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Crystal Structure of Human Arginase I Complexed with Thiosemicarbazide Reveals an Unusual Thiocarbonyl μ-Sulfide Ligand in the Binuclear Manganese Cluster

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Arginase is a 105 kDa homotrimer containing a binuclear manganese(II) cluster in each subunit required for the hydrolysis of L-arginine to yield L-ornithine and urea.[1] Two isozymes, arginase I and arginase II, have been identified in humans and the amino acid sequences of these isozymes are related by 60% identity. In recent years, increasing attention has focused on arginase as a potential therapeutic target owing to the overexpression of these isozymes in a variety of diseased tissues and organs, for example, the airway of asthma patients,[2] the spinal cord fluid in an animal model of multiple sclerosis,[3] and the corpus cavernosum of diabetic men suffering from erectile dysfunction.[4]

The first X-ray crystal structure of an unliganded mammalian arginase was that of rat arginase I, which revealed a Mn^{2+}−Mn^{2+} cluster bridged by a nonprotein ligand interpreted as a μ-hydroxide ion that functions as a nucleophile in catalysis.[5] The subsequently determined structure of rat arginase I complexed with the boronic acid substrate analogue 2(*S*)-amino-6-boronohexanoic acid (ABH)[6] revealed the binding of the tetrahedral boronate anion form of the inhibitor, which mimics the tetrahedral transition state.[7] Recently determined crystal structures of human arginases I and II complexed with ABH and/or the related boronic acid substrate analogue *S*-(2-boronoethyl)-L-cysteine (BEC)[8] revealed similar inhibitor binding modes.[9,10]

Despite the high affinity of ABH binding to human arginase I (K_d = 5 nM),[10] consideration of ABH as a possible drug candidate for the treatment of human diseases linked to arginase hyperactivity is tempered by the relative scarcity of boron-containing drugs.[11] Thus, we have continued to explore new functional groups for manganese coordination in the design and development of new arginase inhibitors. We now report the X-ray crystal structure of human arginase I complexed with thiosemicarbazide determined at 1.95 Å resolution.

For structure determination, human arginase I was overexpressed in *E. coli*, purified, and crystallized as described[10,12] with the exception that the protein solution contained 1.4 mM thiosemicarbazide. The structure was refined to final R_{twin} and $R_{free/twin}$ values of 0.169 and 0.219, respectively. The structure of unliganded human arginase I was also determined at 1.90 Å resolution and refined to final R_{twin} and $R_{twin/free}$ values of 0.198 and 0.244, respectively. Complete experimental details are reported in the Supporting Information.

The root-mean-square (rms) deviation of 314 Cα atoms between unliganded rat arginase I and unliganded human arginase I is 0.64 Å, indicating that these enzymes are quite similar in structure (as expected by their amino acid sequence identity of 87%). However, interesting differences are evident in active site solvent structure

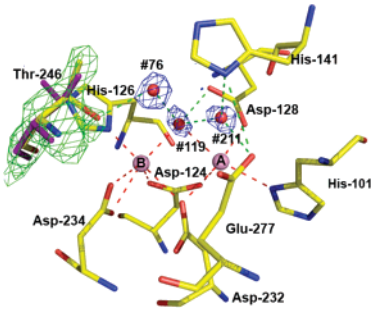

Figure 1. Omit electron density map of unliganded human arginase I calculated with Fourier coefficients $|F_{obs/A}| - |F_{calcd/A}|$ for twin domain A, and phases from the refined enzyme model less the atoms of T246 (contoured at 2.7σ, green) and water molecules #76, #119, and #211 (contoured at 3.0 σ, blue). The T246 conformation in rat arginase I (magenta) is superimposed.

and appear to result from an alternative conformation of T246: in human arginase I, the T246 hydroxyl group of this residue is oriented toward the manganese ions and forms a hydrogen bond with solvent molecule #76, which in turn forms a hydrogen bond with the metal-bridging hydroxide ion (solvent molecule #119); in rat arginase I, the T246 hydroxyl group is oriented away from the manganese ions (Figure 1).

Interestingly, the metal-bridging hydroxide ion is also within hydrogen-bonding distance of solvent molecule #211, which also forms a hydrogen bond with H141. Solvent molecule #211 also interacts weakly with Mn^{2+}_A, but the Mn^{2+}_A−O separation of 2.8 Å is too long to be considered an innersphere coordination interaction. That solvent molecule #211 forms a hydrogen-bonded bridge between the metal-bridging hydroxide ion and H141 is consistent with the proposed role of H141 as a proton shuttle in the regeneration of the nucleophilic metal-bridging hydroxide ion from a metal-bridging water molecule.[5] In other words, solvent molecule #211 may serve as a "proton wire" to facilitate proton transfer in catalysis.[13]

The binding of thiosemicarbazide to human arginase I does not cause any significant structural changes in the active site, and the rms deviation is 0.39 Å for 313 Cα atoms between the structures of the thiosemicarbazide-complexed and unliganded enzymes. However, a significant structural change is observed in the manganese coordination polyhedra: the C═S moiety of thiosemicarbazide bridges Mn^{2+}_A and Mn^{2+}_B with coordination distances of 2.6 and 2.4 Å, respectively (Figure 2). These metal coordination distances are consistent with other metalloprotein crystal structures. A search of the Protein Data Bank (PDB)[14] retrieves 62 unique cysteine−Mn^{2+} interactions in 18 protein structures, 12 unique methionine−Mn^{2+} interactions in 6 protein structures, and 3 unique S−Mn^{2+} interactions involving non-protein groups, for example,

