Published in final edited form as:

Arch Biochem Biophys. 2007 September 1; 465(1): 82–89.

Expression, Purification, Assay, and Crystal Structure of Perdeuterated Human Arginase I, ^{★,★★}

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Abstract

Arginase is a manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to yield L-ornithine and urea. In order to establish a foundation for future neutron diffraction studies that will provide conclusive structural information regarding proton/deuteron positions in enzyme-inhibitor complexes, we have expressed, purified, assayed, and determined the X-ray crystal structure of perdeuterated (i.e., fully deuterated) human arginase I complexed with 2-amino-6-boronohexanoic acid (ABH) at 1.90 Å resolution. Prior to the neutron diffraction experiment, it is important to establish that perdeuteration does not cause any unanticipated structural or functional changes. Accordingly, we find that perdeuterated human arginase I exhibits catalytic activity essentially identical to that of the unlabeled enzyme. Additionally, the structure of the perdeuterated human arginase I-ABH complex is identical to that of the corresponding complex with the unlabeled enzyme. Therefore, we conclude that crystals of the perdeuterated human arginase I-ABH complex are suitable for neutron crystallographic study.

Keywords

protein crystallography; enzyme-inhibitor complex; isotopic labeling

Arginase catalyzes the hydrolysis of L-arginine to yield L-ornithine and urea [1]. In humans there are two isozymes, arginase I and arginase II, that share ~60% sequence identity. Ornithine is the biosynthetic precursor of polyamines that facilitate cellular proliferation and tumor growth [2]. Additionally, arginase I is implicated in tumoral immune evasion, and arginase inhibitors therefore have significant chemotherapeutic potential [2].

*This work was supported by NIH grant GM49758.

* * Atomic coordinates of perdeuterated human arginase I complexed with 2(S)-amino-6-boronohexanoic acid have been deposited in the Research Collaboratory for Structural Bioinformatics (<http://www.rcsb.org/pdb>) with the following accession code: 2PLL.

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The crystal structure of unlabeled arginase I from rat liver (*Rattus norvegicus*) was solved 10 years ago [3] at 2.1 Å resolution, and since then more than 40 additional arginase structures have been determined and deposited in the Protein Data Bank (<http://www.rcsb.org>; [4]). These structures include those of the human isozymes arginase I [5] and arginase II [6], arginase from *Bacillus caldovelox* [7], arginase from *Thermus thermophilus* (PDB accession codes 2EF4, 2EF5, and 2EIV), and several complexes of rat arginase I with different ligands and inhibitors, as well as various mutants.

Arginase adopts an α/β fold consisting of a central parallel β -sheet flanked on both sides by several α -helices [3]. Surprisingly, the subsequent structure determination of histone deacetylase revealed a fold topologically identical to that of arginase [8–10], which has recently been classified as “Rossmannoid” [11–13]. All mammalian arginases are heterologous trimers with C_3 symmetry. However, the bacterial arginase is a hexamer in which two trimers associate in face-to-face fashion with overall D_3 symmetry.

The structure of unlabeled rat arginase I reveals that the metal cluster required for catalysis is located at the bottom of a ~15 Å-deep cavity in each monomer, and a metal-bridging hydroxide ion functions as a nucleophile in catalysis [3]. The metal-bridging hydroxide is also required for the binding of 2(*S*)-amino-6-boronoheptanoic acid (ABH), a reactive substrate analogue that undergoes nucleophilic attack by this hydroxide ion to yield a tetrahedral boronate anion that mimics the tetrahedral intermediate in catalysis [14]. Recently, we demonstrated that ABH and the related analogue *S*-(2-boronoethyl)-L-cysteine (BEC) are highly potent inhibitors of human arginase I with K_d values of 5 and 270 nM, respectively [5]. Analysis of the 1.29 Å resolution structure of the human arginase I-ABH complex reveals that nanomolar affinity is a consequence of strong metal-coordination and hydrogen bond interactions [5]. Furthermore, this structure reveals new mechanistic inferences on the catalytic function of H141, which appears to be stabilized as the positively charged imidazolium group. This residue may accordingly serve as a general acid to facilitate collapse of the tetrahedral intermediate in catalysis.

