Archives of Biochemistry and Biophysics 496 (2010) 101–108

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Inhibition of human arginase I by substrate and product analogues ☆,☆☆

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ARTICLE INFO

Article history:
Received 25 November 2009
and in revised form 8 February 2010
Available online 12 February 2010

Keywords:
Metalloenzymes
Enzyme inhibition
Crystallography
SPR
ITC

ABSTRACT

Human arginase I is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to generate L-ornithine and urea. We demonstrate that *N*-hydroxy-L-arginine (NOHA) binds to this enzyme with K_d = 3.6 μM, and nor-*N*-hydroxy-L-arginine (nor-NOHA) binds with K_d = 517 nM (surface plasmon resonance) or $K_d \approx 50$ nM (isothermal titration calorimetry). Crystals of human arginase I complexed with NOHA and nor-NOHA afford 2.04 and 1.55 Å resolution structures, respectively, which are significantly improved in comparison with previously-determined structures of the corresponding complexes with rat arginase I. Higher resolution structures clarify the binding interactions of the inhibitors. Finally, the crystal structure of the complex with L-lysine (K_d = 13 μM) is reported at 1.90 Å resolution. This structure confirms the importance of hydrogen bond interactions with inhibitor α-carboxylate and α-amino groups as key specificity determinants of amino acid recognition in the arginase active site.

Human arginase I is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to generate L-ornithine and urea. This reaction is the key step of the urea cycle in the liver that allows for the excretion of nitrogenous waste resulting from protein catabolism; healthy adults excrete approximately 10 kg urea per year [1–4]. In extrahepatic tissues arginase serves to regulate L-arginine concentrations for other metabolic pathways. For example, arginase activity can decrease L-arginine concentrations utilized by nitric oxide synthase to generate NO; arginase inhibitors can increase L-arginine concentrations and thereby enhance NO biosynthesis and NO-dependent physiological processes such as smooth muscle relaxation [5]. Accordingly, arginase is a pharmaceutical target for the treatment of diseases associated with aberrant smooth muscle physiology such as erectile dysfunction [6,7] and asthma [8,9].

Among substrate analogue inhibitors of arginase, boronic acid and *N*-hydroxyguanidinium derivatives exhibit the highest affinity (selected inhibitors are shown in Table 1) [7,10–19]. The crystal structures of rat arginase I complexed with 2(*S*)-amino-6-boronohexanoic acid (ABH[1]) [6], dehydro-ABH [17], and *S*-(2-boronoethyl)-L-cysteine (BEC) [7], human arginase I complexed with ABH and BEC [18], and human arginase II complexed with BEC [20] reveal that the planar boronic acid moiety of the inhibitor undergoes nucleophilic attack by the metal-bridging hydroxide ion to yield a tetrahedral boronate anion that mimics the tetrahedral intermediate and its flanking transition states in catalysis. In contrast, the low resolution crystal structures of rat arginase I complexed with *N*-hydroxy-L-arginine (NOHA) and an analogue bearing a side chain shortened by one methyl group (designated nor-NOHA) [21] indicate that the N–OH moiety of the inhibitor displaces the metal-bridging hydroxide ion. Although interactions with the binuclear manganese cluster are strikingly different for the best *N*-hydroxyguanidinium and boronic acid inhibitors, each binds with comparable affinity. With regard to product binding, early studies indicated that L-ornithine and L-lysine are competitive inhibitors of rat and bovine arginase I [22–24]. Moreover, hydroxylation of the side chain amino group of L-lysine yields a potent inhibitor of bovine arginase I with K_i = 4 μM [14]. Here, too, the N–OH group likely displaces the metal-bridging hydroxide ion of the native enzyme.

Given the appreciable inhibitory activity observed for amino acid derivatives of the substrate L-arginine and the product L-ornithine, and given the growing pharmaceutical importance of human arginase isozymes, we now report affinity measurements and X-ray crystal structures of the complexes between human arginase I and nor-NOHA, NOHA, and L-lysine. Importantly, crystals of human arginase I afford 1.55 and 2.04 Å resolution structures of complexes with nor-NOHA and NOHA, respectively, which are significantly improved in comparison with previously-determined structures of rat arginase I complexed with these inhibitors at 2.8 and 2.9 Å resolution, respectively [21]. The crystal structure of the complex with inhibitor L-lysine is determined at 1.90 Å resolution. The higher resolution structures of complexes with human

☆ This work was supported by NIH grant GM49758.

