

The Second Shell Metal Ligands of Human Arginase Affect Coordination of Nucleophile and Substrate

*Everett M. Stone^a, Lynne Chantranupong^a, and George Georgiou^{*a,b}*

^aDepartments of Chemical Engineering, Biomedical Engineering, Molecular Genetics and Microbiology and
Institute for Cell and Institute for Molecular and Cell Biology, University of Texas, Austin, TX 78712

*Address correspondence to: George Georgiou, Ph.D., 1 University Station, C0800
Austin, Texas 78712-1084, Phone: (512) 471-6975, Fax: (512) 471-7963, E-mail gg@che.utexas.edu

Supporting Information:

Experimental Procedures

Library 1 was constructed using the following primers: H101-Forward `5-ggttttagcggtgatNRSagcttagcaattggc, H101-Reverse `5- GCCAATTGCTAAGCTSYNATCACCGCCTAAAACC, S230-Forward `5-cgtccaatccatctgNNSStc gatgttgacg, S230-Reverse `5- CGTCAACATCGAASNNCAGATGGATTGGACG, and the two specific end primers described previously ^[6]. Library 2 was constructed with the following primers: H126-Forward `5-gggttgacgccNRSacggatatcaatac, H126-Reverse `5-GTATTGATATCCGTSYNGGCGTCAACCC, D181-Forward `5-cattggcttacgtNNSgtcgaccagg, D181-Reverse `5- CCTGGGTCGACSNACGTAAGCCAATG and the two specific end primers described previously. Library 3 was made using D124-F `5- ggtgtcattgggttNRSgcccatacggatatac, D124-R `5-GATATCCGTATGGGCSYNAACCCAAATGACACC, W122-F `5-cagatttaggtgtcattNNSgttgaccccatac and W122-R `5- GTATGGGCGTCAACSNNAATGACACCTAAATCTG oligos. Library 4 used the described oligos for position D124, plus D232-F `5-ccatctgagcttcNRSgttgacggcttagac D232-R `5-GTCTAAGCCGTCAACSYNGAAGCTCAGATGG. Library 5 was constructed with the specific end primers the D181 forward and reverse primers and the S230 forward and reverse primers described previously. Libraries were constructed using the aforementioned primers, an E.coli codon-optimized hArgI gene as the template ^[6], dNTPs, buffer and DNA polymerase (Finnzymes, Woburn MA) by overlap extension PCR.

Figure S1: Representative pH profile of k_{cat}/K_M of Co-hArgI, S230G (○), and S230C (●).

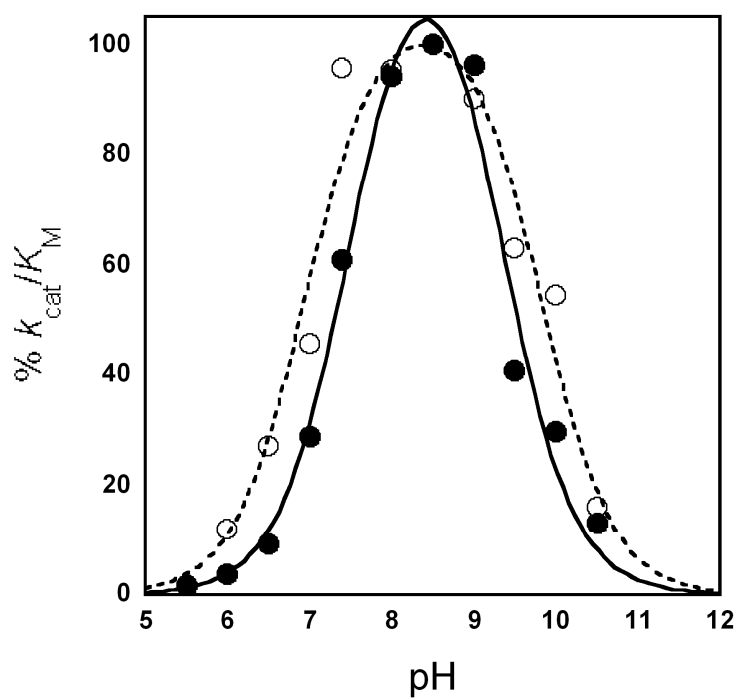


Table S1. Library (1) variants isolated from saturation mutagenesis of hArgI residues H101 & S230

2 nd shell pos S230	1 st shell pos H101		
	Asn	Asp	His
Cys	6		111
Gly			85
Thr			85
Gln			66
Ala			32
Asp			31
Leu			30
Met			29
His		28	
Val			16
Ile			11

Values are % Activity Relative to Co-hArgI

Table S2. Library (2) variants isolated from saturation mutagenesis of hArgI residues H126 & D181

	2 nd shell pos D181	1 st shell pos H126		
		Gln	Asn	His
	Ser			128
	Asn			118
	Thr			113
	Glu			100
	Ala	10		97
	Leu			83
	Cys			82
	His			80
	Gly			73
	Val			65
	Gln		25	57
	Tyr			48
	Met			48
	Lys			34
	Arg			31
	Trp			16

Values are % Activity Relative to Co-hArgI

Table S3. Library (3) variants isolated from saturation mutagenesis of hArgI residues W122 & D124

Table S3: % Relative <i>V/K</i> of Clones Isolated from Library (3)	
2 nd shell pos W122	1 st shell pos D124
	Asp
Asn	1
Thr	1
Ala	2
Gly	9
Val	35

Values are % Activity Relative to Co-hArgI

Table S4. Library (5) variants isolated from saturation mutagenesis of hArgI residues D181& S230

Table S4: % Relative V/K of Clones Isolated from Library (5)		
aa found at Pos. D181	aa found at Pos. S230	% Activity of Co-hArgI
Asn	Gly	113
Ser	Gly	105
Glu	Ala	100
Ala	Thr	79
Thr	Gly	70
Ala	Cys	70
Gly	Gly	67
Ala	Gly	66
Glu	Gly	65
Lys	Gly	65
Asn	Ile	64
Leu	Gln	63
Ser	Thr	63
His	Thr	61
Met	Gln	60
Ser	Leu	52
Ala	Leu	48
Leu	Gly	47
Arg	Thr	44
Gln	Ala	41
Tyr	Ala	35
Cys	Ala	35
His	Ala	34
Val	Gly	34
Val	Thr	34
Thr	Thr	34
Leu	Ala	31
Thr	Ala	30
Gly	Thr	29
Ser	Ile	28
Val	Ala	27
Phe	Asn	25
Ala	Ala	24
Met	Cys	21
Lys	Cys	20
Tyr	Gly	20
Arg	Gly	19
Ile	Ala	19
Gln	Val	16
Leu	Glu	16
Arg	Glu	14
Thr	Glu	11
Ser	Val	7
Leu	Asp	6
Thr	Val	6