The structure of the human arginase I-ABH complex is the highest resolution structure of an arginase ever determined. Intriguingly, the positions of some putative ordered hydrogen atoms are evident in electron density maps, including the acidic proton of the H141 imidazolium group [5]. However, the limiting resolution of the X-ray crystal structure determination (1.29 Å) is near the threshold of reliability for the determination of hydrogen atom positions due to the weak electron density of the hydrogen atom. Therefore, we are now preparing for the neutron diffraction study of the human arginase I-ABH complex in order to locate active site hydrogen/deuterium atoms important for inhibitor binding and catalysis. In order to enhance the signal-to-noise ratio of measurable neutron diffraction in our forthcoming experiments, we have prepared perdeuterated (i. e., fully deuterated) human arginase I and we have determined the X-ray crystal structure of its complex with ABH at 1.90 Å resolution from hemihedrally twinned crystals. The structure of perdeuterated human arginase I complexed with ABH is very similar to the structure of the unlabeled human arginase I-ABH complex, including the positions of solvent molecules interacting with ABH. Activity measurements demonstrate that perdeuteration does not significantly affect the catalytic function of human arginase I. This contrasts with activity measurements of perdeuterated glutathione *S*-transferase [15] and alkaline phosphatase [16], in which catalytic activity increases 1.4-fold and decreases 1.8-fold respectively, upon perdeuteration.

Materials and methods

Expression and purification of perdeuterated human arginase I

For protein expression in perdeuterated media, the full length cDNA of human arginase I was subcloned in a pET-24a (Novagen) vector that confers kanamycin resistance. Perdeuterated human arginase I was obtained by heterologous expression in *Escherichia coli* BL21(DE3) at the ILL-EMBL Deuteration Facility in Grenoble, France. Cells were grown in minimal medium: 6.86 g L⁻¹ (NH₄)₂SO₄, 1.56 g L⁻¹ KH₂PO₄, 6.48 g L⁻¹ Na₂HPO₄·2H₂O, 0.49 g L⁻¹ diammonium hydrogen citrate, 0.25 g L⁻¹ MgSO₄·7H₂O, 1.0 ml L⁻¹ (0.5 g L⁻¹ CaCl₂·2H₂O, 16.7 g L⁻¹ FeCl₃·6H₂O, 0.18 g L⁻¹ ZnSO₄·7H₂O, 0.16 g L⁻¹ CuSO₄·5H₂O, 0.15 g L⁻¹ MnSO₄·4H₂O, 0.18 g L⁻¹ CoCl₂·6H₂O, 20.1 g L⁻¹ EDTA), 5 g L⁻¹ glycerol, 40 mg L⁻¹ kanamycin [17,18]. For preparation of fully deuterated medium, mineral salts were dried out in a rotary evaporator (Heidolph) at 333K and labile protons were exchanged for deuterons by dissolving in a minimal volume of D₂O and re-dried. Perdeuterated d₈-glycerol (Euriso-Top, France) was used as a carbon source. Adaptation of BL21(DE3) cells to deuterated minimal medium was achieved by a multi-stage adaptation process [18]. Typically, 1.5 L of deuterated medium was inoculated with 100 mL preculture of adapted cells in a 3 L fermenter (Labfors, Infors). During the batch and fed-batch phases the pH was adjusted to 6.9 (by addition of NaOD) and the temperature was adjusted to 303 K. The gas-flow rate of sterile filtered air was 0.5 L min⁻¹. Stirring was adjusted to ensure a dissolved oxygen tension (DOT) of 30%. The fed-batch phase was initiated when the optical density at 600 nm reached 6.0. D₈-glycerol was added to the culture to keep the growth rate stable during fermentation. When OD₆₀₀ reached 12, arginase overexpression was induced by the addition of 1 mM IPTG and incubation continued for 24 h. Cells were then harvested, washed with 10 mM HEPES (pH 6.4), and stored at 193 K. Perdeuterated human arginase I was purified as described for the unlabeled enzyme [19] and all buffers used during purification were made with H₂O. Protein purity was assessed by SDS-PAGE and the molecular weight of perdeuterated human arginase I was determined by MALDI mass spectrometry.