☆☆ Atomic coordinates of human arginase I complexed with N^{ω}-hydroxy-nor-L-arginine (nor-NOHA), N^{ω}-hydroxy-L-arginine (NOHA), and L-lysine have been deposited in the Research Collaboratory for Structural Bioinformatics (http://www.rcsb.org/pdb) with the following accession codes: 3KV2, 3LP7, 3LP4.

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[1] *Abbreviations used:* ABH, 2(*S*)-amino-6-boronohexanoic acid; BEC, *S*-(2-boronoethyl)-L-cysteine; NOHA, *N*-hydroxy-L-arginine; nor-NOHA, nor-*N*-hydroxy-L-arginine.

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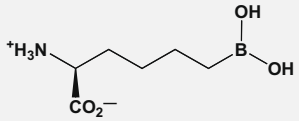
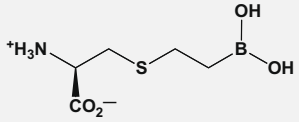
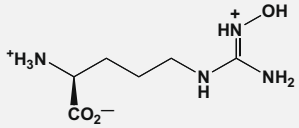
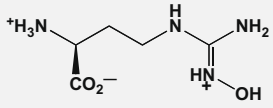
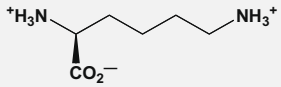
doi:10.1016/j.abb.2010.02.004

Table 1
Arginase inhibitors.

Inhibitor	Structure	Affinity, rat arginase I (nM) (K_d or K_i, pH 8.5)	Affinity, human arginase I (nM) (K_d, pH 8.5)
2(*S*)-amino-6-boronohexanoic acid (ABH)		100[a]	5[b]
(*S*)-(2-boronoethyl)-L-cysteine (BEC)		2200[c]	270[b]
N^{ω}-hydroxy-L-arginine (NOHA)		10,000[d]	3600[e]
N^{ω}-hydroxy-nor-L-arginine (nor-NOHA)		500[d]	517[e]; 47, 51[f]
L-lysine		1,100,000[g]	13,100[e]

[a] Ref. [10].
[b] Ref. [18].
[c] Ref. [7].
[d] Ref. [12].
[e] This study, surface plasmon resonance determination.
[f] This study, isothermal titration calorimetry determination.
[g] Ref. [24].

Table 2
Data collection and refinement statistics.

Human arginase I complex	nor-NOHA	NOHA	L-lysine
Data collection			
Resolution, Å	50.0–1.55	50.0–2.04	50.0–1.90
Total/unique reflections measured	135,232/90,987	81,841/40,118	117,869/49,010
R_{merge}[a,b]	0.032 (0.302)	0.089 (0.186)	0.081 (0.433)
$I/\sigma(I)$[a]	14.4 (2.0)	28.9 (8.7)	17.4 (2.5)
Completeness (%)[a]	98.2 (96.2)	98.6 (91.8)	97.1 (98.9)
Refinement			
Reflections used in refinement/test set	85,281/4185	39,744/1587	46,106/1897
R_{twin}[c]	0.144	0.124	0.155
$R_{twin/free}$[c]	0.178	0.174	0.204
Protein atoms[d]	4782	4782	4782
Water molecules[d]	302	269	209
Inibitor atoms[d]	24	26	20
Manganese ions[d]	4	4	4
R.m.s. deviations			
Bond lengths, Å	0.006	0.006	0.006
Bond angles, °	1.34	1.29	1.33
Average B-factors, Å²			
Main chain	18	26	23
Side chain	20	28	26
Manganese ions	13	17	18
Inhibitors	16	41	27
Solvent	23	30	25

[a] Number in parentheses refer to the outer 0.1 Å shell of data.
[b] $R_{merge} = \Sigma|I - \langle I\rangle|/\Sigma I$, where I is the observed intensity and $\langle I\rangle$ is the average intensity calculated for replicate data.
[c] $R_{twin} = \Sigma||[|F_{calc/A}|^2 + |F_{calc/B}|^2]^{1/2} - |F_{obs}||/\Sigma|F_{obs}|$ for reflections contained in the working set. $|F_{calc/A}|$ and $|F_{calc/B}|$ are the structure factor amplitudes calculated for the separate twin domains A and B, respectively. R_{twin} underestimates the residual error in the model over the two twin-related reflections by a factor of approximately 0.7. The same expression describes $R_{twin/free}$, which was calculated for test set reflections excluded from refinement.
[d] Per asymmetric unit.