Reassessment of the Reaction Mechanism for the Heme Dioxygenases

Nishma Chauhan,[†] Sarah J. Thackray,[‡] Sara A. Rafice,[†] Graham Eaton,[†] Michael Lee,[†] Igor Efimov,[†] Jaswir Basran,[§] Paul R. Jenkins,[†] Christopher G Mowat,[‡] Stephen K. Chapman,[*,‡] and Emma Lloyd Raven[*,†]

[†] *Department of Chemistry, University of Leicester, University Road, Leicester, LE1 7RH, U.K.,* [‡] *EaStCHEM, School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, U.K, and* [§] *Department of Biochemistry, University of Leicester, Lancaster Road, Leicester, LE1 9HN, UK.*

Supporting information

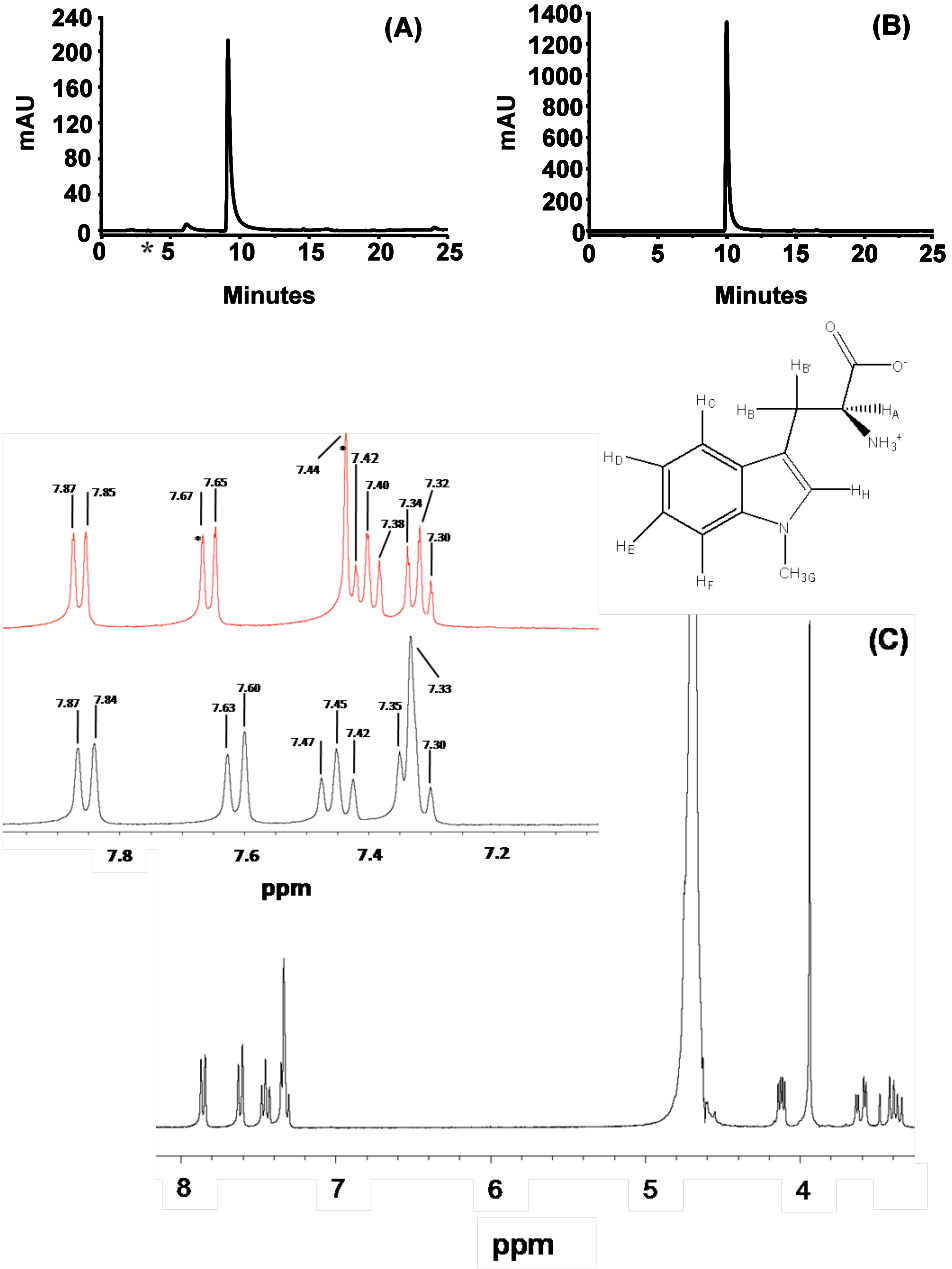

Figure S1. Chromatogram of commercially available 1-Me-L-Trp (A) before and (B) after purification by HPLC. The HPLC retention time for L-Trp (data not shown) was found to be identical to the contaminating species labeled * in (A). (C) NMR spectrum of purified 1-Me-L-Trp from (B). ^{1}H NMR (300 MHz, D_2O/CD_3CN) δ 3.33-3.65 (m, 2H, H_B + $H_{B'}$), 3.93 (s, 3H, H_G), 4.12 (dd, 1H, J = 8.2 Hz, 4.7 Hz, H_A), 7.33 (s + t, 2H, J =7.5 Hz, H_H + $H_{D/E}$), 7.45 (t, 1H, J =7.5 Hz, $H_{D/E}$), 7.61 (d, 1H, J =8.5 Hz, $H_{C/F}$), 7.85 (d, 1H, J =8.1 Hz, $H_{C/F}$). Inset: ^{1}H NMR spectra of commercially available L-Trp (red) and purified 1-Me-L-Trp (black) expanded between the 7-8 ppm region. * indicates the peaks that have shifted significantly in respect to the spectrum of 1-Me-L-Trp and therefore confirm the removal of L-Trp from purified 1-Me-L-Trp. HPLC conditions: wavelength - 280 nm; column - RP C18, 250 x 4.6 mm; eluant - H_2O:MeCN, 90:10 v/v; flow rate - 1 ml/min. NMR conditions: machine – Bruker DPX300; basic frequency – ^{1}H = 300.03 MHz; spectral width 16 ppm.