Crystallization and X-ray diffraction data collection

The complex between perdeuterated human arginase I and ABH was crystallized by the sitting drop vapor diffusion method at 294 K. An initial Index Screen (Hampton Research) identified fourteen different conditions under which crystals appeared overnight. In particular, because of the formation of fewer nuclei and bigger crystals, one condition was most suitable for growing larger crystals. Drops containing 4.0 µL of enzyme solution [3.5 mg/mL perdeuterated human arginase I, 0.1 mM MnCl₂, 1.4 mM ABH, 50.0 mM bicine (pH 8.5)] and 4.0 µL of precipitant buffer [0.1 M Bis-Tris-HCl (pH 5.5), 10–20% (wt/vol) PEG-3350] were equilibrated against a 1 mL reservoir of precipitant buffer. Rod-like crystals appeared in approximately 1–2 days and grew to typical dimensions of 0.2 mm × 0.2 mm × 0.5 mm in one week. These crystallization conditions differed slightly from those initially employed in the crystallization of the unlabeled enzyme in terms of protein concentrations, pH, and the variety of PEG used as a precipitant [5].

Prior to X-ray diffraction data collection, exchangeable protons were substituted with deuterons by equilibrating crystals in precipitant buffer prepared with D₂O and 100 mM Bis-Tris-DCI (pD 6.6) for three days. Crystals were cryoprotected in mother liquor containing 28% glycerol-d₈ prior to flash-cooling and yielded diffraction data to 1.90 Å resolution at the Brookhaven National Laboratory (Upton, NY) on beamline X29A (λ=1.000 Å, 100 K) using an ADSC Quantum 315 detector. Data reduction was achieved with Mosflm [20]. X-ray diffraction intensities measured from these crystals exhibited symmetry consistent with the apparent space group P6 (unit cell parameters a = b = 90.8 Å, c = 69.5 Å). The analysis of measured intensities revealed deviations from ideal Wilson statistics with $\langle I^2 \rangle / \langle I \rangle^2 \approx 1.5$

(statistical analyses were performed using the Merohebral Crystal Twinning Server at the UCLA-DOE Institute for Genomics and Proteomics, <http://www.doe-mbi.ucla.edu>), indicative of perfect hemihedral twinning (i.e., twin fraction = 0.5) that convoluted the true crystallographic $P3$ symmetry. The unit cell parameters were characteristic for a twinned crystal, in that the c -axis of the twinned crystal was one-half the length of the c -axis of untwinned rat arginase I crystals belonging to space group $P3_2$ [3]. Similar crystal twinning complications were encountered in the structure determinations of unlabeled (i.e., non-deuterated) human arginase I complexed with ABH [5], the E256Q unlabeled human arginase I-BEC complex (PDB accession code 1WVB), the rat arginase I-ABH complex [14], the rat arginase I-BEC complex [21], and the E101H rat arginase I-BEC complex [22].

Structure determination and refinement

The structure of perdeuterated human arginase I was solved by molecular replacement using the program *Phaser* [23] with chain A of the human arginase I-ABH complex (PDB accession code 2AEB, less inhibitor atoms and water molecules [5]) used as a search probe against twinned data. This yielded a solution that effectively satisfied one-half of the information in the diffraction pattern. The optimal solution for the positioning of two monomers in the asymmetric unit of twin domain A yielded a total log-likelihood gain of 3442 [24], a rotation function Z score (RFZ) = 16.6, and a translational function Z score (TFZ) = 25.5, for the first monomer, and RFZ = 15.7 and TFZ = 50.0 for the second monomer, using data in the 24 – 2.5 Å resolution range. The Eulerian angles for the orientations of monomers A and B in the asymmetric unit of twin domain A were $\alpha = 162.7^\circ$, $\beta = 0.2^\circ$, $\gamma = 317.2^\circ$ and $\alpha = 123.4^\circ$, $\beta = 2.0^\circ$, $\gamma = 183.1^\circ$, respectively, and the corresponding translational searches yielded orthogonal coordinates $x = -48.80$ Å, $y = 78.99$ Å, $z = -68.04$ Å and $x = 47.60$ Å, $y = -19.99$ Å, $z = -105.27$ Å. The positions of monomers A and B in twin domain B could be generated by application of the twofold twin operator parallel to the c -axis (i.e., corresponding to the hemihedral twin law for space group $P3$).

