Mn^{2+}_B$ (magenta, contoured at 12.5 σ), or BEC (green, contoured at 3.5 σ).

**References**

1. Colleluori D. M., Ash, D. E. (2001) *Biochemistry* 40, 9356-9362.

2. Morrison, J.F., Walsh, C.T. (1988) *Adv. Enzymol. 61,* 201-301.