Communication

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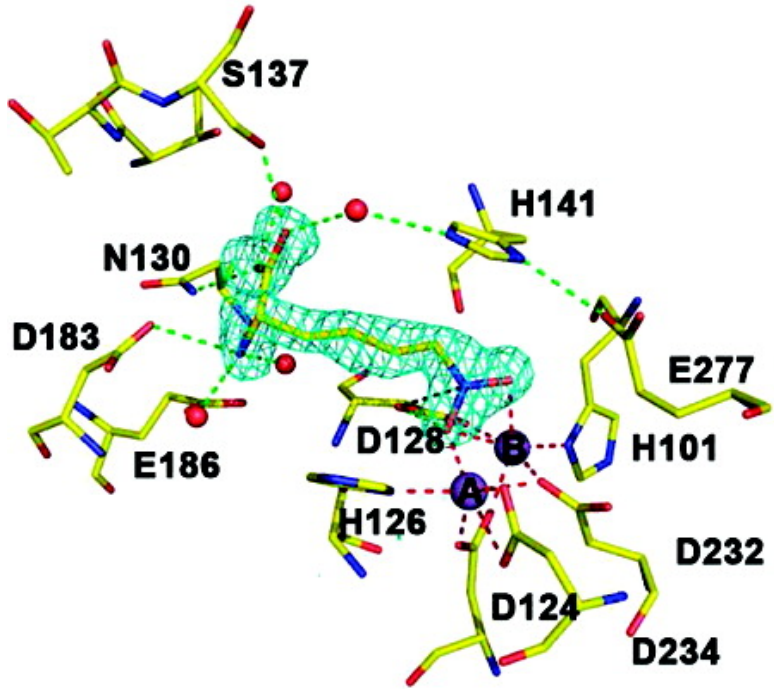

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(*S*)-2-Amino-6-nitrohexanoic Acid Binds to Human Arginase I through Multiple Nitro−Metal Coordination Interactions in the Binuclear Manganese Cluster

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Arginase, an enzyme catalyzing the hydrolysis of L-arginine to form L-ornithine and urea, plays an important role in regulating L-arginine homeostasis.[1] Arginase competes for its substrate with NO synthase, an enzyme that performs a five-electron oxidation of L-arginine to produce L-citrulline and nitric oxide (NO). Thus, inhibition of arginase can result in higher cellular concentrations of L-arginine for NO biosynthesis. The inhibition of arginase may be a promising therapeutic approach for the treatment of diseases in which upregulated arginase activity compromises NO-dependent physiological processes, e.g., erectile dysfunction,[2] asthma,[3] and atherosclerosis.[4]

Arginase is highly specific for the binding of amino acids bearing intact α-amino and α-carboxylate groups due to a precise constellation of hydrogen bond interactions that mediate enzyme−substrate and enzyme−inhibitor recognition in the active site.[2a,5] These interactions account for the stringent catalytic specificity observed for intact L-arginine.[6] To date, numerous amino acid derivatives have been tested for inhibitory activity against arginase: the best reported so far are amino acids bearing boronic acid[7] and *N*-hydroxyguanidinium side chains.[8] Since none of these inhibitors have yet been approved for clinical use, the search for new inhibitors with superior therapeutic profiles continues. Here we report an unusual functional group capable of manganese coordination in the arginase active site: (*S*)-2-amino-6-nitrohexanoic acid (ANH, Figure 1) binds to human arginase I through multiple nitro−Mn^{2+} interactions.

ANH was synthesized according to a previously reported procedure in four steps.[9] ANH binds to human arginase I with K_d = 60 μM as determined by surface plasmon resonance (details of affinity measurements are provided in the Supporting Information). Interestingly, (*S*)-2-aminoheptanedioic acid (AHD, Figure 1), which is isosteric with ANH, exhibits a dramatic 500-fold decrease in binding affinity with K_d = 30 mM. While the carboxylate moiety of AHD ought to serve as a stronger metal ligand due to its negative charge, it appears that the binuclear manganese cluster of arginase is better suited to accommodate the neutral zwitterionic nitro moiety of ANH.

For the X-ray crystal structure determination, human arginase I was overexpressed in *E. coli*, purified, and crystallized as described,[5b] except that 2 mM ANH was added to the crystallization buffer. Crystals diffracted to 1.6 Å resolution at the Brookhaven National Laboratory, and the structure was solved by the technique of molecular replacement using the program Phaser[10] with chain A of unliganded human arginase I (PDB accession code 2ZAV, less solvent molecules[11]) used as a search probe against twinned data. The structure was refined to final R_{twin} and $R_{twin/free}$ values of 0.147 and 0.184, respectively. Full details of the structure determination are reported in the Supporting Information.

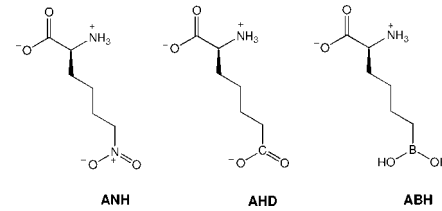

Figure 1. Human arginase I inhibitors (*S*)-2-amino-6-nitrohexanoic acid (ANH), K_d = 60 μM; (*S*)-aminoheptanedioic acid (AHD), K_d = 30 mM; and (*S*)-2-amino-6-boronohexanoic acid (ABH), K_d = 5 nM.