In order to calculate electron density maps, structure factor amplitudes ($|F_{\text{obs}}|$) derived from twinned data ($|I_{\text{obs}}|$) were deconvoluted into structure factor amplitudes corresponding to individual twin domains A and B ($|F_{\text{obs}/A}|$ and $|F_{\text{obs}/B}|$, respectively) using the structure-based algorithm implemented in CNS [25,26]. This approach utilized the initial molecular replacement model to estimate the crystallographic intensities $I_{\text{obs}/A}$ and $I_{\text{obs}/B}$ corresponding to individual twin domains A and B. Difference electron density maps calculated with Fourier coefficients $|F_{\text{obs}/A}| - |F_{\text{calc}/A}|$ or $|F_{\text{obs}/B}| - |F_{\text{calc}/B}|$ and phases derived from the respective model (twin domain A or twin domain B) were used to guide the adjustment of the protein and solvent atoms iteratively with refinement. The protein models in twin domains A and B were refined simultaneously against observed structure factor amplitudes derived from twinned intensities by minimizing the expression $\Sigma[|F_{\text{calc}/A}|^2 + |F_{\text{calc}/B}|^2]^{1/2} - |F_{\text{obs}}|$. Rigid body refinement of the initial molecular replacement model yielded $R_{\text{twin}}/R_{\text{free/twin}} = 0.256/0.242$.

Iterative cycles of refinement using torsion angle dynamics with a starting temperature of 5000 K in the initial stages of the refinement, model building, and minimization using CNS [26] and O [27] improved the protein structure as monitored by R_{twin} and $R_{\text{free/twin}}$. Strict noncrystallographic symmetry restraints were used during refinement and released with the appropriate weighting scheme as judged by $R_{\text{free/twin}}$ and the residual Fourier map as refinement progressed. Group B-factors were utilized during refinement. In the final stages of refinement the majority of water molecules were automatically fit into residual Fourier density using a cutoff of 3.0 σ , which improved R_{twin} and $R_{\text{free/twin}}$ to the values reported in Table 1. A total of 320 water molecules were included in the refinement. The omit electron density map calculated for the perdeuterated arginase I-ABH complex revealed clear peaks corresponding to the unlabeled inhibitor ABH, which was fit and refined with full occupancy to an average

B-factor of 20 Å², consistent with the average B-factor of 16 Å² calculated for the entire protein. Disordered segments at the N- and C-termini (M1-K4 and N319-K322) were absent in the experimental electron density and were omitted from the final model.

The quality of the final model was checked using the software MolProbity (<http://molprobity.biochem.duke.edu>) and Verify3d [28]; the root-mean-square (r.m.s.) and mean distance of models were calculated using the program Top3d, [29]. Finally, figures were made using the program Pymol [30].

Enzyme assay

Perdeuterated human arginase I was assayed for arginase activity using a modified version of the radioactive assay reported by Rüegg and Russell [31]. A typical reaction mixture in H₂O contained 50 mM bicine (pH 8.5), 100 µM MnCl₂, and 0.05 µM L-[guanidino-¹⁴C]arginine in a total volume of 40 µL per each reaction tube. The L-[guanidino-¹⁴C]arginine (specific activity 1.9 GBq mmol⁻¹) was purchased from PerkinElmer Life Sciences. Typically, 5 µL of increasing concentrations of unlabeled L-arginine were added to each 40 µL reaction mixture such that the final L-arginine concentrations were 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, 30.0 and 40.0 mM. Reactions were initiated by the addition of 5 µL of a solution containing 0.47 µM arginase to each reaction mixture. After 5 min, reactions were stopped by the addition of 400 µL of stop solution (0.25 M acetic acid (pH 4.5), 7 M urea, and 1:1 (v/v) slurry of Dowex 50 W-X8 in water) and then vortexed. Reaction samples were then gently mixed for 10 min and centrifuged at 6000 rpm for 4 min. A total volume of 3 mL of Ecoscint solution was added to 200 µL from each supernatant for liquid scintillation counting using a Beckman model LS5000E counter. The counts-per-minute were measured three times for each reaction mixture and averaged. As a control, the same assay was conducted with unlabeled human arginase I. The K_M and V_{max} values were determined from Lineweaver-Burke plots. A similar experiment was performed for the activity measurement of the perdeuterated protein in D₂O with minor modifications: perdeuterated human arginase I was dialyzed against a buffer solution containing 50 mM bicine (pD = 8.5), 100 µM MnCl₂ and the L-arginine solutions were prepared in D₂O.

Mass spectra determination

The extent of perdeuteration in human arginase I was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) using an Applied Biosystems Voyager DE PRO MALDI TOF Mass Spectrometer with an accelerating voltage of 25000 V.

