Published in final edited form as:

J Am Chem Soc. 2007 May 23; 129(20): 6388–6389. doi:10.1021/ja071567j.

Crystal structure of human arginase I complexed with thiosemicarbazide reveals an unusual thiocarbonyl μ -sulfide ligand in the binuclear manganese cluster

Luigi Di Costanzo, Michael E. Pique, and David W. Christianson*

Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, and The Scripps Research Institute MB-5, 10550 North Torrey Pines Road, La Jolla, CA 92037

Abstract

The crystal structure of the human arginase I-thiosemicarbazide complex reveals an unusual thiocarbonyl μ -sulfide ligand in the binuclear manganese cluster. The C=S moiety of thiosemicarbazide bridges $Mn^{2+}A$ and $Mn^{2+}B$ with coordination distances of 2.6 Å and 2.4 Å, respectively. Otherwise, the binding of thiosemicarbazide to human arginase I does not cause any significant structural changes in the active site. The crystal structure of the unliganded enzyme reveals a hydrogen bonded water molecule that could support proton transfer between a μ -water molecule and H141 to regenerate the nucleophilic μ -hydroxide ion in the final step of catalysis.

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 due to the overexpression of these isozymes in a variety of diseased tissues and organs, e.g., the airway of asthma patients², the spinal cord fluid in an animal model of multiple sclerosis³, 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-borohexanoic 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 $R_{\text{free/twin}}$ 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 $R_{\text{twin/free}}$ values of 0.198 and 0.244, respectively. Complete experimental details are reported in the Supplementary Information.

The root-mean-square (r.m.s.) deviation of 314 C σ 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 and appear to result from an alternative conformation of T246: in human arginase I, the T246 hydroxyl group of this residue is oriented towards 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 Mn²⁺_A, but the Mn²⁺_A-O separation of 2.8 Å is too long to be considered an inner-sphere 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 r.m.s. deviation is 0.39 Å for 313 C σ 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 Mn²⁺_A and Mn²⁺_B with coordination distances of 2.6 Å 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-Mn²⁺ interactions in 18 protein structures, 12 unique methionine-Mn²⁺ interactions in 6 protein structures, and 3 unique S-Mn²⁺ interactions involving non-protein groups, e.g., thiophosphate derivatives. The average S-Mn²⁺ 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 Mn²⁺, 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-NH₂ 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 Mn²⁺ 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 colleagues.⁵ The binding affinity of urea is weak ($K_d \sim 1$ 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---Mn²⁺ interactions shown in Figure 2 highlight the potential of thiosemicarbazide as a useful fragment²¹ for the design of amino acid thiosemicarbazide inhibitors that will be described in due course.²²

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank the NIH for grant GM49758 and D.W.C. thanks the Sandler Program for Asthma Research for a Senior Investigator award. Finally, we thank Dr. Hyunshun Shin for suggesting the study of thiosemicarbazide and Prof. Francisco Centeno for the gift of the human arginase I plasmid.

References

- Christianson DW. Acc Chem Res 2005;101:191–201. [PubMed: 15766238]
- Zimmermann N, King NE, Laporte J, Yang M, Mishra A, Pope SM, Muntel EE, Witte DP, Pegg AA, Foster PS, Hamid Q, Rothenberg ME. J Clin Invest 2003;111:1863–1874. [PubMed: 12813022]
- Xu L, Hilliard B, Carmody RJ, Tsabary G, Shin H, Christianson DW, Chen YH. Immunology 2003;110:141–148. [PubMed: 12941151]
- Bivalacqua TJ, Hellstrom WJ, Kadowitz PJ, Champion HC. Biochem Biophys Res Commun 2001;283:923–927. [PubMed: 11350073]
- Kanyo ZF, Scolnick LR, Ash DE, Christianson DW. Nature 1996;383:554–557. [PubMed: 8849731]
- Baggio R, Elbaum D, Kanyo ZF, Carroll PJ, Cavalli RC, Ash DE, Christianson DW. J Am Chem Soc 1997;119:8107–8108.
- Cox JD, Kim NN, Traish AM, Christianson DW. Nat Struct Biol 1999;6:1043–1047. [PubMed: 10542097]
- Kim NN, Cox JD, Baggio RF, Emig FA, Mistry SK, Harper SL, Speicher DW, Morris SM Jr, Ash DE, Traish A, Christianson DW. Biochemistry 2001;40:2678–2688. [PubMed: 11258879]
- Cama E, Colletuori DM, Emig FA, Shin H, Kim SW, Kim NN, Traish AM, Ash DE, Christianson DW. Biochemistry 2003;42:8445–8451. [PubMed: 12859189]
- Di Costanzo L, Sabio G, Mora A, Rodriguez PC, Ochoa AC, Centeno F, Christianson DW. Proc Natl Acad Sci 2005;102:13058–13063. [PubMed: 16141327]
- Robertson JG. Biochemistry 2005;44:5561–5571. [PubMed: 15823014]
- Mora A, del Ara Rangel M, Fuentes JM, Soler G, Centeno F. Biochim Biophys Acta 2000;1476:181–190. [PubMed: 10669784]

13. a) Cui Q, Karplus M. *J Phys Chem* 2003;B107:1071–1078. (b) Lu D, Voth GA. *J Am Chem Soc* 1998;120:4006–4014. (c) Xu J, Voth GA. *Proc Natl Acad Sci* 2005;102:6795–6800. [PubMed: 15857953]
14. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. *Nucleic Acids Res* 2000;28:235–242. [PubMed: 10592235]
15. Allen FH. *Acta Cryst B* 2002;58:380–388. [PubMed: 12037359]
16. Simonov YA, Lozan VI, Kravtsov VK, Gerbeleu NV, Bologna OA, Malinovskii TI. *Zh Neorg Khim* (Russ J Inorg Chem) 1992;37:1519–1524.
17. Campestre C, Agamennone M, Tortorella P, Preziuso S, Biasone A, Gavuzzo E, Pochetti G, Mazza F, Hiller O, Tschesche H, Consalvi V, Gallina C. *Bioorg Med Chem Lett* 2006;16:20–24. [PubMed: 16242329]
18. Cox JD, Cama E, Colletuori DM, Pethe S, Boucher JL, Mansuy D, Ash DE, Christianson DW. *Biochemistry* 2001;40:2689–2701. [PubMed: 11258880]
19. Among the first row transition metals, the Mn^{2+} ion is relatively “hard” yet is thiophilic in comparison with the “harder” Mg^{2+} ion; see (a) Reed GH, Poyner RR. *Met Ions Biol Syst* 2000;37:183–207. [PubMed: 10693135] (b) Crowley JD, Traynor DA, Weatherburn DC. *Met Ions Biol Syst* 2000;37:209–278. [PubMed: 10693136]
20. Reczkowski RS, Ash DE. *Arch Biochem Biophys* 1994;312:31–37. [PubMed: 8031143]
21. Hajduk PJ, Greer J. *Nature Rev Drug Discov* 2007;6:211–219. [PubMed: 17290284]
22. The atomic coordinates of the human arginase I-thiosemicarbazide complex and unliganded human arginase I have been deposited in the Protein Data Bank with accession codes 2PHO and 2PHA, respectively.

Figure 1. Omit electron density map of unliganded human arginase I calculated with Fourier coefficients $|F_{\text{obs}}/A| - |F_{\text{calc}}/A|$ 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 3.0σ , blue). The T246 conformation in rat arginase I (magenta) is superimposed.

Figure 2.

(a) Stereoview of simulated annealing gradient maps showing thiosemicarbazide (3.1σ contour, magenta) and its electron-rich sulfur atom (7.3σ 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.