The binding of ANH does not cause any significant conformational changes in the active site, and the rms deviation is 0.24 Å for 312 Cα atoms in comparison with the unliganded enzyme. However, important structural changes are observed in the manganese coordination polyhedra (Figure 2a): oxygen atom O1 of the ANH nitro group displaces the metal-bridging hydroxide ion present in the unliganded enzyme[11] and bridges Mn^{2+}_A and Mn^{2+}_B (average coordination distance = 2.3 Å), and oxygen atom O2 of the nitro group chelates Mn^{2+}_A (average coordination distance = 2.2 Å). Adopting the nomenclature used to describe carboxylate−metal coordination,[12] the nitro group of ANH is a *syn*−*anti* bidentate bridging ligand in the binuclear manganese cluster. The nitrogen atom of the nitro group is 3.0 Å away from the carboxylate oxygen atom of D128, which could reflect a favorable electrostatic interaction if D128 is ionized. However, since the same carboxylate oxygen of D128 is also 2.7 Å away from the O1 atom of the ANH nitro group, it is possible that D128 is protonated to accommodate a hydrogen bond interaction (albeit poorly oriented).

As observed in the binding of other amino acid inhibitors to human arginase I,[2a,5] the α-carboxylate and α-amino groups of ANH are anchored to the active site of arginase by three direct and four water-mediated hydrogen bonds. Additionally, a weaker hydrogen bond interaction may occur between oxygen atom O1 of the nitro group and the carboxylate group of E277 (O···O separation = 3.3 Å). Interestingly, superposition of ANH with the best known arginase inhibitor (*S*)-2-amino-6-boronohexanoic acid (ABH, Figure 1)[7a] as found in the structure of its complex with human arginase I[5b] demonstrates significant overlap (Figure 2b). In the structure of each enzyme−inhibitor complex, inhibitor oxygen atom O1 bridges Mn^{2+}_A and Mn^{2+}_B and inhibitor oxygen atom O2 chelates Mn^{2+}_A. However, unlike the trigonal planar nitro group, boronic acids bind to arginase as tetrahedral boronate anions.

The Cambridge Structural Database contains 120 structures with nitro−metal coordination interactions (3 with Mn), and none of these structures exhibits a *syn*−*anti* bridging mode. The Protein Data Bank contains two structures with nitro−metal

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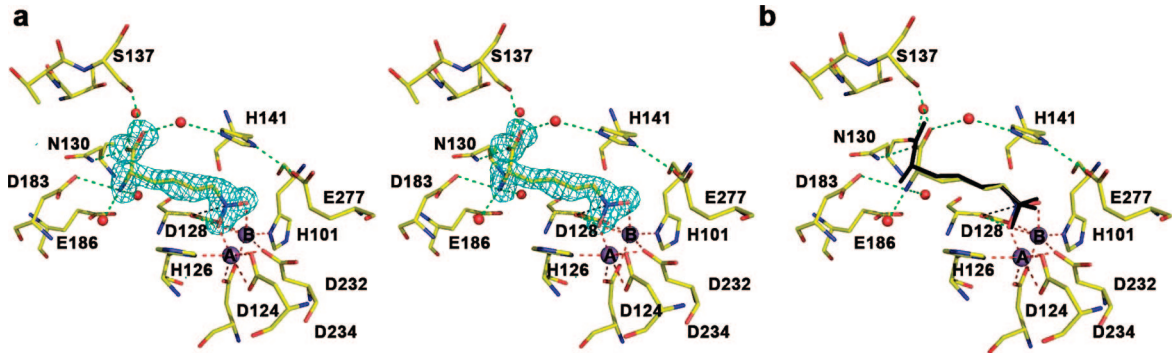

Figure 2. (a) Stereoview of a simulated annealing omit map calculated with Fourier coefficients $|F_o|-|F_c|$, in which ANH was omitted from the structure factor calculation (3.5 σ contour, cyan). The map shows ANH bound in the active site of human arginase I (monomer A). Dashed lines indicate manganese coordination (red), hydrogen bond (green), and electrostatic interactions (dark gray). Atom color codes: carbon (yellow), oxygen (red), nitrogen (blue), manganese (violet). (b) Superposition of the arginase I−ANH complex and ABH (black) as observed in the human arginase I−ABH complex (PDB accession code 2AEB[5b]).

coordination interactions. One of these structures is the complex between carboxypeptidase A and 2-benzyl-3-nitropropanoic acid, where the inhibitor nitro group coordinates to Zn^{2+} with *syn* monodentate geometry.[13] Interestingly, the nitro containing compound ($K_i = 0.15\ \mu M$) binds more tightly than its carboxylate analogue, 2-benzylsuccinate ($K_i = 0.45\ \mu M$). The second structure is the complex between *p*-nitrobenzoate and *p*-aminobenzoate *N*-oxygenase, which reveals coordination of the nitro group to Fe^{2+} with *anti* monodentate geometry.[14] In this structure, however, the possibility of backward binding, i.e., with metal coordination by the carboxylate and not the nitro group, cannot be ruled out.

While the nitro group is perhaps generally regarded as an unfavorable hydrogen bond acceptor or metal ligand due in part to the superacidity of its conjugate acid ($pK_a \approx -12$),[15] it is notable that when substituted for the carboxylate group of the glutamate side chain via unnatural amino acid mutagenesis, an isosteric nitro group can preserve ligand binding and catalytic functions.[16] Given that the substitution of a metal-coordinating nitro group for a metal-coordinating carboxylate group in carboxypeptidase A and arginase inhibitors strengthens enzyme−inhibitor affinity, it is possible that incorporation of the zwitterionic nitro moiety as a neutral isosteric substitution for carboxylate should be more widely explored as a possible approach for enhancing protein−ligand affinity.

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Supporting Information Available: Experimental procedures, CSD and PDB search parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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