Results

Perdeuterated human arginase I is easily expressed in *E. coli*, yielding approximately 5 mg of protein from 2 g of cell paste. A similar yield is obtained for the expression of unlabeled human arginase I. The purity of the protein preparation is ~98% as determined by SDS-PAGE analysis (data not shown). MALDI mass spectra indicate that the molecular weight of the perdeuterated protein is 37,026 when prepared in D₂O buffer (Figure 1). For reference, the theoretical molecular weight of unlabeled human arginase I is 34,735 Da and that of perdeuterated human arginase I is 37,236 Da, so the extent of perdeuteration is calculated to be [(37,026–34,735)/(37,236–34,735)]×100% = 92%. The molecular weight of the perdeuterated protein is 36,801 Da when transferred to H₂O buffer (Figure 1), and its theoretical molecular weight is 36,701 Da with all exchangeable deuterons substituted by protons. From these data, the extent of perdeuteration is calculated to be [(36,081–34,735)/(36,701–34,735)]×100% = 105%, indicating that not all exchangeable deuterons are substituted by protons in the protein sample transferred to H₂O buffer. For example, some exchangeable deuterons are deeply embedded

in the structure and not easily accessible to solvent. Regardless, we conclude that perdeuterated human arginase I is almost completely deuterium-labeled and therefore suitable for neutron diffraction experiments.

Perdeuterated human arginase I exhibits a K_M value of 7.6 mM at pH 8.5 for L-arginine hydrolysis in H_2O buffer and 7.7 mM in D_2O buffer (the unlabeled enzyme exhibits a K_M value of 8.3 mM). Perdeuterated human arginase exhibits $V_{max} = 1.5 \times 10^{-3} \mu\text{mol}\cdot\text{s}^{-1}$, in both H_2O buffer and D_2O buffer, which is not significantly different from the value of $1.9 \times 10^{-3} \mu\text{mol}\cdot\text{s}^{-1}$ measured for the unlabeled enzyme. Thus, perdeuterated human arginase I does not exhibit a solvent isotope effect. We conclude that the perdeuteration of the protein has a negligible effect on catalytic activity.

The asymmetric unit in the structure of perdeuterated human arginase I complexed with ABH complex contains two monomers from two different trimers related by a noncrystallographic two-fold screw axis parallel to the crystallographic c -axis (Figure 2). Each monomer contains two manganese ions and one ABH molecule bound as the tetrahedral boronate anion. The root-mean-square (r.m.s.) deviation is 0.25 Å for 314 C α atoms between the structures of unlabeled and perdeuterated human arginase I-ABH complexes [5] (Figure 3). Despite the substitution of all protons with deuterons, the binuclear manganese cluster in the perdeuterated human arginase I-ABH complex is nearly identical in structure to that of the unlabeled human arginase I-ABH complex [5]. The tetrahedral boronate anion of ABH coordinates to the metal cluster (Figure 4).

A simulated annealing omit map of ABH reveals that the trigonal planar boronic acid moiety of the inhibitor undergoes nucleophilic attack by the metal-bridging deuteroxide anion to yield a tetrahedral trideuteroxyl boronate anion (Figures 4 and 5). The interactions of the boronate anion are identical to those observed in the unlabeled enzyme complex with ABH [5], and the chemistry accompanying inhibitor binding to the perdeuterated enzyme is identical to that accommodating inhibitor binding to the unlabeled enzyme. Therefore, we conclude that perdeuteration has not compromised chemical function in the active site. Boronate deuteroxyl group O1 symmetrically bridges Mn^{2+}_A and Mn^{2+}_B with an average metal-oxygen separation of 2.3 Å, boronate deuteroxyl group O2 interacts with Mn^{2+}_A (2.5 Å) and hydrogen bonds with His-141 and Glu-277, and boronate deuteroxyl group O3 interacts with Thr-246 through an intervening solvent molecule. The α -carboxylate and α -amino groups of ABH are anchored to the active site of human arginase I by three direct and four deuterium oxide-mediated hydrogen bonds with protein residues, and their positions are comparable to those of waters molecules observed in the unlabeled enzyme complex with ABH [5] (Figure 6).