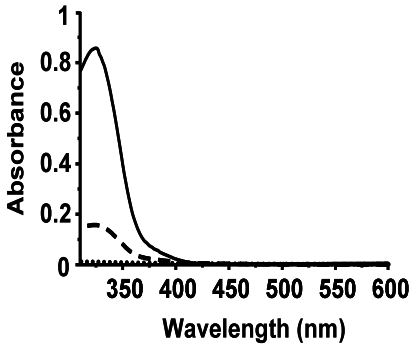

Figure S2. UV-visible spectrum of *N*-formyl-kynurenine (solid line) and *N*-formyl-methylkynurenine (dashed line) showing absorption bands at 321 nm. Spectra were obtained after isolation of the compounds from steady state assays with hIDO. Parallel experiments carried out with 1-Me-L-Trp in the absence of enzyme show no product formation at 321 nm (dotted line).

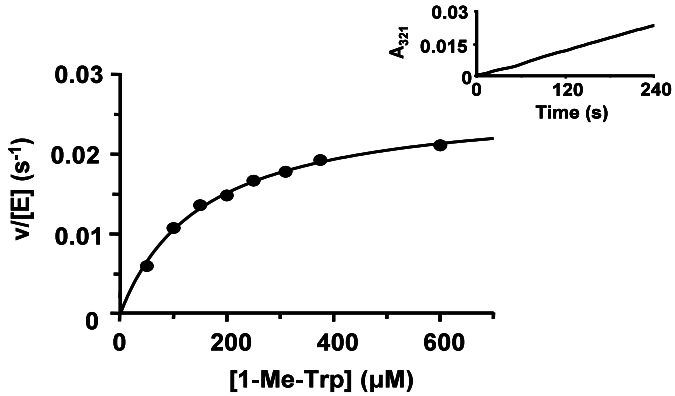

Figure S3. Steady state oxidation of 1-Me-L-Trp by hIDO. Solid line shows a fit of the data to the Michaelis-Menten equation. Inset: Time-dependence of absorbance changes at 321 nm, which report on *N*-formyl-methylkynurenine formation. Conditions: Tris/HCl buffer, pH 8.0, 25.0 °C. Reactions in the presence and absence of enzyme (Figure S2) confirm that product formation is due to enzymatic oxidation alone.

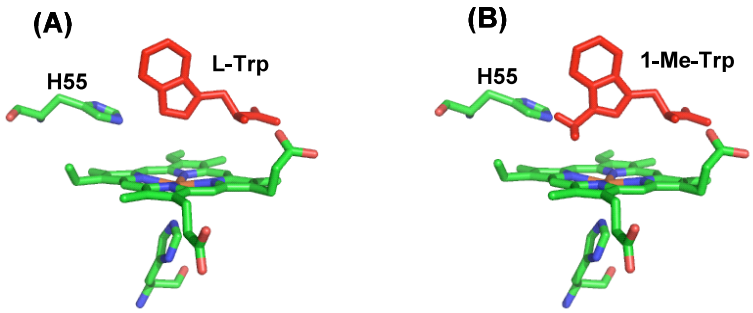

Figure S4. (A) Crystal structure of *Xc*TDO in complex with (A) L-Trp[8]; (B) A model of 1-Me-L-Trp binding to *Xc*TDO generated by overlay of 1-Me-L-Trp on the L-Trp coordinates in (A).

Table S1. Inhibition data (K_i) for inhibition of L-Trp oxidation by 1-Me-L-Trp (dash indicates no measurable activity).

[a] The high K_M in this case meant that a reliable measure of K_i was not accessible.

	Variant	K_i (μM)
hIDO	native	3.4 ± 0.4
	S167A	2.2 ± 0.2
hTDO	native	-
	H76S	[a]
*Xc*TDO	native	-
	H55A	150 ± 10
	H55S	400 ± 50