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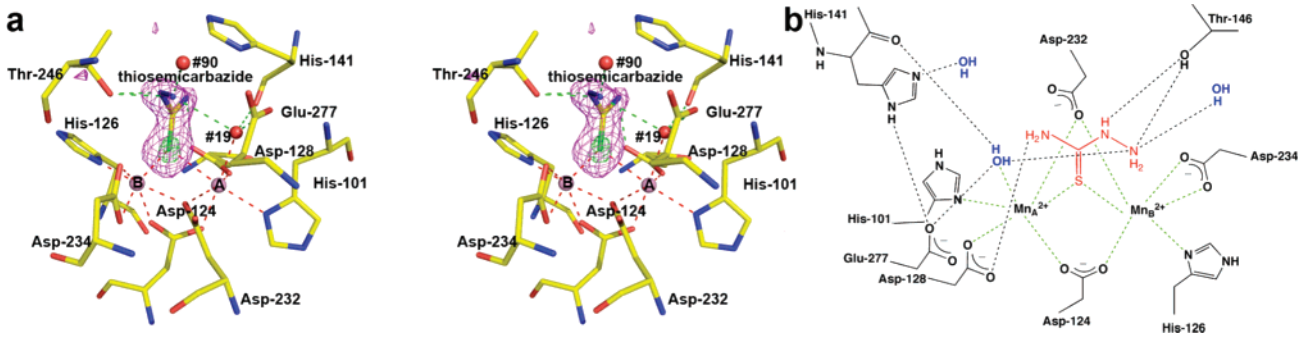

Figure 2. (a) Stereoview of simulated annealing gradient maps showing thiosemicarbazide (3.1 σ contour, magenta) and its electron-rich sulfur atom (7.3 σ contour, green) bound to human arginase I. Dashed lines indicate manganese coordination (red) and hydrogen bond (green) interactions. Atom color codes: carbon (yellow), oxygen (red), nitrogen (blue), manganese (pink), sulfur (green). (b) Summary of intermolecular interactions.

thiophosphate derivatives. The average S−Mn^{2+} coordination distance is 2.6 ± 0.2 Å for cysteine ligands and 2.8 ± 0.1 Å for methionine ligands. Additional geometric data are reported in Figure S1 of the Supporting Information.

Analyses of the PDB and the Cambridge Structural Database (CSD)[15] indicate that the human arginase I−thiosemicarbazide complex is only the second crystal structure ever determined of a thiosemicarbazide complexed with Mn^{2+}, the first such complex being aqua-2,2′-bipyridyl)-(thiosemicarbazidediacetato-O,O′,S)-manganese(II) sesquihydrate (CSD accession code YARSEP) in which the manganese-sulfur coordination distance is 2.6 Å.[16]

Further analysis of the CSD (see Supporting Information) yields a total of 68 structures of thiosemicarbazide complexes with the following metal ions: Mn (1), Fe (3), Co (6), Ni (22), Cu (6), Zn (7), Rh (3), Ag (8), Cd (6), Pt (1), Hg (2), Pb (2), and Bi (1). The thiosemicarbazide S−C−N−N dihedral angle is occasionally distorted in these structures, and out-of-plane deviations of up to 21° are observed. At 64°, the distortion of the S−C−N−N dihedral angle of thiosemicarbazide bound to human arginase I is even more pronounced (Figure 2). This distortion appears to facilitate the formation of numerous direct and water-mediated hydrogen bonds. Comparable distortions of O−C−N−O dihedral angles are observed for *N*-hydroxyurea inhibitors of matrix metalloproteinases and may similarly be facilitated by intermolecular hydrogen-bond interactions.[17]

Given that the N−OH groups of N-hydroxy-L-arginine and *N*-hydroxy-nor-L-arginine displace the metal-bridging hydroxide ion of unliganded rat arginase I,[18] it is surprising that the N−NH_2 group of thiosemicarbazide does not do likewise. It is especially surprising that the electron-rich sulfur atom of thiosemicarbazide is preferred for manganese coordination given the fact that the electron-rich sulfur atom of thiosemicarbazide is a relatively soft ligand and Mn^{2+} is a relatively hard metal ion.[19]

Regardless, insofar that thiosemicarbazide is an analogue of urea, it is notable that this structure provides the first experimental evidence in support of a metal-bridging mode for the urea product as first proposed by Kanyo and collegues.[5] The binding affinity of urea is weak ($K_d \approx 1$ M),[20] and isothermal titration calorimetry similarly indicates weak affinity for thiosemicarbazide with $K_d >$ 0.1 mM (i.e., beyond the detection threshold), so the K_d value of thiosemicarbazide likely resides somewhere between these values. Nevertheless, we conclude that the unusual C=S−Mn^{2+} interactions shown in Figure 2 highlight the potential of thiosemicarbazide as a useful fragment[21] for the design of amino acid thiosemicarbazide inhibitors that will be described in due course.[22]

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

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