Discussion

Despite the substitution of nearly all hydrogen atoms with deuterium atoms, the three-dimensional structure of the perdeuterated human arginase I-ABH complex is essentially identical to that of the unlabeled enzyme-inhibitor complex. The essentially complete deuteration of the enzyme has essentially no effect on catalysis in H_2O or D_2O , with V_{max} and K_M values comparable to those of the unlabeled enzyme in H_2O . Although crystals of the unlabeled human arginase I-ABH complex typically grow to a large size (1.8 mm $\times$ 0.3 mm $\times$ 0.3 mm, volume = 0.16 mm³), it has not been possible to significantly improve the resolution limit of X-ray diffraction beyond that of 1.29 Å previously reported [5]. Therefore, the neutron crystallographic structure determination of the human arginase I-ABH complex represents the sole possible approach for the conclusive analysis of protonation/deuteration states of catalytic residues such as His-141.

Even though the pioneering neutron crystal structure determinations of myoglobin and trypsin were reported decades ago [32,33], there are only 36 protein structures solved by neutron crystallographic methods [34] and 23 are currently deposited in the PDB [4]. This is a very small number in comparison with the total number of 42,082 X-ray crystal structures currently deposited in the PDB. Crystal volume generally represents the “bottleneck” for neutron crystallographic experiments at currently available neutron sources, and macromolecules with larger unit cells require an even larger crystal volume in order to measure diffraction [34]. However, protein perdeuteration improves the neutron diffraction signal measurable from smaller crystals with volumes of $\sim 0.1 \text{ mm}^3$ [34,35], with 40-fold reductions in background scattering [34,36]. For example, crystals of perdeuterated human aldose reductase with volume $\approx 0.15 \text{ mm}^3$ yield 2.2 Å resolution neutron diffraction data [35]. Since (1) perdeuterated human arginase I is fully catalytically active, (2) the X-ray crystal structure of perdeuterated human arginase I-ABH complex is essentially identical to that of the unlabeled enzyme complex, and (3) crystals of the perdeuterated human arginase I-ABH complex typically grow to a volume of 0.16 mm^3 , we conclude that the neutron structure determination of this complex is now feasible and will be studied in due course.

Acknowledgements

We thank the National Synchrotron Light Source at the Brookhaven National Laboratory (beamline X29A) for beamline access and Prof. Francisco Centeno for sharing the initial plasmid of human arginase I. F.M. acknowledges support from the NC Agricultural Research Service and DOE/BES.

References

- Christianson DW. *Acc Chem Res* 2005;101:191–201. [PubMed: 15766238]
- Muller AJ, Scherle PA. *Nat Rev Cancer* 2006;6:613–625. [PubMed: 16862192]
- Kanyo ZF, Scolnick LR, Ash DE, Christianson DW. *Nature (London)* 1996;383:554–557. [PubMed: 8849731]
- Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K, Feng Z, Gilliland GL, Iype L, Jain S, Fagan P, Marvin J, Padilla D, Ravichandran V, Schneider B, Thanki N, Weissig H, Westbrook JD, Zardecki C. *Acta Cryst D Biol Crystallogr* 2002;58:899–907. [PubMed: 12037327]
- Di Costanzo L, Sabio G, Mora A, Rodriguez PC, Ochoa AC, Centeno F, Christianson DW. *Proc Natl Acad Sci USA* 2005;102:13058–13063. [PubMed: 16141327]
- 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]
- Bewley MC, Jeffrey PD, Patchett ML, Kanyo ZF, Baker EN. *Structure* 1999;7:435–448. [PubMed: 10196128]
- Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP. *Nature (London)* 1999;401:188–193. [PubMed: 10490031]
- Somoza JR, Skene RJ, Katz BA, Mol C, Ho JD, Jennings AJ, Luong C, Arvai A, Buggy JJ, Chi E, Tang J, Sang BC, Verner E, Wynands R, Leahy EM, Dougan DR, Snell G, Navre M, Knuth MW, Swanson RV, McRee DE, Tar LW. *Structure* 2004;12:1325–1334. [PubMed: 15242608]
- Vannini A, Volpari C, Filocamo G, Casavola EC, Brunetti M, Renzoni D, Chakravarty P, Paolini C, De Francesco R, Gallinari P, Steinkühler C, Di Marco S. *Proc Natl Acad Sci USA* 2004;101:15064–15069. [PubMed: 15477595]
- Burroughs AM, Allen KN, Dunaway-Mariano D, Aravind L. *J Mol Biol* 2006;361:1003–1034. [PubMed: 16889794]
- Aravind L, Iyer LM, Koonin EV. *Curr Opin Struct Biol* 2006;16:409–419. [PubMed: 16679012]
- Rossmann MG, Moras D, Olsen KW. *Nature (London)* 1974;463:194–199. [PubMed: 4368490]
- Cox JD, Kim NN, Traish AM, Christianson DW. *Nat Struct Biol* 1999;6:1043–1047. [PubMed: 10542097]
- Brockwell D, Yu L, Cooper S, McClelland S, Cooper A, Attwood D, Gaskell SJ, Barber J. *Protein Sci* 2001;10:572–580. [PubMed: 11344325]

16. Rokop S, Gajda L, Parmerter S, Crespi HL, Katz JJ. *Biochim Biophys Acta* 1969;191:707–715. [PubMed: 4903503]
17. Meilleur F, Contzen J, Myles DA, Jung C. *Biochemistry* 2004;43:8744–8753. [PubMed: 15236583]
18. Artero JB, Hartlein M, McSweeney S, Timmins P. *Acta Cryst D Biol Crystallogr* 2005;61:1541–1549. [PubMed: 16239733]
19. Mora A, del Ara Rangel M, Fuentes JM, Soler G, Centeno F. *Biochim Biophys Acta* 2000;1476:181–190. [PubMed: 10669784]
20. Leslie, AGW. *Crystallography Computing 5*. In: Moras, D.; Podjarny, AD.; Thierry, JC., editors. *From Chemistry to Biology*. Oxford: 1991. p. 50–61.
21. Kim NN, Cox JD, Baggio RF, Emig FA, Mistry SK, Harper SL, Speicher DW, Morris SM, Ash DE, Traish AM, Christianson DW. *Biochemistry* 2001;40:2678–2688. [PubMed: 11258879]
22. Cama E, Emig FA, Ash DE, Christianson DW. *Biochemistry* 2003;42:7748–7758. [PubMed: 12820884]
23. McCoy AJ, Grosse-Kunstleve RW, Storni LC, Read RJ. *Acta Cryst D Biol Crystallogr* 2005;61:458–464. [PubMed: 15805601]
24. Read RJ. *Acta Cryst D Biol Crystallogr* 2001;57:1373–1382. [PubMed: 11567148]
25. Redinbo MR, Yeates TO. *Acta Cryst D Biol Crystallogr* 1993;49:375–380. [PubMed: 15299512]
26. Brünger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. *Acta Cryst D Biol Crystallogr* 1998;54:905–921. [PubMed: 9757107]
27. Jones TA, Zou JY, Cowan SW, Kjeldgaard M. *Acta Cryst A* 1991;47:110–119. [PubMed: 2025413]
28. Eisenberg D, Luthy R, Bowie JU. *Methods Enzymol* 1997;277:396–404. [PubMed: 9379925]
29. Collaborative Computational Project Number 4. *Acta Cryst D Biol Crystallogr* 1994;50:760–763. [PubMed: 15299374]
30. DeLano, WL. *The PyMOL Molecular Graphics System*. 2002.
31. Rüegg UT, Russell AS. *Anal Biochem* 1980;102:206–212. [PubMed: 7356155]
32. Schoenborn BP. *Nature (London)* 1969;224:143–146. [PubMed: 5343513]
33. Kossiakoff AA, Spencer SA. *Nature (London)* 1980;288:414–416. [PubMed: 7432541]
34. Meilleur F, Myles DA, Blakeley MP. *Eur Biophys J* 2006;35:611–620. [PubMed: 16897039]
35. Hazemann I, Dauvergne MT, Blakeley MP, Meilleur F, Haertlein M, Van Dorselaer A, Mitschler A, Myles DA, Podjarny A. *Acta Cryst D Biol Crystallogr* 2005;61:1413–1417. [PubMed: 16204895]
36. Shu F, Ramakrishnan V, Schoenborn BP. *Proc Natl Acad Sci USA* 2000;97:3872–3877. [PubMed: 10725379]
37. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. *J Appl Cryst* 1993;26:283–291.

Figure 1. MALDI mass spectra of perdeuterated human arginase I prepared in D₂O (right) and H₂O (left); ionized species with charges 1+ and 2+ are evident in the spectra of samples prepared in H₂O.

Figure 2.

The asymmetric unit of twin domain A of hemihedrally twinned perdeuterated arginase I-ABH contains two monomers (red) related by a twofold screw axis parallel to the crystallographic *c*-axis. Each monomer completes a separate trimer by application of the crystallographic threefold-axis corresponding to space group *P*3 (blue monomers). Twin domain B is generated by the application of the twofold twin operator parallel to the *c*-axis.

Figure 3.

Least-squares superposition of monomer C α traces of the perdeuterated human arginase I-ABH complex (blue) and the unlabeled (i. e., non-deuterated) human arginase I-ABH complex (red) (pdb accession code: 2AEB). The active site Mn²⁺ ions are represented as spheres and ABH is shown as a color-coded stick figure. The atoms of ABH are color-coded as follows: carbon (yellow), oxygen (red), nitrogen (blue), and boron (green).

Figure 4.

(a) Simulated annealing omit map contoured at 2.6σ generated with Fourier coefficients $|F_{obs}/A| - |F_{calc}/A|$ for twin domain A and phases calculated from the refined perdeuterated human arginase I-ABH complex less the nonhydrogen atoms of ABH. Atoms are color-coded as follows: carbon (yellow), oxygen (red), nitrogen (blue), manganese (pink) and boron (light green). (b) Perdeuterated human arginase I-ABH hydrogen bond interactions. Dashed lines indicate manganese coordination (red) and hydrogen bond (green) interactions of ABH. For clarity only the deuterium oxide molecules (red spheres) interacting with ABH are indicated.

Figure 5.

Scheme summarizing intermolecular interactions in the perdeuterated human arginase I-ABH complex. Manganese coordination interactions are indicated by green dashed lines, and hydrogen bonds are indicated by black dashed lines. The dideuteroxyl boronic acid moiety of ABH is proposed to undergo nucleophilic attack by the metal-bridging deuteroxide ion to yield a tetrahedral trideuteroxyl boronate anion.

Figure 6.

Superposition of perdeuterated human arginase I-ABH complex (blue) and unlabeled human arginase I (red) structures. Selected active site residues, water/deuterium oxide molecules, Mn^{2+} ions, and ABH are shown. Notably, the positions of solvent molecules interacting with ABH are nearly perfectly conserved.

Table 1

Data Collection and Refinement Statistics

	Perdeuterated Human Arginase I-ABH complex
Data collection	
Resolution, Å	69.5 - 1.90
Total reflections measured	65304 (9534)
Unique reflections measured	46244 (6862)
R_{merge}^a	0.089 (0.37) ^b
$I/\sigma(I)$	7.5 (2.0) ^b
Completeness (%)	91.5 (93.1) ^b
Multiplicity	1.4 (1.4) ^b
Refinement	
Reflections used in refinement, test	42382/1908
$R_{\text{twin}}/R_{\text{twin/free}}^c$	0.141 (0.235)/0.201 (0.298)
Protein atoms ^d	4776
Water molecules ^d	359
ABH atoms ^d	26
Manganese ions ^d	4
r.m.s. deviations	
Bond lengths, Å	0.007
Bond angles, °	1.4
Dihedral angles, °	22.7
Improper dihedral angles, °	0.9
Average B-factors	
Main chain	15
Side chain	17
Manganese ions	11
ABH	20
Deuterium oxide molecules	22
Ramachandran plot^e	
Allowed (%)	88.2
Additionally allowed (%)	11.2
Generously allowed (%)	0
Disallowed (%)	0.6

^a $R_{\text{merge}} = \sum \sum |I_{hi} - \langle I_h \rangle| / \sum \sum \langle I_h \rangle$, where I_{hi} is the scaled intensity for reflection h in data set “ i ” and $\langle I_h \rangle$ is the mean intensity of reflection h from replicate data.

^bNumber in parentheses refer to the outer 0.1 Å shell of data.

^c $R_{\text{twin}} = \sum [|F_{\text{calc}}/A|^2 + |F_{\text{calc}}/B|^2]^{1/2} - F_{\text{obs}} / \sum F_{\text{obs}}$ for reflections contained in the working set. $|F_{\text{calc}}/A|$ and $|F_{\text{calc}}/B|$ 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 [25]. The same expression describes $R_{\text{twin/free}}$, which is calculated for test set reflections excluded from refinement.

^dPer asymmetric unit.

^eRamachandran plot statistics calculated for non-proline and non-glycine residues using PROCHECK [37].